Handbook of Depression and Anxiety

Second Edition Revised and Expanded

edited by

Siegfried Kasper
Johan A. den Boer
J. M. Ad Sitsen

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Series Introduction

Depression and anxiety, both externally experienced and internally perceived, are part of the normal human repertoire of response to stress. In my opinion, those who never experience such feelings are seriously ill, unable to recognize or respond appropriately to the importance of danger and loss. On the other hand, the capacity to tolerate a "normal expectable" level of each is a sign of mental health. Unfortunately, however, many of us are unable to withstand the impact of the usual vicissitudes of life and are overwhelmed by excessive stress or chronic strain. The stressors may be as sudden as the events of 9/11, the diagnosis of a life-threatening disease, or the loss of someone we love, or they may be an accumulation of the ordinary stresses of life at work and at home, with family and friends. Symptoms of depression and/or anxiety may develop, sometimes assorted into identifiable syndromes and, at times, representing specific disease states.

The chapters of this volume provide us with background information, such as the conceptual history of our understanding of anxiety and depression, their epidemiology and genetics. They also provide insight into our current knowledge of the physiology and pathology of both anxiety and depression, and information about up-to-date treatment approaches for both acute and chronic presentations.

This volume should be kept on our desks, readily accessible for consultation when we need help with understanding the difficult issues that bring people to us in search of relief. It is a "vade-mecum"—a useful manual of what we now know about the biological and pharmacological treatments of depression and anxiety, and an invaluable resource for all who care for the afflicted, important for general practitioners as well as for psychiatrists and others in the mental health fields. The internationally renowned group of contributors illustrate a growing consensus that anxiety and depression are both symptom and syndrome, and, in some instances, disease. It is time to apply this understanding to the practice of medicine.

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Preface

Anxiety and depression are complex emotional states in which cognitive evaluations and affective and physiological responses are involved. Considering the complexity of these emotions, which can be described in several scientific languages at different levels of analysis, there is no doubt that multiple neuronal systems in the brain are implicated in the pathophysiology of these closely related disorders.

On a phenomenological level, there is a high degree of overlap between symptoms of anxiety and depression as well as a high degree of comorbidity. This does not imply, however, that both syndromes are merely different phenotypical expressions of a genetically based constitution sharing similar biological underpinnings. The question of whether anxiety and depression result from similar or different biological abnormalities cannot be answered using one research strategy. On the one hand, psychophysiological research provides evidence suggesting that anxiety and depression are clearly distinct disorders; on the other hand, some anxiety disorders and depressive syndromes respond to similar pharmacological interventions. Thus, these conflicting findings from different biomedical fields of research present a challenge to all of us involved in the study of these very common and often debilitating psychiatric illnesses.

Some investigators argue that distinguishing different subtypes among depressive syndromes and anxiety disorders is a somewhat artificial practice. They reason that a syndrome exists based upon a common underlying propensity toward ''nervousness.'' This (dimensional) viewpoint implies that the different diagnostic categories could be merely phenotypical manifestations of an underlying vulnerability to anxiety or depression.

Over the last decade, our knowledge about the biological underpinnings of depression and anxiety disorders has increased substantially, particularly for anxiety disorders, and new insights are continually emerging from widely disparate fields, such as epidemiology, genetics, immunology, psychophysiology, and psychopharmacology. This volume brings together these different disciplines and reviews the state of the art in research on anxiety and depression and their interrelatedness.

We consider ourselves fortunate to have succeeded in getting so many internationally renowned contributors, who lend to this volume their knowledge and expertise in

these disciplines. We hope that scientists and clinicians from many fields will be inspired by the exciting findings presented in this book.

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1

A Conceptual History of Anxiety and Depression

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I. INTRODUCTION

For more than 2500 years, physicians have distinguished the clinical conditions we call affective or anxiety disorder from such everyday feelings as fear, restlessness, and despondency. Contrary to what might be expected, case descriptions from the past often bear remarkable resemblances to patients encountered in modern-day clinical practice. Whether one consults Aristotle, Galen, Burton, or the 19th-century alienists, images of a suggestive reality are evoked, images in which we can easily recognize the depressive, anxious, and melancholic individual of our own era. These are similarities in symptomatology and course, as well as in the distinction between normality and pathology.

On the other hand, there are also considerable disparities in language and frame of reference, conceptualization, and interpretation. From the time of Hippocrates until well into the 17th century, the description and interpretation of anxiety and depression were dominated by the doctrine of bodily fluids (humors). And, until quite recently, all manner of ideas involving neural energy overshadowed discussions of phenomena such as neurasthenia, inhibition, and motor agitation.

These disparities have traditionally been given particular emphasis. The undeniably impressive growth of our knowledge is seen as evidence of the superiority of contemporary explanatory models. Conversely, ideas that were current from antiquity until the 19th century are considered to be of no more than historical interest—simply a fanciful mythology for enthusiasts. The history of medicine has become a somewhat antiquated study, pursued by a handful of specialists.

This may or may not be considered regrettable. However, these disparities once again become relevant at a deeper level of discussion for clinicians as well as for scientific investigators. I refer here to the level of medicine's basic concepts and to the cultural and ideological strata from which these concepts derive their meaning.

A consideration of the foundations of medicine helps, for example, to put into perspective the already overly strict separation of symptoms and explanatory models. Symptoms are not natural phenomena in that they are not the invariable expressions of an unchanging biological substratum. Whatever one's concept of disease, what the patient says is always based upon interpretation, at least to a certain extent. That interpretation can be colored by whatever is considered to be normal or abnormal in a particular culture. Explanatory models, on the other hand, are not simply conjured up out of the blue. They are neither arbitrary nor coincidental, but are instead closely linked to whatever paradigms are currently fashionable in the various branches of science. Moreover, they are always interpretations of a reality that is already interpreted by the afflicted person and those around him.

Accordingly, we cannot pretend that depression and anxiety are natural phenomena that have consistently been expressed and experienced in the same way down through the centuries. The view according to which only the explanatory models have changed and not the phenomena themselves should be rejected. Concentrating purely on differences at the level of the explanatory models can easily turn the history of medicine into a study of scientific folklore, as if, with the passage of time, only the explanatory models have undergone change and not the signs and symptoms of the disorders. Notwithstanding the above-mentioned similarities in clinical picture and course, the symptoms of anxiety and depression also have changed (i.e., their relevance to what counts as disease and their meaning as an expression of disease).

Seen from this point of view, the study of the history of medicine suddenly becomes extremely relevant to a clear understanding of all sorts of current explanatory models. The medical history of anxiety and depression is, therefore, not simply concerned with internal scientific development. It also involves the interplay of cultural changes and psychopathological phenomena, including the scientific interpretations of such phenomena. In this chapter, several leading concepts in the history of anxiety and depression will be summarized. Instead of delving into historical detail, the emphasis will be on concepts and, particularly, on the paradigm shifts associated with the changes in conceptual content. Anyone interested in the detail is referred to the existing literature on the history of medicine, particularly to the outstanding studies of Jackson [1] and Klibansky et al. [2]. Also of interest are the studies by Ackerknecht [3], Beek [4], Berrios [5,6], Flashar [7], Foucault [8], Gardiner et al. [9], King [10], Leibbrand and Wettley [11], Lewis [12], Roccatagliata [13], Rosen [14], Starobinski [15], and Zilboorg [16].

II. NOMENCLATURE

Before commencing with our historical review, a few comments about terminology. First of all, it should be realized that the generally accepted distinction between anxiety and depression is of comparatively recent vintage. The first nonphobic form of anxiety to take its place in the description of disease did so as recently as the middle of the 19th century. Flemming's Über Praecordialangst, which dates from 1848, was cited by Schmidt-Degenhard [17] as the first medico-psychiatric text exclusively devoted to a nonphobic form of anxiety.

Of course, this does not mean that subtle variations in the spectrum of anxiety and depressive disorders had not been observed and described prior to this. Evidently, however, there was no recognition of the need for a systematic distinction between anxiety and depression. For a long time, both were encompassed by the broad concept of melancholia. Since the *Corpus Hippocraticum* (5th-century B.C.), fear and despondency have been referred to as the prominent characteristics of melancholia.

The terms melancholia (Greek: melaina cholè, black bile) and hypochondria (Greek: hypochondrios, under the breastbone) are therefore of ancient vintage. The same applies to the concept of mania. The word depression (Latin: deprimere, to press down) gradually came into use during the 18th century [18].

Unlike the term phobia (Greek: phobos, fear), the term anxiety has neither Greek nor Latin origins. The word anxiety (German: angst, worry) probably derives from the Indo-Germanic root angh, which means to narrow, to constrict, or to strangulate [19–21]. This root reappears in the Greek word anchein, which means to strangle, to suffocate, or to press shut. The root angh has also survived in Latin, for example, in angor (suffocation; feeling of entrapment) and anxietas (shrink back fearfully; being overly concerned). Fear derives from the German stem freisa or frasa. The term panic, on the other hand, has a Greek background, namely, Pan or Panikos, the Greek god of the forest and of shepherds, who was thought to have caused panic amongst the Persians at Marathon.

The boundaries of the different terms are rather vague. This is particularly true of the term melancholia, which covers practically all forms of nonorganically determined psychopathology. In summary, however, it can be stated that despondency is a central element in numerous terms for depression, whereas in terms referring to anxiety the emphasis is often on sensations of tightness and constriction in the region of the chest and throat.

III. A HISTORY OF THE CONCEPTS OF ANXIETY AND DEPRESSION BASED UPON THE CONCEPT OF MELANCHOLIA

A. Ancient Greece and Rome

Western psychiatry, just like somatic medicine, has its roots in Greek natural philosophy. In this philosophy, the traditional explanations of mental illness, based upon the supernatural, gradually diminish in significance. Clinical observation and reasoning become established practice. Natural philosophers attempt to elucidate the universal principle behind observed phenomena. They observe heaven and earth, the orbits of heavenly bodies, and the course of the seasons, as well as the cycle of ascension, splendor, and decline in the living and the nonliving worlds. They are dissatisfied with demonological explanations of mental illness, such as those found in the works of Homer, for example.

This does not mean that moments of speculation become a thing of the past (let us consider, for example, the *Corpus Hippocraticum*). This work consists of a series of 70 medical texts dating from the 5th century B.C., which are attributed to Hippocrates and his pupils. The *Corpus* contains the earliest formulation of the theory of the four humors or bodily fluids. This humoral theory was a modified version of the view first encountered in the works of Empedocles that the universe is made up of a mixture of four elements: earth, fire, air, and water. Empedocles himself was probably influenced by the Pythagorean school's doctrine of the "harmony of the spheres," which placed strong emphasis on notions such as tuning and equilibrium. According to the humoral theory, disease results from a disturbance in the natural balance (dyscrasia) of the elements.

Blood, yellow bile, black bile, and phlegm are the four bodily fluids or humors distinguished in the Hippocratic texts. These fluids were considered to be influenced by the seasons. Accordingly, blood would increase in the spring, yellow bile in the summer, black bile in the fall, and phlegm in the winter. In addition, each of the humors was associated with a pair of primary qualities. Thus blood was associated with heat and wetness, yellow bile with heat and dryness, black bile with dryness and cold, and phlegm with cold and wetness (see Fig. 1).

To the Greek physician, disease was caused by a disturbance in the natural balance of the bodily fluids. This balance was influenced by all sorts of factors, such as seasonal changes, climate, geographical conditions, age, mental effort, as well as eating and drinking habits. The Greeks were well aware, for example, of the link between depressive phenomenon and the fall. In addition to these factors, certain people were temperamentally predisposed to melancholia. The term temperament refers to a personal's humoral constitution. Due to an excess of black bile, or to an increased susceptibility of the black bile to heat or cold, some people could have a natural tendency toward melancholia.

This suggests that the balance between the humors reflects a much broader biopsychological and ecological equilibrium. This is indeed the case. The ancient concept of disease must be seen against the background of the then popular idea of a fundamental likeliness of macrocosm and microcosm. Universe at large is a well-ordered macrocosm. Its changes are reflected at the level of microcosm—the individual body, for instance. This theme was to dominate the concept of disease for at least two millennia. It left no room for the principle of linear (unidirectional) causality, which began to dominate medicine in the middle of the 18th century, nor can it be equated with the late 19th-century concept of homeostasis, since this concept presupposes the idea of internal feedback, a notion that is quite foreign to the ancient Greeks. In antiquity, disease was seen as a disorder reflected on all levels of existence, rather than as the consequence of an internal disorder. The excess of black bile in melancholia was the analogue of changes in the seasons, in dietary habits, and in psychological constitution [22,23]. The origins and conclusion of disease were not confined to the relative isolation of the body. Instead, disease reflected changes on various levels within the macrocosm.

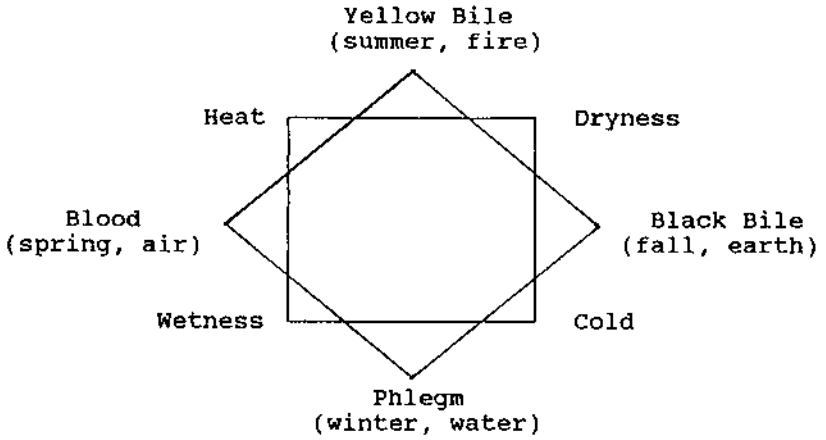

Figure 1 The four humors and their relation to the seasons, the elements, and the primary qualities.

The first-century reader may suspect that there are conceptual problems here; however, none seemed to exist for the Greek physician-philosopher, who seemed quite uninterested in the question of how all these different processes interacted with one another, and chose instead to ignore the problem. Some have suggested, for example, that the Greek outlook could not accommodate a psychogenic cause of mental illness. This is factually incorrect, since the literature of that time includes many examples of scholars becoming depressed through excessive study, and of melancholics consumed by feelings of guilt, hatred, or grief over a lost love. In addition, there is also a conceptual misunderstanding here, such as occurs whenever modern ways of thinking begin to dominate the interpretation of humoral pathology. The humors are then reduced to purely biological phenomena (comparable to neurotransmitters) and the lovelorn state, or that of being overworked, to mere matters of psychology. Greek physicians undeniably thought of the melainè cholè as a substance that was both visible and tangible, even though they had never actually seen it. Nevertheless, they persisted in associating this unseen substance with all kinds of effects at the psychological and behavioral levels. From a first-century point of view, this association could be seen as a metaphor. To the Greek physician, however, the notion of atrabiliousness (black bitterness) was a condensation of all sorts of very real experiences and perceptions. In short, even though the emphasis lay on what is now referred to as the biological component, the psychological connotation still was implied by the terms for the bodily fluids.[1] According to Aretaeus, bile means anger and black, much or furious:

> . . . in certain of these cases, there is neither flatulence nor black bile, but mere anger and grief, and sad dejection of mind; and these were called melancholics, because the terms bile and anger are synonymous in import, and likewise black with much and furious (Aretaeus; via Jackson [24]).

For all that, black bile was the last substance to be ranked amongst the true bodily fluids. Initially interpreted as a breakdown product of yellow bile, black bile was first described as a natural constituent of the body in the *Corpus Hippocraticum*. Its change in status can probably be attributed to the dark-colored urine and feces observed in malaria sufferers and in patients with hepatic disease or gastric bleeding.

However, more than five centuries were to pass between this reference to black bile in the Hippocratic texts and the first summarized description of its effects. This summary, which can be found in the medical works of Galen (131–201 A.D.), was to serve as a model for medical thinking for centuries to come.

Galen owed a great deal to the work of Rufus of Ephesus (circa 100 A.D.), who we must thank for a description of various melancholic delusions, amongst other things. One such delusion was that of being an earthen pot, another was that of lacking a head. Rufus also influenced Arabic medicine and, through it, the medicine of the Middle Ages. It was Rufus from whom the great Ishaq ibn Imran, of 10th-century Baghdad, reputedly derived his ideas about melancholia. The latter's work was to become the direct source for De Melancholia by Constantinus Africanus (11th century), a text that enjoyed great authority during the Middle Ages and the Renaissance [25]. The distinction between the three forms of melancholia, which he may have derived from Rufus, was considered by Galen to be of particular significance. He distinguished the following forms:

1. A generalized form of melancholia, with the blood being full of black bile
2. A cerebral form of melancholia, which only affected the brain
3. An hypochondrical form of melancholia involving the organs of the upper abdomen [26–28]

The first form, unlike the second, could be accompanied by other phenomena (e.g., discoloration of the skin, cirrhosis of the liver, and the accumulation of fluid). The mental manifestations of both the generalized and the cerebral forms were due to obstructed blood vessels in the brain, as a result of a thickening of the blood. Galen suspected that this obstruction led to a blockage of the channels through which the so-called pneuma animalis flowed. This pneuma was an etherlike substance, made up of small, lightweight, highly animated particles. From ancient times until well into the 17th century, it has been associated with all kinds of mental functions, including perception and imagination. In other cases, according to Galen, black bile caused cerebral tissue damage, leading to the impairment of intellectual functions in particular. In the third form of melancholia, disease symptoms were not interpreted as resulting from black bile as such. Instead they were caused by a vapor emanating from this fluid, as a result of local warming in the hypochondrium. This smoky vapor, according to Galen, rose up into the brain, obscuring thought. It was this mental obscuration that explained the anxiety seen in melancholics. Galen compared it to the darkness of night, which induces a causeless fear in many people:

> As external darkness renders almost all persons fearful, with the exception of a few naturally audacious ones or those who were specially trained, thus the color of the black bile induces fear when its darkness throws a shadow over the area of thought [in the brain] [29].

Apart from generating this vapor, such local warming also converted one of the other bodily fluids to black bile, thereby producing an excess. Under circumstances such as this, melancholia would be characterized by heat rather than coldness. According to some later writers of the Galenic school, this explained motor restlessness and behavioral disorders, an interpretation with which Burton concurred in 1621.

In terms of treatment, it was the distinction between the three forms of melancholia that became of primary importance. Phlebotomy, the preeminent therapy for the generalized form, was ineffective in the treatment of the other two forms of melancholia, which required alternative measures. Mention is made of changes in eating and drinking habits, the use of emetics and laxatives, and attaining a correct balance between rest and physical exercise.

Galen was aware that, while the manifestations of anxiety and depression are tremendously varied, the heart of melancholia consists of despondency and anxiety, especially the fear of death:

> Although each melancholic patient acts quite differently than the others, all of them exhibit fear or despondency. They find fault with life and people; but not all want to die. For some the fear of death is of principal concern during melancholy. Others again will appear to you quite bizarre because they dread death and desire to die at the same time.
>
> Therefore, it seems correct that Hippocrates classified all their symptoms into two groups: fear and despondency. Because of this despondency patients hate everyone whom they see, are constantly sullen and appear terrified, like children or uneducated adults in deepest darkness [30].

The link between melancholia and mania had already been established by Aretaeus of Cappadocia, who lived around 150 A.D. [31]. However, in the work of Galen, this link is conspicuous by its very absence. In the Hippocratic texts, the term mania was frequently used when referring to mental illness in general, even though the link with the action of black bile had already been established. In the centuries that followed, mania and melancholia gradually became delineated as disorders having a certain periodicity, but with

contrasting outward expressions. Nevertheless, we must exercise caution, and not be over hasty in identifying these ailments with the present-day, bipolar disorder. The term melancholia still has very wide connotations, incorporating many different forms of psychosis and all kinds of neurotic symptoms. Mania, on the other hand, specifically refers to the various forms of emotional restlessness and motor excitation.

In fact, mania and melancholia together encompass virtually the entire field of prolonged psychopathology, that is, chronic diseases not associated with fever. The third form of mental illness, phrenitis, is both acute and associated with fever. The obvious comparison here is with delirious visions and acute psychoses. Epilepsy had a place all its own. Viewed by the Greco-Roman world as a "sacred disease," it includes forms that are transitional between classic epilepsy and melancholia.

B. The Middle Ages

During the Middle Ages, ideas about anxiety and depression actually changed very little. Scholars continued to build upon the foundations created by the Hippocratic–Galenic school. For many centuries, Alexandria, with its enormous library, was the center of research and contemplation. One area of Byzantine medicine, as it is known, is particularly worthy of mention, particularly with respect to the work of compilers such as Oribasius of Pergamon (325–403), Alexander of Tralles (525–605) and Paul of Aegina (625–690). These scholars classified existing ideas from many different sources, without adding any significant contributions of their own.

At about the same time, the work of the Greek authors began to be translated into the Semitic languages by Christians who had fled the Byzantine Empire, as well as by Arab authors. In this way Arabic medicine came to assimilate its Byzantine inheritance, in addition to influences from India and even China. By the end of the first millennium, writers from the Eastern Caliphate (Baghdad), such as Rhazes (865–923) and Avicenna (980–1037), had produced medical treatises of their own. Avicenna's Canon of Medicine, in particular, was to dominate medical ideas for centuries to come. From Persia came another significant figure, Ishaq ibn Imran (early 10th century), who has been referred to previously. His work on melancholia inspired the great and influential treatises on the subject by the encyclopedist Constantinus Africanus (1020?–1087). Originally from North Africa, Constantinus subsequently lived in Salerno and later moved to Monte Cassano. His work reflects that of Ishaq, in that he also devoted an extraordinary amount of consideration to psychogenic causes of melancholia. Later on, famous scholars from the Western Caliphate (Spain), such as Averroes (1126–1198) and Maimonides (1135–1204), also exerted an influence on medicine. In the late Middle Ages, however, authors dealing with melancholia mainly referred to the works of Avicenna and Constantinus [32]. In the late Middle Ages, medical knowledge was mainly concentrated in monasteries and in cathedral schools. Moreover, various university medical schools were founded, the best known of these being Montpellier, Bologna, and Padua. In addition to continued classification, some scholars now began to apply morality to humoral pathology [33]. This led to melancholics being described by some as degenerate, along with phlegmatics and cholerics. Sanguinics, on the other hand, were considered to represent man, as God had intended him to be, at the Creation. Melancholia was also associated with acedia, a type of listlessness and restless boredom, accompanied by a longing for change of environment. As long ago as the 4th century, Cassianus described this condition in the monks of desert monasteries not far from Alexandria. The afternoon demon would appear around the sixth

hour. It bred in the monks a loathing for their own cells, a disdain of the other brothers, and a slothful unwillingness to take part in the routine activities of monastic life. Acedia, in the guise of Sloth, was to become one of the Seven Deadly Sins of the Middle Ages [34,35]. One particular development in the continuing systematization of humoral pathology was an accentuation of the difference between two forms of melancholia. In additon to melancholia as a result of an excess of natural black bile, a second form was discerned, caused by an excess of unnatural black bile. Unnatural black bile was thought to be produced by the combustion, or degeneration, of one of the four bodily fluids.

In cases of an excess of natural black bile, the characteristics of the melancholic temperament became more prominent. In such patients, mediation gave way to brooding. Their previously sincere and caring attitude toward life plunged into one of anxiety and gloom. Those afflicted would stare at a single point, be incommunicative, and avoid all contact. Beek, whose *Waanzin in de Middeleeuwen* (Madness in the Middle Ages) is, unfortunately, not available in an English translation, summarizes the writings of many authors as follows:

> An excess of melancholic humor, which is thick and sediment-like, imparts a red color to the head. The patient also experiences a feeling of heavy-headedness. He tastes a bitter-sweet taste, the sediment of the humor. The pulse is weak and the veins full. The urine is thick and red-colored [36,37].

Although the combustion or degeneration of black bile presents a broadly similar picture, there is the added element of preoccupation with death:

> They become agitated about funerals. Believing that they are about to die, they lie on graves and collect the bones of the dead. The pulse is hard and tense, the urine is lead-colored and thin [38].

The picture that developed as a result of burned yellow bile was one of mania. Patients ranted, raved, and screamed all day long. Referring, once again, to Beek:

> They lie awake. They exhibit excessive movement, jumping and running around. They are reckless and quarrelsome, wanting to beat those around them, preferably with an iron bar. In the grip of the mania, they throw themselves through windows. The choleric temperament is characterized by a lack of inhibition, wild behavior, recklessness, constant motion and rage. Such patients have a lemon-colored complexion, their urine is thin and yellowish, the pulse hard and rapid. It is thought that sufferers do not feel the cold because the combustion of the bile keeps them warm [39].

Conversely, the combustion of blood produces feelings of happiness rather than of sadness:

> They talk and laugh the whole time, wanting to dance and make merry all whole day long. Their temperament type is one of happiness, laughter and loquacity. Their urine is thick and reddish brown [40].

Degeneration of phlegm, on the other hand:

> . . . induces apathy, inertia and absent-mindedness. Feeling heavy-headed, these patients neither move nor laugh, nor do they feel joy. In people with this type of temperament, inertia, drowsiness and forgetfulness come to the fore. Characterized by a moist mouth and nostrils, and a pale white complexion, they are referred to as lymphatics. Believing themselves to be fish, they ask for water all day long and pine for river, or the sea. Their pulse is small and weak, their urine pale, whitish and of medium thickness [41].

In tracing the origins of melancholia, factors other than the humors (combusted or otherwise) must be taken into consideration. These were the complexio (the temperament and primary qualities) of the brain and the condition of the rest of the body. A brain that is overly dry, or excessively cold, has an increased susceptibility to disease. A weak heart lowers the threshold against developing melancholia. Particular and frequent mention is made of the relationship between heart and head. Heart palpitations, for example, are the expression of an affection of the heart. Obviously, there is a relationship between the malfunctioning of this organ and a person's state of mind. After all, did not the spiritus vitalis ascend from the heart to the brain, where it influenced the spiritus animalis?

In medieval medicine, the nature of these influences was connected to the localization of functions in the different ventricles. The anterior (or lateral) ventricles were associated with imagination (imaginatio), the median (or third) ventricle with reasoning (ratio), and the posterior (or fourth) ventricle with memory (memoria). Melancholia was usually linked with a disorder of the middle ventricle. Conditions that involve hallucinations and delusions, such as mania and psychotic depression, were based upon disturbances to the equilibrium of the anterior ventricles.

Classification was extended to include therapeutic procedures. Polypharmacy had been popular even in ancient times, with leading roles being played by black hellebore and extracts of mandragora. In addition, regulation of the so-called non-naturalia (external or environmental factors) remained an important part of the therapeutic arsenal. Traditionally, six such factors were identified. These were:

Air: The patient should be kept in a warm, moist environment, the door of his house should preferably face east.

Rest and exercise: The aim should be to reduce sensory excitation and to achieve relaxation. The patient should preferably be nursed in a dark room. The walls should be bare of pictures, since these might overstimulate his imagination. A not overly arduous walk, when the time is ripe, is beneficial for the maintenance of body temperature, as are massages and hot baths. An excess of body heat, however, dries out the body and causes melancholia.

Waking and sleeping: The patient should sleep neither too little nor too much.

Food and drink: This sickness can result both from overeating and from excessive fasting (as seen in the ascetics). Food must be easily digestible. Vegetables such as lentils and beans give rise to flatulence and therefore cause melancholia. Peppery spices, garlic, leeks, and onions must be avoided since they can burn the humors. The same applies to both mature and salted meat, the meat of forest animals, mature cheese, vinegar, and fish. A heavy wine, rich in sediment, causes sickness, whereas a light, young wine can actually raise the spirits.

Retention and excretion: Melancholia can result from the accumulation of those bodily fluids which are normally discharged, such as menstrual blood, sperm, or hemorrhoidal blood. The same is true of feces. Evacuation sometimes requires mechanical assistance, for example, in constipation, the nonappearance of menstruation, or the nonbleeding of hemorrhoids. Coitus is generally to be recommended, although men with low potency should avoid overindulgence. Bathing, whether in a herbal bath or just with plain water, is an important therapeutic agent that can also facilitate evacuation. However, excessively hot baths can induce madness.

Passions of the soul: Excessive fear, hatred, and grief should be avoided. The same applies to excessive study and to intense preoccupation with a particular task. Nevertheless, anxious and inhibited melancholics can actually be cured by either a fierce rage or a sudden shock. Generally speaking, however, these passions should be kept under control. Discussion and philosophical reading can both be useful in calming the patient.[2]

C. Intermezzo: Melancholia as a Characteristic of Genius

We have placed great emphasis on the continuity of the medical debates regarding melancholia. This continuous dialogue spans the centuries, from ancient times until the Middle Ages and, as we shall see, even beyond. There are, in all, three closely related concepts:

1. Natural black bile.
2. The disorder of melancholia, based on either an excess of natural black bile or the combustion of one of the four body humors (melancholia adusta)
3. A chronic predominance of natural black bile in people with a melancholic-type temperament.

In spite of the allegorizing and moralizing interpretations, the concept of melancholia retained its link with humoral pathology. This doctrine, however, was not entirely undisputed in ancient times. The empirical school, for example, considered the theory of bodily fluids to be too speculative. There was also the methodist school, which sought refuge in a much simpler classification of disease (involving the status strictus versus status laxus or, in other words, the respective contraction and relaxation of the so-called internal pores). We also found that, with regard to the temperaments, there was some doubt about the normalcy of the character variations (cf. Note 1).

There is another line that, in terms of the theme of this review, is even more important. This proceeds from Plato and Aristotle, via the Florentine School of the Middle Ages (Marsilio Ficino) and Robert Burton [42] in the Renaissance, to William James [43] in the present. It is a line of thought that regards the melancholic as exhibiting certain traits of genius. There is the famous opening line from the thirtieth book of Aristotle's *Problemata*:

> Why is it that all those who have become eminent in philosophy or politics or poetry or the arts are so clearly of an atrabilious temperament, and some of them to such an extent as to be affected by diseases caused by black bile, as is said to have happened to Heracles among the heroes? [44]

Aristotle seeks a natural explanation for this "madness which comes from the gods." This is in contrast with Plato's Phaedrus [45], which offers a mythological description.[3] Aristotle suggests that the eminence of the poets, politicians, and philosophers in question could be ascribed to an optimum warmth of the black bile. Black bile, by its very nature, was thought to be sensitive to changes of temperature. When cooled, it brought about "apoplexy or torpor or despondency or fear." Heating induced "cheerfulness accompanied by song and frenzy and the breaking forth of sores and the like" [46]. In the case of a man of genius, black bile, which is as such a pathogenic fluid, is in an optimum state. Such a person represents the normal within the abnormal or, in the words of Klibansky, Panofsky, and Saxl, an "eucrasia within an anomaly" [47].

During the Renaissance, a time of revived interest in astrology, this association

between melancholia and genius acquired a special meaning. In antiquity, the planet Saturn had been associated with Kronos, the son of Uranus (the god of Heaven) and Gaia (the Earth goddess), who had been banished to the underworld after the castration of his father. Now, Saturn gradually turned into a symbol of the ambivalence of intellectual and artistic life. According to ancient astrology, Saturn was the planet of gloom, desolation, decline, and old age. However, the Neo-Platonists claimed that Saturn, as the highest planet, was the planet of the elevated, of ecstatic release from early things and happenings [48]. Marsilio Ficino (1433–1499), Neo-Platonist of the Florentine school as well as a priest and physician, depicted Saturn at the heavenly body whose rays influenced the vital spirits of the brain (spiritus animalis), which were thought to connect the physical to the spiritual. This influence was described as a kind of saturation process, one to which those born under the sign of Saturn were especially sensitive. Although enjoying intellectual powers and creative talents far exceeding those of others, there was a tragic element to these people. They spent their lives teetering on the very brink of catastrophe and they were especially susceptible to melancholia. Black bile was identified with the earth, including the very center of the planet itself, which meant that such people tended to have a deeply penetrating understanding of existence. The association with Saturn, the highest of the planets, meant that they aspired to higher planes of thought [49]. The novel element here is the heightening of self-consciousness, the awareness of man's vulnerability to catastrophe and decline. Their view of life took on a nostalgic and tragic tint. This outlook was to recur in a variety of different forms in later periods, as, for example, in the Elizabethan literature of the 16th and 17th centuries (Elizabethan Malady) [50].

D. The Renaissance, 17th, and 18th Centuries

The Renaissance was not only an age of heightened self-awareness and the era of Homo literatus, with his knowledge of the classics, it was also the time of alchemy. This lent impetus to the interpretation of disease in terms of chemical change, an approach that texts on the history of medicine refer to as iatrochemistry. Substances such as salt, sulfur, and silver became the focus of attention. Paracelsus (1493–1541) was one of the first to apply the newly gathered knowledge to medicine in an attempt to break down the hegemony of humoral pathology. He did not, however, renounce the doctrine of the temperaments and the elements. Melancholia now became associated with the qualities of the chemical elements, which are sharp and acidic. Thomas Willis (1621–1675), renowned for *Two Discourses Concerning the Soul of Brutes*, expounded the theory that, in melancholia, the blood became "salino-sulphureous," causing the spiritus animalis to adopt a different pattern of motion [51].

The high point of 17th century medical literature on melancholia, however, was Robert Burton's *The Anatomy of Melancholy*, published in 1621 [52]. This work, which may seem somewhat bizarre to the modern reader, offered a compilation of all contemporary knowledge on the subject of melancholia. Greatly indebted to ancient medicine and philosophy, Burton punctuated his arguments with references from ancient times. He subscribed to the dichotomy of the passions (derived from Thomas Aquinas), a doctrine that was generally accepted at the time. Here, the passions that predisposed one toward desire (passiones concupiscibiles) were distinguished from those which predisposed one toward rage (passiones irascibiles). "Sorrow" and "fear," emotions (perturbationes) belonging to the latter category, were described by Burton as being directed at the evil that crossed

one's path. Sorrow was related to disaster in the present, fear to disaster in the future. Burton considered sorrow to be a major cause of melancholia, as well as one of its manifestations. It was:

> . . . an inseparable companion, the mother and daughter of melancholy, her epitome, symptom, and chief cause [53].

The same was true of fear, the emotion most able to hold the imagination in thrall [54]. The imagination was:

> . . . medium deferens of the passions, by whose means they work and produce many times prodigious effects [55].

Burton went on to add, however, that the converse was also true, since imagination serves to enhance the impact of emotions.

In discussing the symptoms of melancholia, Burton considered fear and sorrow to be two of a whole list of phenomena affecting those suffering from this condition. However, although these emotions were relatively nonspecific, this did not mean that they were unimportant. Burton was acquainted with many of the forms of anxiety known today. He made reference to fear of death, fear of losing those who are most important to us, and paranoid anxiety. He also cited anxiety based on ideas and delusions of reference, fear associated with depersonalization, delusional depersonalization, and hypochondria. Other forms that are mentioned include agoraphobia (!) and many other kinds of specific phobias, such as fear of public speaking, fear of heights, claustrophobia, anticipatory fear, and hyperventilation [56]. The physiology of emotions was strongly emphasized in René Descartes' *Les Passions de l'Âme* (The Passions of the Soul), which was published about a quarter of a century later [57–59]. This emphasis on physiology had a distinctly mechanistic flavor in keeping with the contemporary trend toward a general mechanization of the world view—a trend in medicine that really only took off in the 18th century and is known as iatromechanics [60]. According to Descartes, passions not only prepare the body but also predispose the soul to desire that for which the body is being prepared. The physical manifestations of emotion therefore provide some degree of insight into the function of emotions.

Fear and anxiety did not rank highly amongst the passions [61]. Descartes considered the emotion of fear to be quite useless. Nevertheless, his descriptions of the processes that give rise to fear are worthy of mention, since they are representative of the 17th- and 18th-century mechanistic view of disease. According to Descartes, the sight of a dangerous animal caused certain particles (the "esprits animaux" or spiritus animalis referred to previously) to be released by the pineal gland. Although invisible, these rapidly moving particles were quite material in nature. They traversed the neural pathways to reach the heart, the leg muscles, and the circulatory system, and gave rise to the physiological component of the fight-or-flight response. The altered distribution of blood then caused a rush of these esprits animaux (animal spirits) to the brain. Here, the pores associated with fear were opened, directing the esprits onward, back toward the periphery. Mental influences were not, therefore, presumed to be involved in the generation of fear. Instead, fear was seen as a complex, but purely physiological, reflex.

Although emotional perception was secondary, consisting of the registration of pineal gland movements, Descartes believed that such registration had a purpose, namely evaluation. It facilitated the perception of objects in terms of their effects upon us, whether beneficial or otherwise.[4] Properly employed, passions predispose the soul to desire those

things which are naturally good for us. In this resigned adaptation to the eternal laws of nature, one can detect the after-effects of the Stoa—after-effects that extend to the implicit morality of certain modern-day forms of psychotherapy aiming at tolerance and cognitive apprehension of the symptoms of anxiety and/or depression.

Descartes' emphasis on mechanics was not accepted by the medical world until the closing years of the 17th century. It enjoyed a brief flowering in the period around 1750 before giving way to other interpretations. Those associated with iatromechanics, as it was called, included men such as Pitcairn, Hoffmann, and Boerhaave (whose work reveals traces of a humoral pathology, interpreted from the point of view of fluid dynamics). Boerhaave and Pitcairn favored a vasocentric approach seeking the origins of melancholia in the modified flow patterns and viscosity changes of liquids in the blood channels. Hoffmann was one of the first to consider a neurocentric approach. He considered nerves to be hollow tubes containing a flowing liquid. Under normal circumstances, this neural fluid was thin and volatile; however, if it thickened to an earthy, slimy consistency, then melancholia developed.

Under the influence of Isaac Newton, men such as Mead and Cullen (who was the first to use the term neurosis) speculated that this neural fluid might also have electrical properties. Cullen and others thought of this neural fluid as a tenuous, highly mobile substance that was related to ether. This etherlike substance did not actually flow, but transmitted motion to the various parts of the body by means of vibration. This caused the fluid hydraulics model of mental illness to be discarded. At the time, there was only a vague notion of electrical phenomena. These were linked to the vitalistic interpretation of disease, the central concepts of which were tone and irritability. Cullen considered the irritability of the nervous system to be responsible for tissue tone [62].

Meanwhile, the clinical description of melancholia remained virtually unchanged. Some still cited the Galenic trio of generalized, cerebral, and hypochondriacal melancholia. However, there were those (Boerhaave, for example) who interpreted this classical triad merely as stages on a continuous scale of severity. Some authors were inclined to uncouple hypochondria from melancholia (of which it was the least serious form) and link it instead with hysteria. According to Sydenham and Lorry, for example, hysteria in women was equivalent to hypochondria in men.

The 18th century was a time bursting with tensions and contrasts, shifts and changes. Humoralism gave way to solidism (the explanation of disease based on the properties of the organs and tissues). Iatrochemistry gave way to iatromechanics, which in turn was replaced by concepts such as tone and irritability. Vasocentric views were replaced by neurocentric views. Meanwhile, vitalistic views of disease blended with speculation on the electrical properties of neural fluids. Each of these various approaches was considered to be compatible, incompatible, or related to one another. The rational framework of the early 18th century clearly offered medicine of that time the requisite intellectual freedom with which to forge its principal concepts.

Toward the end of the century, this all began to change. Pathological anatomy had expanded enormously, and greater emphasis was being given to clinical observation and description. It was a time of sensualism and fascination with sensory perception. Notions such as irritability bear witness to a preoccupation with the hypersensitivity of the nervous system and of the senses. The end of the 18th century saw the final demise of humoralism. During the same period, the notion that melancholia originated in the blood, or in the functions of the liver or spleen, was also dismissed. The central nervous system replaced blood and abdominal organs. Today, the idea of temperament is all that remains of hu-

moralism. Although it has no place in the scientific view of character and personality, it still exists as a metaphorical expression for the experience of despondent people.

E. The 19th Century: Further Disintegration of the Concept of Melancholia

The concept of partial insanity was popular in the nasographical schemes of the 18th century and the first decades of the 19th century [63]. As has been noted, medical scholars of the late 18th century were fascinated with the sensitivity and irritability of the nervous system. This, together with the fast-growing influence of faculty psychology (which sharply distinguished between intellect, will, and imagination), may have contributed to the popularity of the concept of partial insanity. This was not particularly novel, it was more the emphasis of an element of meaning found in descriptions of melancholia dating from ancient times (e.g., those of Aretaeus and Galen). For centuries it had been observed that the thoughts and ideas of the melancholic were confined to a single theme, often to the extent that they became delusional. Melancholia was traditionally considered to be a delirium without fever, accompanied by fear, despondency, and additional phenomena such as restlessness, insomnia, weariness, and discomfort in many parts of the body. In addition, however, frequent reference was made to the monothematic content of the melancholia sufferer's ideas and thoughts [64].

Late 18th-century medicine had a neurocentric orientation, one which tended to focus upon sensitivity and cognitive capabilities. Perhaps this may have influenced the classification of mental illness into disorders in which the powers of judgment were completely incapacitated, and those in which they were partially impaired (partial insanity). Melancholia was considered to fall within the latter category, a view that can be found, for example, in the *Traité Médico-Philosophique sur l'Aliénation Mentale* (Medical-Philosophical Treatise on Mental Disorder) by Philippe Pinel (1745–1826) [65]. Pinel also considered mania to belong to this "melancholia with delirium."

This (temporary) identification of melancholia with partial insanity completed the decline of a concept that for centuries had dominated the description of mental illness. Melancholia was divided up and its various parts were classified under other disorders. A number of things contributed to this redistribution, such as resistance to humoral pathology, and its terminology; more detailed observation (as a side effect of growing institutionalization); and rationally inspired expectations regarding psychology's ability to influence mental illness [66].

The first line of demarcation was the idea of partial insanity itself. Some melancholics exhibit no signs of insanity (delirium) whatsoever; thus none of their thoughts and ideas would be regarded as psychotic in the modern sense. In the *Manual of Psychological Medicine*, which he wrote in collaboration with Bucknill, D. Hack Tuke (1827–1895) distinguished between simple melancholia, in which the intellectual powers were intact, and complicated melancholia, in which they were not. The distinction applied by Henry Maudsley (1835–1918) was essentially the same as Tuke's, if somewhat wider in scope. In addition to melancholia simplex (cf. Bucknill and Tuke's simple melancholia), he distinguished not one, but two, forms of melancholia, both falling within the category of ideational insanity. These were melancholia as a form of general insanity and melancholia as a form of partial insanity. In the first case, intellectual derangement is complete, whereas in the latter case it is only partial. There was also a parallel distinction in mania. However, Maudsley went to great pains to emphasize the provisional nature of this theory [67].

Similarly, in France the significance of the term melancholia declined considerably under the influence of a nomenclature introduced in 1838 by Jean-Etienne-Dominique Esquirol (1772–1840) in *Des Maladies Mentales* [68]. Since ancient times, the meaning of the term melancholia had encompassed both dejection and exultation. Finding this an unsatisfactory state of affairs, Esquirol substituted the term monomania for melancholia. Monomania, which became an equivalent of the term partial insanity, was subdivided as follows:

> 1. monomania, properly so-called, which is indicated by a partial delirium and a gay or exciting passion; this condition corresponded to maniacal melancholy, maniacal fury, or (. . .) melancholia complicated with mania; in fine . . . (to) amenomania; and 2. monomania coresponding to melancholy of the ancients, the tristimania of Rush and the delirium with melancholy of Pinel [69].

Esquirol borrowed the terms amenomenia and tristimania from the work of Benjamin Rush (1745–1813), the father of North American psychiatry. It was Rush who had linked tristimania with hypochondria, alluding to the ancient hypochondriacal form of melancholia rather than to the diluted, 18th-century meaning of the term. Confusingly, besides employing monomania in the broad sense mentioned above, Esquirol also used the term to denote the manialike form of partial delirium. In this way, monomania became the equivalent of the first form of monomania, the partial delirium with the "gay or exciting passion." The second, melancholic form of monomania was denoted by the term lypemania. In addition, Esquirol distinguished mania as a generalized insanity associated with excitement and exultation. He occasionally tended to describe lypemania, monomania (in the strict sense of the word), and mania as having progressive degrees of severity, the greatest derangement occurring in mania and the least in lypemania.

Even in ancient times it was frequently pointed out that melancholia and mania could occur in parallel, in sequence and in combination. However, it was not until 1854 that cyclical mood swings were specifically identified as the distinguishing criterion of a subcategory of manic and depressive patients. In the same year, Jules Baillarger (1809–1890) described la folie à double forme (the insanity with two forms), 10 days later followed by a commentary of Jean-Pierre Falret (1794–1870), in which he also discussed a folie circulaire (circular insanity). Some 40 years later, Kraepelin explicitly harked back to the work of these two French clinicians when he distinguished dementia praecox from manic-depressive psychosis. Nonetheless, even by the middle of the 19th century, the terminological distinction between affective and schizophrenic psychopathology was by no means a fait accompli. Accordingly, Richard von Kraft-Ebing (1840–1902) declared that there were two forms of melancholia, namely simple melancholia and melancholia with stupor. The latter form, also known as melancholia attonita or melancholia stupida, was quite different from the partial delirium of French and English psychiatry. It was related to a condition that, a few decades later, Kahlbaum was to call catatonia, which could also be associated with reduced consciousness. With regard to simple melancholia, von Kraft-Ebing distinguished between a mild form of melancholia that was not associated with delusions, melancholia associated with precordial pain, and melancholia associated with delusions and hallucinations. He placed strong emphasis on psychomotor inhibition in all forms of melancholia, stating that in cases of melancholia attonita, this usually had an organic cause, such as a blockage in the motor neuron pathways. Such inhibition could, however, also be psychogenically induced. In practical terms, there were all kinds of transitional states between the two major forms of melancholia. The difference between

these states lay in the relative degree to which mental and organic components were involved in the origin of the inhibition.

This shows that the ups and downs of 19th-century melancholia, as a concept, were determined by a variety of different factors. In addition to attempts to distinguish new forms and the pursuit of ever-more precise classification, there were also advocates of continuity, who searched for transitional forms between the diverse clinical pictures. An extreme example of this is provided by Wilhelm Griesinger (1817–1868), who strongly defended the concept of a unitary psychosis (Einheitspsychose) in his *Die Pathologie und Therapie der psychische Krankheiten für Ärtzte und Studierende* (The Pathology and Therapy of Mental Diseases for Physicians and Students). In this work, he cited both his mentor, Zeller, and the Belgian alienist Guislain. According to Griesinger, the various clinically defined forms of mental illness could be reduced to the different stages of one and the same disease. The first of these successive states of mental depression was the stadium melancholicum—that is, the deterioration of normal emotions such as grief and jealousy. Next came hypochondria, the mildest form of insanity. This was followed by melancholia in sensu stricto, which, although not necessarily associated with psychotic phenomena, had a greater effect on the personality than did hypochondria. Finally, there was mania, which caused the most pronounced mental derangement of any of the conditions listed here.

F. Emil Kraepelin

The debate about the classification of mood disorders, which continued on into the 20th century, centered around the question of whether or not this group of disorders could be subdivided. However, after 1900, the matter of whether mood disorders constituted a separate category of mental illness was hardly ever discussed.

This remarkable fact can be largely traced back to the work of one man, Emil Kraepelin (1856–1926). It has often been pointed out that the term melancholia (along with related terms such as mania, partial delirium, and monomania) certainly cannot be identified with what we currently refer to as affective disorder. The clinical pictures were always those incorporating a variety of phenomena that would currently be regarded as expressions either of schizophrenia or of a schizophreniform disorder. It is remarkable, to say the least, that this age-old intermingling of psychotic and affective symptomatology should have come to an end at the beginning of this century.

In his early years, Kraepelin worked with the neuroanatomist Flechsig. For a lengthy period, he was also a research worked in Wundt's psychological laboratory. Kraepelin cannot simply be portrayed as a materialist, or as a somatologist; his field of interest was much too comprehensive for that [70]. Nevertheless, the idea that every psychiatric clinical picture could ultimately be reduced to an organic substratum in the brain was kept alive by Kraepelin and many of his contemporaries. They were motivated by the discovery of the cause of dementia paralytica, the confrontation with many cases of alcohol dementia, and the aftermath of the theory of degeneration, formulated by Morel in the middle of the 19th century. Kraepelin accordingly established an anatomical laboratory in Heidelberg. He also brought in Nissl, a histopathologist, to assist him in the visualization of the cerebral cortex. Their collaboration eventually bore fruit in the form of photographs (measuring from 50 to 75 cm) showing general views of the cerebral cortex [71].[5]

However, it was not these efforts that ultimately contributed to the lasting topicality of Kraepelin's work. Possibly influenced by Kahlbaum, Kraepelin became persuaded

about the importance of systematic clinical observation and description. This conviction inspired him to amass a database of all the patients registered in Heidelberg. This database enabled him to follow the medical histories of his patients, in some cases for several decades. It formed the basis for the separation of manic-depressive illness ('das manisch-depressive Irresein') from schizophrenia (dementia praecox), which was first described in the fifth (1896) and sixth (1899) editions of his famous textbook [72]–[74]. Although there was always a bias toward neuroanatomy and localization, it gradually faded into the background and made way for a more functional and strictly empirical approach in which classfication had less to do with diagnosis (i.e., the cause) and more to do with prognosis [75]. It was the course alone (rather than cause, symptoms, or periodicity) that proved decisive in demarcating dementia praecox from manic-depressive illness. Dementia praecox led, by definition, to personality decline (Verblödung; dementia), whereas manic-depressive illness did not.

> (. . .) the universal experience is striking, that the attacks of manic-depressive insanity within the delimitation attempted here never lead to profound dementia (Verblödung, G.G.), not even when they continue throughout life almost without interruption. Usually all morbid manifestations completely disappear; but where that is exceptionally not the case, only a rather slight, peculiar psychic weakness develops, which is just as common to the types here taken together as it is different from dementias in diseases of other kinds [76].

At the onset of the illness, it can be extremely difficult to reach a correct diagnosis. Some things to go by are age at onset (younger than 20 or beyond middle age) and a confirmed family history. There is also the premorbid character that, in the case of manic-depressive illness, is weak, susceptible, dejected, and lacking in self-confidence [77].

Meanwhile, the category of manic-depressive illness was very broad, encompassing much more than the bipolar disorder, as it is called today:

> Manic depressive insanity (. . .) includes on the one hand the whole domain of the so-called periodic and circular insanity, on the other hand simple mania, the greater part of the morbid states termed melancholia and also a not inconsiderable number of cases of amentia. Lastly, we include here certain slight and slightest colorings of mood, some of them periodic, some of them continuously morbid, which on the one hand are to be regarded as the rudiment of more severe disorders, on the other hand pass over without sharp boundary into the domain of personal predisposition. In the course of the years I have become more and more convinced that all the above mentioned states only represent manifestations of a single morbid process [78].

In this definition, the bipolar disorder of our time coincides with "periodic and circular insanity." Here, manic derangement is characterized by the triad of rapid association of ideas, elated mood, and hyperactivity. Depression, on the other hand, is associated with the triad of dejection or anxious moods, inhibition of thought, and reduced spontaneity. In addition to the circular and simple disorders, amentia, and milder mood disorders, Kraepelin also refers to mixed pictures. These cases exhibit characteristics resembling the mixed episodes of contemporary bipolar disorder. He also refers to the so-called "ground states" (Grundzustände; predisposing personality traits), which form the basis for the development of mood disorders [79]. Kraepelin distinguished four ground states: depressive, manic, irritable (erregbare), and cyclothymic. Finally, a distinction was made between this group and the form of melancholia associated with a decline due to the effects of aging (Rückbildungsalter; involution). In the latter case, inhibition was often absent while anxiety and hypochondria were more prominent. Although Kraepelin was initially inclined

to keep this (involutional) melancholia separate from the others, he abandoned this idea after the comprehensive study of this clinical picture by [80]. He subsequently included this form of melancholia within manic-depressive psychosis (das manisch-depressive Irresein). The debate about involutional melancholia was not finally settled until the 1970s when this condition became just another form of depression.

Something that is common to all forms of manic-depressive illness is the absence of an immediate cause, or at least a course that appears to be independent of possible causes. A distinction should therefore be made between psychogenic depression and the conditions referred to above [81]. The most fundamental cause of manic-depressive psychosis is an hereditary, morbid predisposition. According to Kraepelin, of the 990 cases that he studied in Heidelberg, he managed to establish that about 80% involved an hereditary defect [82].

Despite opposition [83,84], Kraepelin's interpretations nevertheless enjoyed great authority in German-speaking regions. For example, this is demonstrated by Eugen Bleuler's textbook, which was published in 1916. In describing the manic-depressive illness as a distinct disorder, this work relies heavily on the questions that Kraepelin had raised on the subject [85]. The 13th edition of this book, published in 1975, exhibits some reservations about the heredity hypothesis and about the possibility of an airtight distinction between dementia praecox, on the one hand, and psychogenic disorders on the other. Nevertheless, it still contains references to the old Kraepelinian classification [86].

G. Critique on Nosology: Reaction Type Versus Endogeny

The situation was different in the United States where, during the first decade of the 20th century, Adolf Meyer (1866–1950) expressed doubts about the value of the course criterion. He distinguished several forms of depression, such as constitutional depression, simple melancholia proper, other forms characterized by neurasthenic malaise or hypochondriacal complaints, depressive deliria, catatonic melancholia, and the so-called "delire de negation," in which the patient believes he is nothing [87]. This was more than just a debate about classification. Meyer was particularly uneasy about the coupling of heredity (endogeny) and manic-depressive psychosis, in the broad, Kraepelinian sense. He viewed this link as nothing more than "neurologizing tautology," which might easily give rise to therapeutic nihilism. Moreover, it did not do justice to the fact that mental illness is an attempt at adaptation, a reaction to the demands of a changing situation. Only when disease is seen as an inadequate attempt at recovery, the search for what he called "modifiable determining factors" could begin. We are then "in a live field, in harmony with our instincts of action, of prevention, of modification and of an understanding doing justice to a desire for directness" [88].

The question remains as to whether Meyer did justice to Kraepelin. In any event, Kraepelin cannot be accused of therapeutic nihilism. Under his direction, the enlarged baths at Heidelberg achieved international fame and were visited by many foreign guests. Nothing was too much trouble for him when it came to experimenting with new methods of treatment. Of greater importance is the conceptual point at issue here. According to Meyer, the debate on classification should not be short-circuited by an appeal to such ideas as endogeny and heredity, about which nothing was empirically established. He did not presume that biological processes should enjoy a privileged position in the list of determining factors for mental illness. For this reason, Meyer substituted the term manic-depressive psychosis with the etiologically neutral term "affective reaction type." Within

this reaction type, he distinguished a manic-depressive type, an anxious type, and simple depressions [89].

Essentially the same view was held by Sir Aubrey Lewis who, in the 1930s, published an authoritative study of 61 cases investigated at the Maudsley Hospital in London [90–92]. According to Lewis, a total reaction of the organism is involved, even in cases where the illness appears to be entirely without cause. Without doubt, there were

> changes in the internal structure of the body, its chemical and vegetative regulation which play a great part in determining its course. But these are only part of the total reaction of the organism, and it is by no means in denial of their fundamental importance in the illness that one refuses them independent and preponderant significance, either aetiological or as part of the process of the illness [93].

According to Lewis, the more closely patients were studied, the less evidence there was for a nosological distinction between autonomous (endogenous) and reactive (psychogenic; situational) depressions. Incidentally, the very same view had already been expressed 8 years earlier by Mapother, Lewis' predecessor, on the basis of impressions obtained in clinical practice.

This established the scope of a debate which, since then, has been repeated with endless variations and which, even now, continues to exert a hold on psychiatry [94,95]. Ironically enough, the seeds of controversy were sown by Kraepelin, the very person who most wanted to distance himself from the speculative impetus within the debate. In spite of his empirical bias, Kraepelin continued to link the clinically descriptive course criterion with the etiological hypothesis of endogeny. Through this, the reference to heredity became an established fact. In practical terms, Kraepelin's coupling of the course criterion with the idea of endogeny raised two mutually related issues, namely: (1) the role of exogenic (biological and nonbiological) factors in the origin of depression and (2) the demarcation of manic-depressive illness with respect to psychogenic depressions and milder variants within the manic-depressive spectrum [96,97].

Both of these issues ultimately proved to be insoluble within the framework of the endogeny/exogeny dichotomy. In practice, it was quite impossible to distinguish between depressions that were psychogenically induced and those in which psychological and situational factors merely played an instigating role. The responsiveness of the illness proved to be of only limited significance as a distinguishing criterion. Meanwhile, it should not be forgotten that, in Kraepelin's time, the term endogeny was also an expression of embarassment. At the end of the 19th century, Möbius introduced the twin concepts of endogeny and exogeny. By about 1900, these concepts corresponded respectively to diseases whose causes were still unknown and those whose causes had been well defined. Causes whose existence were (still) uncertain were described as endogenous. These were attributed, more or less out of embarrassment, to innate personal qualities [98]. Exogenous causes included bacteria, toxins, and tissue injury resulting from brain trauma. In 1910, Bonhoeffer devoted a monograph to these so-called exogenic reactive types, and his name has been linked to them ever since [99].

H. The Influences of Psychology and Psychoanalysis

After 1920, mainly because of changes in the use of the adjective "exogenous," this debate became even more complex. Originally this term was used only in relation to biological factors; now it was extended to include intrapsychic and situational factors as well, thus

highlighting the demarcation between depression and neurosis. New dichotomies consequently arose, such as endogenous/reactive (Lange); autonomous/reactive (Gillespie); endogenous/neurotic; psychotic/neurotic. After World War II, these were supplemented by S (somatic) type/J (justified) type (Pollitt); and vital depression/personal depression (van Praag). Of course, this shift in the debate cannot be dissociated from the influence of Sigmund Freud (1856–1939) and the psychoanalytic school. In one of his early works, the so-called Draft G, Freud had already addressed the neurological explanation of melancholia [100]. In 1917, he published an excellent, authoritative article on the link between grief and melancholia [101]. In this article, he stated that, unlike grief, object loss in melancholia was associated with unresolved feelings of ambivalence and regression of the ego. Anger at being abandoned would then be directed toward the ego, which remained narcissistically identified with the other. This internal "other" was then destroyed. Indeed, Karl Abraham had already demonstrated self-destructive behavior and experiences in depressive patients [102]. From here, a line can be drawn via the work of Melanie Klein to authors such as Lindemann [103] and Bowlby [104–106]. Klein distinguished a depressive position as a phase in early childhood development. Lindemann wrote an influential article on reactions to grief. Bowlby, as is well-known, worked extensively on the relationship between psychopathology and the processes of attachment and separation.

The influence of the theory of emotions, developed by the philosopher Max Scheler (1874–1928) should also be mentioned. Scheler distinguished between four emotional levels or strata: the level of sensory, physical (or vital), psychic, and spiritual feelings, respectively [107]. This theory constitutes part of Scheler's own moral philosophy with emphasis on values as nonsubjective realities that are expressed in the interaction between a person and the environment. Scheler's theory had the advantage of accounting for the fact that people are capable of experiencing more than one mood and/or emotion at a time. A person can be in a dejected mood while at the same time being angry with his neighbor. Kurt Schneider applied this feeling theory to depression by stating that depression is based on a disorder in the vital sphere. Psychic feelings, such as feelings of guilt and inadequacy, whould then be an "understandable reaction" to this vital disorder. The vital feeling of being depressed would co-occur with psychic feelings of guilt and worthlessness. This theory of feelings was held, albeit temporarily, in high esteem. The adjective "vital" for endogenous depression, which until recently was in use in Europe, represents the psychopathological remnant of this theory.

I. Toward the Twin Pillars of DSM-III

Psychoanalysis enjoyed considerable influence for several decades, to such a degree that there was barely any perceptible movement in the classification debate. However, this began to change with the discovery of the mechanisms of action of antidepressants, toward the end of the 1950s. At that time, the effects of lithium had been known for 10 years while ECT had been in use for more than 20 years. The advent of these new therapeutic drugs raised the question of whether the biological active site of these drugs could be linked with specific target symptoms of depression (or mania). Gradually, it was accepted that this was indeed the case. Target symptoms, it was assumed, pointed to a given core disorder in the spectrum of depressive symptomatology. The increasing use of advanced statistical methods also had considerable impact on the debates. It enabled larger groups of patients, taken from adjacent diagnostic categories, to be investigated for clustering of

symptoms (discriminant function analysis) and underlying factors or dimensions (factor analysis).

The outcome of these developments was not immediately obvious [108]. In summary, the classification debate gradually split into two separate debates after 1960. These concerned (1) the distinction between manic-depressive psychosis and other forms of depression; and (2) the distinction between "endogenous" and neurotic depression.

The considerable overlap between these two debates served only to complicate matters, since the classificatory status of endogenous depression was central to both. When highlighting some moments of this debate, mention should first be made of the clinical and genetic studies of Perris [109] and Angst [110]. Both workers found that depressives with a previous history of mania had different hereditary profiles from those with no such previous history. On the basis of these findings, they concluded that the distinction between unipolar and bipolar depressions, which was already defended on clinical grounds by Leonhard in 1959, was valid. This distinction became one of the basic assumptions of the classification of mood disorders in the DSM-III [111] and its subsequent editions [112,113].

Kendell, meanwhile, carried out a retrospective investigation of 1080 patients who had been admitted to Maudsley Hospital [114]. He could find no evidence of a bimodal distribution in the symptom profiles of a heterogeneous group consisting of manic depressive patients, patients with neurotic depression, and patients with involutional melancholia. However, this kind of distribution was found in the Newcastle study. Something common to both studies was the relatively high loading of a "bipolar" factor.

As Kendell himself observed, his investigation did not negate the distinction between psychotic and neurotic depression. These could represent two poles of a continuum, with the psychotic pole displaying greater homogeneity than the neurotic pole. The simple fact that the symptoms of the neurotic side are milder and fewer in number contributed substantially to the reduced tendency toward clustering [115].

The lack of homogeneity at the neurotic depressive pole of the spectrum also found expression in the tri- and tetra-partite divisions of Klein [116] and Paykel [117], respectively. Klein distinguished an "endogenomorphic" depression, as well as a reactive and a (chronic) neurotic form. Paykel distinguished psychotic depressives as well as three other groups: anxious depressives, hostile depressives, and young depressives with personality disorder. Again, remarkably, some degree of consensus existed with respect to the psychotic or "endogenomorphic" end of the spectrum. The limited extent of this consensus was due to the fact that, for the above-mentioned authors, the central issue was not bipolarity (cf. Perris, Angst, and Kendell), but rather vital phenomena and psychotic symptoms. For this reason, it is not surprising that some clinicians opted for a center-periphery instead of a continuum model. Mendels and Cochrane, for example, observed that:

> the so-called endogenous factor might represent the core of depressive symptomatology, whereas the clinical features of the reactive factor may represent phenomenological manifestations of psychiatric disorders other than depression which 'contaminate' the depression syndrome [118].

Ultimately, however, with the advent of DSM-III(R), neither the center-periphery nor the continuum model was to gain the upper hand. Instead, the winner was a twin pillar model, centering around the pillars of bipolar disorder and depressive disorder. In an article that appeared in 1974, Klein articulated an important consideration regarding this shift [119].

He pointed out that endogenomorphic depressions (those which give the impression of having arisen endogenously) occur particularly frequently in the group of neurotic depressives. As a result of epidemiological studies, less emphasis was placed upon the heterogeneity of neurotic depressions. Conversely, greater attention was paid to the chronicity and severity of the disease. Finally, in the 1980s, genetic investigations not only favored the further demarcation of bipolar disorder—something which had already been advocated on the basis of factorial analysis studies—but also the distinction of dysthymia (neurotic depression) as a separate category.

In summary, it can be said that a consensus began to emerge in which the most important demarcation line was drawn between bipolar disorder and unipolar depression. In addition, within the group of unipolar depressive disorders, a subdivision was created, roughly corresponding to the distinction between endogenomorphic and chronic neurotic depression as described by Klein. Ironically enough, the old concept of melancholia was once again called upon for assistance, namely, in the definition of the endogenomorphic (vital) form of major depression.

It should be noted, however, that this consensus was by no means universal. For example, it still has not been decided whether or not the categorical distinction between cyclothymia and dysthymia, on the one hand, and personality disorders, on the other, is an artifact. In addition, the debate about the demarcation between affective and anxiety disorders has become of particular relevance in the past decade. Longitudinal, familiar, and epidemiological studies have demonstrated that there is a high degree of comorbidity between affective and anxiety disorders, both in the course of the illness and in relatives. However, this discussion is beyond the scope of this chapter.

I switch now to a discussion of some highlights from the conceptual history of anxiety and anxiety disorders.

IV. HIGHLIGHTS FORM THE CONCEPTUAL HISTORY OF ANXIETY AND ANXIETY DISORDERS

A. The Demarcation of Agoraphobia

As mentioned in Sec. II, prior to about 1850, anxiety was not considered to be a distinct form of psychopathology in the medical literature. This is of particular importance to the recent debate on the demarcation between affective and anxiety disorders. For hundreds of years, the symptoms of anxiety had simply been seen as part of melancholia. In the course of the past century and the present one, the various forms of anxiety came to be distinguished from depressive disorders, on a variety of grounds. In light of the century-old merging of anxiety and depression, a reconsideration of these grounds is therefore a matter of considerable topical interest. Why the urge to merge the independent status of various forms of anxiety within the total spectrum of psychopathological symptoms? Historically, attention focused initially on phobias. Phobic anxiety, like other symptoms of anxiety, had been described in other terminology in the Hippocratic texts. Burton described agoraphobia, claustrophobia, and fear of public speaking. Errera [120] cites le Camus' Médecine de l'Esprit (Medicine of the Mind) from 1769 [121] and de Sauvages' Nosologie Méthodique (Methodical Nosology from 1770–1771 [122] as being the first medical studies in the field of phobia. The former includes a section on Des aversions (avoidance behavior) while the latter lists many different types of phobias. The term phobia was

occasionally used in a diagnostic context even before 1850, as, for example, in the 1798 work of Benjamin Rush [123].

However, three publications that appeared around 1870 became particularly authoritative. The first of these was a short article by Benedikt entitled Über Platzschwindel (On dizziness on squares) [124]. Here, the author focused on a form of dizziness that, because of its characteristic symptomatology and treatability, in his opinion, merited a separate classification among the various forms of giddiness. This article was to go down in medical history as one of the first descriptions of agoraphobia. As just noted, this is in fact historically inaccurate. Furthermore, the term "Platzschwindel," was invented by Griesinger, not Benedikt. Nevertheless, by according a separate classification to the series of phenomena currently referred to as agoraphobia, Benedikt's article does mark a turning point.

Although Benedikt had observed anxiety in the patients he described, he believed this to be secondary to the dizziness:

> . . . however, as soon as they enter a wider street or (especially) a square, they are overcome by dizziness. They either become terrified of collapsing mentally or else they are gripped by such tremendous fear that they will never dare to pass through such a place again [125].

Two years later, Westphal challenged the view that dizziness was of primary importance [126]. Westphal, who was the first to use the term agoraphobia in a technical sense, believed that anxiety, rather than dizziness, was at the root of this phobia. It was anxiety that caused the dizziness, not the other way around. Westphal's observation was the forerunner of a debate that went full swing more than a century later [127]. This debate centers around the provocative role of bodily sensations and their interpretation in the origin of panic attacks. Westphal based his hypothesis upon clinical observation, rather than on theoretical considerations. He imputed Benedikt's interpretation to the incompleteness of his investigations [128].

Interestingly enough, Westphal himself was very much aware of the fact that the three patients he described were certainly not afraid of streets or squares, as such. He stressed the unfounded nature of their anxiety. Theirs was rather a fear of anxiety itself, an anxiety that only much later is linked to particular situations. Modern-day authors who point out that agoraphobic anxiety is not a fear of streets or squares and that it occurs under all sorts of other circumstances find an ally in Westphal.

B. Anxiety Under Circumstances of War

During the same period, Da Costa published an article on cardiac symptoms in exhausted infantry soldiers during the Civil War [129]. Da Costa, himself a cardiologist, spoke of an "irritable heart." Observations of more than 300 patients led him to believe that this condition was caused by a heightened nervous irritability of the heart, which, in turn, was caused by prior overactivity, such as long marches or physical illness, for example. During auscultation of the heart, Da Costa heard a weak and sometimes split first sound, a pronounced second sound, and sometimes a systolic murmur. This systolic murmur has recently been related to the midsystolic click of mitral valve prolapse. Da Costa spoke of a sound "like the sudden motion of an only slightly elastic or cartilaginous substance" [130].

This classic article by Da Costa continued to stir things up among cardiologists, neurologists, and psychiatrists well into the middle of this century [131]. The debate became particularly intense during and after both world wars when, once again, tens of

thousands of those departing for the front lines were afflicted with the syndrome described by Da Costa. Thomas Lewis gave a figure of 70,000 such cases among British soldiers during World War I, 44,000 of whom subsequently received a war pension [132]. In addition to a report on this subject, which he drew up for the Medical Research Committee in 1917, in 1940 Lewis also published a monograph on the same theme [133]. By that time, many agreed with Lewis that the term "irritable heart" was incorrect, since this placed a one-sided emphasis on heart palpitations and on pain in the region of the heart, which wrongly suggested that the complaints were cardiac in origin. However, despite this consensus, there was still no unanimity about what actually lay behind the syndrome.

This lack of unanimity was also reflected in the nomenclature. Lewis introduced the term effort syndrome in order to emphasize both the intolerance to physical exertion and the syndromal character of the picture described by Da Costa. While the clinical picture was indeed determined by cardiac symptoms, he found that only one-sixth of the patients actually suffered from heart disease. Other terms that came into use were soldier's heart [134], war neurosis [135], Da Costa syndrome [136], neurocirculatory asthenia, and heart neurosis.

Following rejection of the cardiac hypothesis, the controversy over etiology mostly centered on whether this picture was determined by psychic factors [137,138] or whether it was a syndrome that could develop in more than one way [139–142].

Mackenzie, for example, blamed the confusion surrounding the condition (which he termed soldier's heart) on consideration of some individual symptoms to the exclusion of all else, and on the fact that this disorder had been named after its most prominent symptom (in this case, heart palpitations and pain in the region of the heart). Because of this, less prominent symptomatology tended to be disregarded. Mackenzie states that, according to the "law of associated phenomena," local disorders are usually accompanied by so-called reflex symptoms in other parts of the body (see Sec. IV. D). Moreover, long before the formulation of the attribution theory, he already emphasized the influence of medical terms on the way in which disease is perceived. By designating the systolic murmur associated with effort syndrome as an aortic or mitral valve defect, physicians could turn their patients into invalids. Intolerance to exertion would then wrongly be labeled as heart disease.

The debate was settled provisionally by two collaborative studies by a number of specialists from a section of the Maudsley Hospital, which was rehoused at Mill Hill School during World War II. A special unit had been set up at the school for the purpose of studying patients with effort syndrome. One of these studies was an award-winning work by Maxwell Jones, who later achieved fame as a champion of the therapeutic community [143]. In the other study, cardiologist Paul Wood concluded that the symptoms of Da Costa syndrome were also prevalent in peace time and that they closely resembled the symptoms of anxiety neurosis [144]. Although constitution, heavy exertion, and previous infectious diseases could all be precipitating factors, effort syndrome was ultimately explicable in terms of (and maintained by) a neurotic mechanism. It affects those who, in their youth, "clung too long to their mothers' skirts" [145] and who, either due to parental overconcern or to comments by their physician, learned to interpret various (normal) physiological changes as signs of physical impotence or even of danger.

Like Mackenzie, Wood placed great emphasis on the suggestibility of the patients, particularly on their capacity to interpret, in a negative way, the normal physiological changes that occur during physical exertion. Wood's interpretation was partly based upon physiological experiments that had demonstrated that peripheral sensation in effort syn-

drome was founded neither upon local abnormalities like hypersensitivity of the central nervous system (such as in hyperadrenalism), nor hyperventilation. Therefore, in his opinion, there was no specific pathophysiological mechanism that could be held responsible for the physical symptoms. However, there did exist a specific psychological mechanism that involved the association of exertion with all sorts of imaginary physical catastrophes. Wood believed that this association was mediated by an emotion, usually anxiety, although patients were generally not consciously aware of this. Therefore, the therapy consisted of a form of psychoeducation in which people were informed of the physical manifestations of emotions and of the fact that physical sensations were not, by definition, indicative of a disorder in any particular organ.

Maxwell Jones concurred with this. He developed a form of group psychoeducation, using groups of about 100 patients, which later evolved into the first therapeutic community. The aim of therapy was to teach patients to adopt a different attitude toward their symptoms. In addition, Jones stressed the reality of intolerance to exertion, for which extensive physiological studies failed to provide an explanation. He therefore spoke of an effort phobia. Jones' investigations had demonstrated that effort phobic patients quit exerting themselves long before they reach their physiological limit, as expressed by a slightly smaller increase in the lactate level relative to a normal control group following the subjective maximum of exertion.

The debate can be summarized by stating that the variation in nomenclature was determined not only by all sorts of theoretical views concerning the causation of physical sensations, but also by the immediate military importance of identifying and treating those suffering from war neurosis. Wars have contributed greatly to our knowledge not only of anxiety disorders but also, for example, of traumatic neuroses and terror psychoses [146,147]. In addition, they have propelled this knowledge in a specific direction. Lewis' choice of the term effort syndrome was significant, as was his involvement in the development of exercise programs to increase the exertion tolerance of the soldiers he was treating. Both can be seen as a direct reflection of the military importance of the capacity to deliver physical effort [148]. Lewis' effort syndrome is a splendid example of social influences affecting psychiatric diagnosis. Regarding subsequent developments, it can be noted that the special relationship between anxiety phenomena and the heart persisted even after 1950. While consideration was being given on the sidelines to the psychiatric mode of interpretation, the somatic approach continued to play a dominant role. The 1960s saw the development of somatic concepts such as hyperkinetic heart syndrome and hyperdynamic beta-adrenergic condition. In the 1980s and 1990s, the relationship between anxiety disorders and mitral valve prolapse has been the subject of debate, as has the so-called fatigue syndrome [149,150].

C. Anxiety Psychosis

The frequent occurrence of anxiety with psychotic symptoms did not, of course, go unnoticed by 19th-century psychiatry. Wernicke, however, was the first to use the term anxiety psychosis. In Wernicke's opinion, frightening cognitions, hallucinations, delusions, and delusory ideas were the result, rather than the cause, of the emotion of anxiety. He explained the psychotic phenomena seen in anxiety psychosis by the intensity of the anxiety itself. The reverse was true of alcohol hallucinosis, where anxiety was the result of the hallucinations. Melancholia also differed from anxiety psychosis. So-called agitated melancholia, on the other hand, which was actually a variant of anxiety psychosis, was totally

unrelated to melancholia [151]. The motor expressions that appeared in anxiety psychosis were interpreted by Wernicke as complications, rather than as direct consequences of anxiety. In the ensuing debate, criticism was leveled at the term anxiety psychosis [152,153]. Some questioned the worth of a classification that was based on the content of cognitions. Anxiety-dominated cognitions were not restricted to anxiety psychosis; they also occurred in a variety of other psychiatric disorders. Wernicke himself had already made a distinction between anxiety psychosis, on the one hand, and anxiety in paranoid delusions and in delusions of reference, on the other. The distinguishing criterion—namely, that the cognition in anxiety psychosis must be a direct consequence of anxiety—proved not to be unequivocally applicable in practice.

Meanwhile, Specht's interpretation differed considerably from that of Forster. Specht felt that anxiety psychosis was a mixed (Kraepelinian) form of manic depressive psychosis in which motor agitation was an expression of the manic component. Like Wernicke, he drew a sharp distinction between anxiety and agitation on the basis that the former frequently occurred in depression, while the latter was commonly a feature of mania. Specht's view was that anxiety psychosis involved motor agitation as an expression of the manic component, accompanied by the anxiety and inhibition of thought as a result of the depressive component. For Wernicke, anxiety was the central characteristic of anxiety psychosis; for Specht it was agitation.

Forster, on the other hand, felt that the symptoms of anxiety psychosis could best be seen either as a variant of melancholia (melancholia agitata) or as the early stage of another disorder. Not only did Forster not want to separate anxiety from agitation, he also resisted Kraepelin's separation of manic depressive psychosis from dementia praecox. In his opinion, anxiety was not so much an emotion that was difficult to define, but more a complex series of cognitions that cannot be expressed in words. This formal characteristic, i.e., a special type of cognitive complexity, was determined by the "fundamental disorder" that Forster placed at the level of the organic cerebral substratum [154].

In the ninth edition of Kraepelin's textbook, anxiety psychosis was still included under the Emotionelle Symptomenkomplexe (emotional symptom complexes). It was defined as a dysphoric condition that cannot be sharply distinguished from depression. It was associated with anxiety, motor restlessness, and psychotic symptoms [155]. As a symptom complex, it could occur in all types of psychiatric disorders, including manic depressive psychosis and dementia praecox. It could also be age-related, as in presenility and senility.

Two questions dominated this somewhat confusing debate surrounding anxiety psychosis. These concerned the relationships between emotions and cognition, and between anxiety and (psycho)motor agitation. On the first point, Wernicke considered the anxiety emotion to be dominant, whereas Forster placed the cognition in the leading role. Specht's reference to the Kraepelinian idea of the manic depressive mixed condition suggested a preference for the view that the affective component was dominant. On the second point, both Wernicke and Specht made a sharp distinction between anxiety and agitation. With regard to a distinguishing criterion for anxiety psychosis, Wernicke emphasized the anxiety, Specht the agitation. Forster allowed anxiety, agitation, and desperation to intermingle, since he believed that there were insufficient empirical grounds for a sharp distinction between anxiety disorders, manic depressive disorders, and psychotic disorders. Basically Forster rejected the traditional classification (derived from faculty psychology) into thinking disorders, feeling disorders, and disorders affecting the function of the will [156].

In the post-1910 literature, two publications are worthy of mention: G.E. Störring's Zur Psychopathologie und Klinik der Angstzustände (On the psychopathology and treatment of anxiety states) [157] and K. Conrad's Die beginnende Schizophrenie (incipient schizophrenia) [158]. Although neither work includes the term anxiety psychosis, both point out the fundamental significance of anxiety in the origin of psychosis. Both go on to describe a period of depersonalization, anxiety, and anxious moods which often precedes the onset of psychosis. Conrad used the term trema to denote this anxious delusory mood. Störring described how this anxious delusory mood could lead to so-called objectivation of anxiety, which nowadays is called projection. Feelings of anxiety are no longer experienced internally, but transform into perceptions of a dreadful and mysteriously changed world. In the case of psychosis, the background to this symptom is a disorder that affects the sense of identity. Psychotics are no longer able to perceive themselves as the source of meaningful experiences and activities. Feelings lose their natural bond with the I. As a consequence, they take on an enigmatic and indeterminate character. While the patient does not necessarily experience anxiety subjectively, the world nevertheless changes in an obscure way and appears to be terrifying, threatening, and gruesome. Sometimes anxiety is experienced in flashes, in which case, according to Störring, it makes sense to speak of a delusory affect rather than of a delusory mood.

Meanwhile, with the virtual disappearance of the term anxiety psychosis from clinical usage, interest in anxiety symptoms in the context of psychosis had also faded [159]. However, studies pertaining to the occurrence of panic attacks in schizophrenia and in schizophreniform disorders are still published from time to time.

D. Neurasthenia

In the second half of the 19th century a new concept, neurasthenia, gained ground. George M. Beard, the American advocate of this idea, considered neurasthenia to be a functional disorder characterized by a deficiency of "nervous force." This deficiency could express itself in a multitude of symptoms, particularly at the level of the central nervous system, the digestive tract, and the reproductive tract [160,161]. Although not highly prominent among these symptoms, morbid fear and phobia were nevertheless ranked among the most difficult symptoms to cure [162,163]. Beard used analogies for nervous exhaustion such as that of a furnace lacking in fuel and of a battery losing its charge [164]. Central to the concept of neurasthenia was the lack of the strength and reserve to fight the disturbances of nervous function caused by stress. Beard's neurasthenia concept was closely linked with his vision of American society, which supposedly caused much greater overexcitation of the central nervous system than did European society. "American nervousness," one of Beard's favorite synonyms for neurasthenia, was a typical product of an industrial society in which the upper classes were doomed to a hectic lifestyle.

Beard experienced just as little difficulty with the conceptual difference between the physical depletion of energy and the psychic feeling of exhaustion as did Freud a decade later [165]. He had observed that not only did neurasthenia patients tend to survive their own physicians, but also they were capable of considerable mental effort. However, this did not cause him to reconsider the difference between subjective feelings of exhaustion and an actual deterioration in achievements resulting from a lack of physical reserves. On the contrary, he stressed that, in a functional sense, there was actually something amiss, such as a hyperemia of the cerebrum, the stomach, or the prostate, for example.

Due to a lack of resistance, the functional disorder (which initially occurred locally) became transmitted to other regions of the body (irradiation). It therefore had no opportunity to develop into a permanent local abnormality. In Beard's opinion, this was not the case in healthy people where occasionally local overexcitation could even result in death. In the case of neurasthenia, the local functional disorder never exceeded the threshold of intensity beyond which permanent defects could develop. Irradiation not only explained the variable and migratory course, but also the multiplicity of symptoms. Among the symptoms included by Beard were the "irritable heart," all kinds of phobias, compulsions, impotence, hyperesthesia, and a huge range of physical sensations.

The irradiation of the local functional disorder occurred reflectively, and Beard thought that the sympathetic nerve played an important role here. This hypothesis of reflective nerve impulse transport was one of the three basic assumptions in Beard's concept of neurasthenia. In addition to the reflex theory, there was the idea of the electrical nature of nerve excitation and the law of conservation of energy [166].

Beard himself believed that it was open-minded observation that led him to the discovery of neurasthenia, and his descriptions do indeed bear testimony to his extraordinary attention to detail. He would take even the most idiosyncratic, subjective sensations quite seriously. The fact that neurasthenia had not been previously described was, in his opinion, due to the fact that neurasthenic patients are not found in hospitals or mental institutions. They should instead be sought elsewhere, neurasthenia being a disease of the street [167]. Nevertheless, Beard overestimated his inductive powers, as is demonstrated by the above-mentioned three basic assumptions and the role they played in his work. These assumptions constituted the guiding principle on which he based his attempts to forge a whole out of the positively exorbitant diversity of observations. Moreover, it was quite common in those days to think of psychic disorders in terms of an excess or a deficiency of (nervous) energy. Furthermore, as we have previously seen, ideas such as asthenia and irritability were already fashionable a century earlier [168,169]. In 1848, W.B. Carpenter explicitly suggested the idea of a close relation between nervous energy and electricity. Thinkers such as Spencer, Fechner, and Darwin subsequently elaborated this idea still further. Meanwhile, in the therapeutic sphere, the process of electrification became quite popular [170]. Nor was Beard the first to see a connection between lifestyle and functional changes in the central nervous system. The previously mentioned theory of degeneration, which was very popular on the European continent at the time, provides still more far-reaching examples. It is true to say that the fascination with the relationship between nervous energy and electrical phenomena was not unconnected with developments in the natural sciences. Its origin, however, lay in the romantic period. The intellectuals of the romantic period are known to have been strongly captivated by the living world's organic urge to develop and evolve. Early in the 19th century, the concept of natural force encompassed not only physical forces, such as motion and heat, but also biotic and psychic forces, such as the life force, growth energy, and the urge toward further development. It is therefore not the case that the concept of physical energy was initially discovered by physicists and only later applied, in a metaphorical sense, to psychic symptoms. In the second half of the 19th century, the prevailing climate of thought, which was still dominated by the influence of the romantic period, swung in a materialistic and mechanistic direction. This transformation, which was associated with such names such as H. Helmholtz, E. du Bois-Reymond, E.W. Brücke, and C. Ludwig, was triggered by the discovery of the law of conservation of energy by Robert Mayer in 1842. It resulted

in a differentiation between physical and psychic force, which suddenly breathed new life into the psychophysical problem. At the end of the 19th century, there was yet another swing, this time back in a neoromantic direction, whereby all sorts of vitalistic concepts gained new ground. Beard's concept of nervous force seemed to fit in with this neoromantic pattern of a vitalistic mixture of psychic and physical forces. In summary, it can be said that both Beard's description of neurasthenia, as well as the temporary popularity of this concept, cannot be understood from a purely medical perspective. Instead, one must consider the interaction between medical observations, theoretical opinions, philosophical traditions of thinking, and various social changes. However, the fact that medicine concerned itself with neurasthenic patients at all was, to a great extent, a social phenomenon. When social pressure became too much for an individual's resilience, neurasthenia offered a medical excuse for taking it easy.

E. Psychasthenia

One of the most remarkable studies in the history of the classification of anxiety is Pierre Janet's Les obsessions et la psychasthénie (The obsessions and psychasthenia), dating from 1903 [171]. This work, written in an elegant and still readable style, not only offers an overview of all possible manifestations of pathological anxiety, it also contains numerous vivid descriptions of conditions that are known today as depersonalization, somatoform disorder, hypochondria, stereotyped movement disorder, and chronic fatigue syndrome.

Janet argues against the tendency of many of his colleagues to divide symptom clusters into separate diagnostic entities. Indeed, he presents a classification of his own, by making a distinction between three types of psychasthenia: obsessive thoughts, irresistable movements (compulsions, tics, outbursts of temper as a result of the inability to complete the compulsions), and visceral anxiety (generalized anxiety, panic, phobias, and even pain syndromes). These types, in their turn, are subdivided into various clinical states. Janet nevertheless emphasizes the close ties between these states. In the course of their illness, many patients show symptoms of conditions belonging to different types. Moreover, suppression of the target symptoms of one type often leads to the emergence of symptoms belonging to another type of psychasthenia. Blocking of the obsessions, for instance, heightens the anxiety and may induce compulsive behavior. Resisting one's compulsions, on the other hand, often leads to cardiac palpitations and the sensation of suffocation.

The real innovative element of Janet's study, however, is his attempt to fit his numerous observations in a general theory of psychological functioning. In his Introduction, Janet declares his sympathy with the French psychologist Ribot, who was one of his intellectual fathers and who had made a plea for the close collaboration between medicine and psychology. Common to all patients, says Janet, is a disturbance in psychological functioning, the so-called psychasthenic state or psychasthenia. This state is characterized by three distinctive features, namely: (1) a sense of incompleteness (sentiment d'incomplétude); (2) a diminishing or loss of the sense (or function) of reality (la fonction du reel); and (3) exhaustion [172].

It is not easy to perceive exactly what Janet meant with the first two of these features. Roughly speaking, the sense of incompleteness refers to the subjective feeling that something is missing in one's actions, feelings, or intellectual functioning. It is a sense of being incapable and unsuccessful. Whatever one does, it seems useless and incomplete. Doubt,

hesitance, and endless rumination dominate one's activities. Depersonalization, feelings of doubleness and unreality, restlessness, apathy and disgust complete this list of manifestations.

With regard to the second feature, the diminishing of the sense (or function) of reality, it is at first sight even harder to imagine what Janet had in mind. Citing Spencer, he defines it as "the coefficient of reality of a psychological fact" [173]. Rephrasing this statement, one could say that certain classes of psychic functioning can be assessed with respect to their degree of reality (i.e., to a certain quality of psychic functioning in relation to actual tasks and circumstances). In sum, the function of reality refers to the capacity to be present, spontaneous, and effective, particularly in the domain of voluntary action, attention, and perception.

Janet, after all, discerns five hierarchical levels of psychological functioning: the function of reality at the upper level; then indifferent activities (routine acts and vague perceptions); the imagining function (memory, imagination, abstract reasoning, and day dreaming) and visceral emotional reactions; and finally, at the lowest level, involuntary muscular movements. The quality of psychological functioning is determined by the so-called psychological tension, the psychic correlate of the nervous energy, to which Beard and Freud had alluded to. Lowering of this tension initially leads to a lack of attention, concentration, and other synthetic mental functions—in other words, to a loss of "la fonction du réel" and subsequently to a disruption of routine activities at the second level. The psychasthenic state is the result of precisely this lowering of psychic tension (abaissement de la tension psychologique) [174].

From this, it will become clear that anxiety is by no means the central symptom in Janet's account of the psychasthenic state. Anxiety occurs when psychic functioning is disturbed from the upper level down to the fourth level, that of the visceral emotional reactions. Anxiety, consequently, belongs to the most elementary of the mental functions:

> Underneath the anger, fear and love, there is an emotion, that is not specific any more, that is a sum-total of vague respiratory and cardiac complaints, which don't evoke in the mind the idea of any inclination or any particular action. That emotion is called anxiety, the most elementary of the mental functions (translation by the author) [175].

Clearly, psychasthenia encompasses a broad range of clinical phenomena, including the anxiety disorders of our time. The psychasthenic state, however, is determined by a breakdown of only the highest level of psychic functioning. This implies that even in the case of phobias, obsessive-compulsive disorder, and panic attacks, a central role should be assigned to feelings of unreality, incompleteness, ineffectiveness, and depersonalization, and not to feelings of fear and anxiety. Emotions and emotion theory play only a secondary role in Janet's description and explanation of these disorders.

Janet does not deny the occurrence of panic attacks in some cases of psychasthenia [176]. But these can only be accounted for by the assumption of a temporary and more severe collapse of the psychological tension, leading to disturbances at the third and fourth level. Fear, on the other hand, is a more complex and differentiated emotion, involving psychic activity of the higher levels, such as imagination, perception and goal-directed behavior. Fear as such, however, is the expression of activity at the fourth level of psychic functioning.

From a psychological point of view, Janet was far ahead of his time, by pointing to the importance of disturbances in the domain of attention and perception and their

relation to the sense of self. Psychology and psychiatry had to wait until the 80s before attentional bias became a topic of some interest in empirical research of the anxiety disorders.

F. Anxiety Neurosis

The history of the classification of anxiety disorders since the time of Beard can be seen as a peeling away of layers of the concept of neurasthenia. Anxiety neurosis was the first stratum to be laid bare under its surface. Next came all sorts of classificatory subdivisions within anxiety neurosis [177].

Kahlbaum's successor, Hecker, initiated the above-mentioned process in a classic article on anxiety states in neurasthenia [178]. He had noticed that the anxiety attacks experienced by many neurasthenia sufferers were not accompanied by any subjective feeling of anxiety. There were also patients who did not show anything like the full range of physical symptoms. Hecker used the term "larvirt" (larval; larvalike) to denote this absence of a feeling of anxiety. The term "abortiv" was indicative of the interrupted, incomplete nature of the attack in terms of the somatic symptomatology. The picture described by Hecker bears a strong resemblance to the so-called limited symptom attacks in present-day literature on panic disorder. Citing Lange, a Dane who had formulated an interpretation of emotions that was practically identical to that of William James, Hecker stated that the absence of subjective anxiety in the attack was based on a kind of misperception. The physical symptoms were simply not recognized as expressions of anxiety. However, it was also possible for an attack to commence with just one of the somantic symptoms before radiating to other parts of the body. The way in which Hecker described this irradiation betrays a relationship with Beard's reflex theory.

In 1895, Sigmund Freud, with reference to Hecker, joined the critics of Beard's broad concept of neurasthenia. However, in being more explicit about pathogenesis, Freud went a step further than Hecker [179,180]. He believed that demarcation of neurasthenia was essential since anxiety neurosis, because of its different pathogenesis, required different treatment. Neurasthenia was a disorder of the way in which the so-called somatic-sexual excitation was released, whereas anxiety neurosis was primarily a disorder in the psychic processing of such excitation. In the case of anxiety neurosis, Freud imagined that there was a buildup of pressure on the walls of the male seminal vesicles. When this pressure exceeded a given threshold, it was transformed into somatic energy and transmitted, via neural pathways, to the cerebral cortex. Under normal conditions, sexual fantasy groups became charged with this energy, leading to sexual excitement (libido) and the pursuit of release. Anxiety neurosis involved a blockage in the psychic processing of this somatic sexual tension. Such a blockage might arise through abstinence, for example, or due to the use of coitus interruptus, or because sexual fantasies had simply failed to take shape. Somatic sexual tension was thus deflected away from the psyche (the cortex) and directed to subcortical paths, finally expressing itself as inadequate actions, which occurred most characteristically during an anxiety attack.

The pioneering article in which Freud detached anxiety neurosis from neurasthenia includes a description of the symptomatology of the various forms of anxiety that is still valid today [181]. Freud cited anxious expectation as the core symptom of anxiety neurosis. He also distinguished between specific phobias agoraphobia, free-floating anxiety, and anxiety attacks. The latter were spontaneous in nature and were described as a purely

somatic phenomenon [182]. The aforementioned distinctions anticipated the now generally accepted classification of specific phobias, agoraphobia, generalized anxiety, and panic disorders. Freud was not alone in anticipating DSM-IV. As the authors of DSM-IV have acknowledged, striking similarities are also to be found in the sixth edition of Kraepelin's textbook [183,184]. Furthermore, it is interesting that Freud considered agoraphobia to be characterized by a fear of panic attacks, and not by fear of streets or squares per se: ". . . ce que redoute ce malade c'est l'événement d'une telle attaque . . ." (. . . what the patient fears is the occurrence of such an attack . . .) [185].

Freud's reputation was not based upon his interpretations of anxiety neurosis, however. His second theory of anxiety, in which anxiety was interpreted as a signal of inner threat, would ultimately have much greater influence [186].

This second theory had already announced its arrival by around 1895, albeit in a somatic guise. Freud asked why nonprocessed sexual excitation should express itself specifically in the form of anxiety. In answering this question, a glimpse is afforded of something that would later be more explcitly developed as a theme. Unlike real anxiety, which was based on the perception of an external threat, neurotic anxiety was a reaction to inner threat. The core of this inner threat was an inability to process endogenously created (sexual) excitation [187]. On another occasion, Freud put it as follows: "Anxiety is the sensation of the accumulation of another endogenous stimulus, the stimulus to breathing . . ." [188].

It is sometimes forgotten that elements of the above hypothesis also appeared in Freud's signal theory. There, also, the basis of all anxiety was biological helplessness (i.e., the helplessness of the child with respect to its own drive impulses) [189]. Although the signal theory also concerns the satisfaction of needs, it does not relate primarily to sexual needs but rather to those associated with the instinct for self-preservation [190]. Object loss, the most clear-cut threat recognized by this instinct, becomes the psychological prerequisite for inducing the ego to give off a small quantum of anxiety in order to restore a favorable balance of pleasure and displeasure. The threat of object loss remains linked to the biological state of being at the mercy of one's drive impulses. This linkage is mediated by remembrance symbols that, via separation and birth, ultimately refer to an archaic inheritance of hereditary anxiety responses. In anxious patients, the symptom of gasping for air is no longer seen as a mitigated orgasm but rather as the rudiment of the cry of a newborn child [191].

In a negative sense, Freud's second theory of anxiety was of great significance within the classification debate. His statement that anxiety was the "loose change" of the neurotic conflict illustrates the nosologically nonspecific character gradually adopted by anxiety in his interpretations.

This was partly the reason why the classification of anxiety symptoms did not exactly receive top priority in the period between 1930 and 1960. This is not, of course, meant to detract from Freud's exceptional merits. In the field of anxiety theory, these merits lay particularly in the concept of anxiety as a reaction to an inner threat. This idea, which was without precedent in Freud's days, permanently changed the face of psychiatry. Freud thereby gave a wholly individual treatment to the fundamental distinction between (object-less) anxiety and (object-linked) fear, a theme that for the rest was to find its way into psychiatry via another route.[6]

Freud's approach was not limited to the psychoanalytic school. In its interpretation of anxiety, cognitive psychology (in the style of Beck) built upon Freud's pioneering concept of anxiety as an inner threat [192,193].

G. Clinical Studies

After 1900, despite the almost universal recognition of the central position of anxiety in psychopathology, relatively few psychiatric monographs were devoted exclusively to anxiety and anxiety disorders. One exception was the previously mentioned, exhaustive study by Störring. Several authors occupied themselves with conceptual questions based on clinical observations. Goldstein [194] and Kronfeld [195] produced splendid articles that incorporated some fundamental concepts. Some other names that should be mentioned in this context are those of Hoche [196], Kornfeld [197], and Oppenheim [198]. We already discussed Janet's, "Les obsessions et al psychasthénie" (Obsessions and psychasthenia); however, his "De l'angoisse à l'extase" (From anxiety to ecstasy) from 1926 is worthy of special mention too [199].

Next, reference should be made to several studies arising from particular theoretical points of view. These include not only the psychoanalytical studies by Stekel [200], Bitter [201], and Riemann [202], but also the anthropological studies of von Gebsattel [203–205] and Tellenbach [206]. In addition, studies exclusively devoted to a particular form of anxiety, such as the innumerable publications on "Schreckneurosen" and "Schreckpsychosen" (from the German Schreck: terror), were carried out in the periods around both world wars [206–208].

Seen from a broader perspective, the conceptual framework within which the debate on pathological anxiety took place was specifically determined by the intellectual legacy bequeathed by scientists and philosophers such as Charles Darwin, W.B. Cannon, William James, Sigmund Freud, and Max Scheler. It is no simple matter to illustrate just how the various interpretations and schools of thought influenced one another. Accordingly, I will restrict my discussion to two themes, the role of bodily perceptions in the origin of anxiety and the further determination of the distinction between anxiety and fear.

With regard to the first theme, there seemed to be a significant resistance among clinicians to the James-Lange theory of emotions. Bodily changes, according to this theory, instead of resulting from subjective feelings, were actually the cause of the emotional coloring of sensory perceptions. Perception became emotion via the awareness of bodily changes [210]. James himself was responsible for the subsequent confusion as to whether a temporal-causal relationship existed between bodily changes and emotional perceptions, or whether both occurred simultaneously. It was usually assumed (probably not entirely correctly) that James was postulating a temporal sequence between bodily changes and emotional perceptions. Clinicians, who also adopted this interpretation, criticized him on this point by pointing out the immediacy of the experience of anxiety. According to Störring, the experience of anxiety was not mediated by prior bodily perception. However, he was not entirely logical on this point since he consistently spoke of anxiety as a processing of, or a reaction to, sensations associated with specific organs [211]. Both Kornfeld (not to be confused with Kronfeld, who has been mentioned previously) and Hoche lodged objections, on descriptive grounds, against the idea of a temporal sequence between bodily change and emotional perception. Kornfeld, who himself suffered from neurasthenic complaints and panic attacks, enlivened his article with a description of one of his nightly panic attacks. On awakening, he would first experience a severe feeling of anxiety without really knowing why he was frightened. Shortly afterward, he would become aware of bodily sensations such as a feeling of constriction in the region of the heart, difficulty in breathing, paraesthesia, and cold akra. This led him to conclude that a sharp distinction should be made between feelings of anxiety and the perception of bodily changes. Al-

though Hoche thought along similar lines, like Hecker he formulated a sort of interoceptive explanation for anxiety, in which anxiety was based on the misinterpretation of bodily sensations. Kraepelin and Lange's handbook ultimately rejected James' theory of emotions on theoretical grounds, both because of its psychophysical dualism and its disregard for central regulatory processes. An emotion such as fear of suffocation could be both somatic and psychological in origin. According to Kraepelin and Lange, the origin of this fear of suffocation (whether lack of oxygen, hypercapnia, acidosis, or frightening events) was irrelevant to the quality of the emotion itself. In all cases, the central issue was a threat to the patient's existence as a biological entity rather than any perception of bodily changes [212]. It should be noted, howver, that James was too much of a Darwinist to be accused of psychophysical dualism.

In summary, it can be said that clinical psychiatrists mainly resisted the James-Lange theory because of their support for the primacy of clinical observation. Nevertheless, the antitheoretical sentiment in these convictions sometimes led to all sorts of nonexplicit theoretical views being smuggled in through the back door.

In discussing the second theme, that of the further determination of the distinction between anxiety and fear,[6] consideration should first be given to Kurt Goldstein's observations of patients with organic brain damage. The majority of Goldstein's patients were victims of the World War I. He observed that, when faced with overly complex tasks, these patients displayed a catastrophic reaction consisting of a wide range of physiological and psychomotor symptoms. Goldstein believed that even though it was not subjectively experienced as such, this condition could best be interpreted as an expression of anxiety.

While Goldstein's patients were unaware of the fact of their anxiety, the appearance of their physical symptoms coincided with the failure to accomplish their tasks. Strictly speaking, their anxiety was neither a reaction to failure nor a reaction to an awareness of failure. Anxiety—and this was the essence of Goldstein's interpretation—was quite literally the actual manifestation of failure. Goldstein concludes that generally spoken anxiety was the expression of a frustrated urge for self-realization.

This reference to the urge for self-realization was particularly popular among those contemporary authors who drew their inspiration from vitalism. Although similar references can also be found in Freud's later work [213], it was actually the colossal presence of Charles Darwin, behind the scenes, which inspired this line of thought. However, Goldstein was not thinking of the survival of the species, or that of the individual, in purely Darwinian terms. The urge toward self-realization was more than a purely biological reality. It also found expression, for example, in the productive creativity shown by children and adults in mastering the world. Anxiety was referred to as the "Erschütterung (des) Bestandes der Persönlichkeit" (disruption of the stability of personality) [214]. Ultimately, however, Goldstein failed to fully clarify the conceptual status of the propensity for self-realization. The task of formulating more explicitly things that Goldstein merely hinted at fell to other authors. Here we find a scheme in which personality was divided into an impersonal substructure and a personal superstructure. The substructure was described in vitalistic terms while the superstructure was analyzed in terms derived from existentialist phenomenology. Examples of this can be found in work by Arthur Kronfeld, Felix Krueger, Philipp Lersch, and, to a lesser extent, H.C. Rümke. According to Kronfeld, anxiety was based upon a deterioration of the personal superstructure. In extremely succinct terms, this disintegration was expressed as psychotic anxiety. However, the type of anxiety that Kronfeld initially had in mind was existential rather than psychotic:

> Anxiety is the mental expression of the existential annihilation of the integrity ("Einheitsform") of the person. Its archetype is the fear of death, the anxiety related to vital destruction [215].

Such statements only become comprehensible when it is realized that Kronfeld rejected the link between anxiety and threat. Anxiety, in the true sense of the word, is not the counterpart of safety, but of meaning, of being a person, of "one-ness," of living. The anxious person need not necessarily have a subjective awareness of this, however. Death has significance long before it becomes a subjective reality. According to Kronfeld, the biological aspect of anxiety consisted of life fleeing from death. Such flight is in vain, however, and therein lies the source of all fear of life and of anxiety about fate. Therefore, with anxiety, it is necessary to pose the question of meaning [216]. Kronfeld's criticism of the biologically inspired interpretation of anxiety was that it failed to do this.

In uncoupling anxiety from threat, Kronfeld may have gone a little too far, but his remark that indescribable anxiety often has the quality of fear of living receives daily confirmation from the mouths of borderline patients.

H. From Dimension to Category

As has already been noted, the study of anxiety was not a high priority in the period from 1930 to 1960. In addition to the previously mentioned influence of psychoanalysis, which described anxiety as a nonspecific phenomenon, the assumption that anxiety occupied a low position in the hierarchy of psychiatric symptoms also had a part in this [217]. According to this line of thought, not only did anxiety occur in practically all psychopathological syndromes, it also marked the lower boundary of psychopathology, where this bordered on normality.

Jablensky adds to this that classification had traditionally been an area of interest for institutional psychiatry [218]. An explanation for the relative neglect of the classification of anxiety disorders was that, as a rule, patients with neurotic anxiety were never hospitalized. This status quo gradually changed toward the end of the 1950s. I shall summarize a number of these developments.

The psychophysiological investigation of emotions continued along the lines of the James-Lange theory. In this context, Ax attempted to draw a distinction between the emotions of anxiety and anger on the basis of their peripheral physiological symptoms [219].

The anxiolytic effect of benzodiazepines was discovered, resulting in a flood of research into the effects of these chemicals on the central nervous system [220].

J. Wolpe introduced systematic desensitization as a form of behavior therapy, thereby giving new impetus to the treatment of people with anxiety disorders [221].

The British psychiatrist M. Roth described a form of depersonalization associated with severe anxiety and phobic phenomena [222]. This was the so-called "phobic anxiety-depersonalization syndrome." Although it usually developed in the wake of a psychotrauma, this picture could sometimes occur spontaneously. The EEGs of just under one-sixth of all patients revealed the presence of temporal-epileptic symptoms.

Finally, at the end of the 1950s, D.F. Klein (who has been referred to previously) discovered that panic attacks in agoraphobic patients could be blocked using Imipramine [223,224]. This marked the beginning of a great flood of experimental, pharmacological, clinical, longitudinal, epidemiological, genetic, and familial research into the existence and course of panic disorder.

With this boom in psychopharmacological research, increasingly stringent criteria for the definition of psychiatric syndromes were drawn up. This was principally for the sake of comparability between research groups. Thus, psychopharmacological and biological psychiatric research constituted a powerful impetus for the development of the Feighner Criteria [225]. These, together with the Research Diagnostic Criteria [226] formed the basis of the DSM-III and its further editions. The emphasis on descriptive precision led to the demarcation of various forms of anxiety and to an abandonment of the concept of neurosis, which was considered to be too vague. The depressive neurosis of DSM-II (1968) became dysthymia in DSM-III, falling under the affective disorders. Two types of hysterical neurosis, hypochondria and depersonalization, were classified under somatoform and dissociative disorders, respectively. Neurasthenic neurosis was discarded. Anxiety neurosis, phobic neurosis, and obsessive-compulsive neurosis were combined under the heading of anxiety disorders. Post-traumatic stress disorder, a newcomer, was added to the anxiety disorders [227,228]. The anxiety neurosis was subsequently split up into panic disorder and generalized anxiety disorder, while the phobic neuroses were divided up into agoraphobia, simple phobias, and social phobia [229,230].

In spite of the nontheoretical nature of DSM-III, this change nevertheless heralded in a fundamentally different approach to the psychopathology of anxiety. DSM-III bode farewell not only to the psychodynamic conflict model, but also to a broader tradition in which anxiety was associated with disorders in personality structure. It was replaced by a finely grained description and classification of more superficial symptomatology. Anxiety was no longer regarded as a consequence of the personality structure but primarily as a symptom in and of itself. This resulted in a switch from the predominantly dimensional or dispositional approach, which characterized the neurosis model, to a typological or categorical approach to psychopathology.

Panic disorder represents the most outstanding example of this development, becoming "a microcosm of many of the classic controversies surrounding psychiatric research" [231]. Biological, psychoanalytic, behavioral, and cognitive explanatory models now competed for priority in this debate [232–236]. At the moment, the question of what exactly should be classified (patients? theoretical constructs? diseases?) is once again highly relevant [237].

V. SUMMARY AND CONCLUSION

The preceding chapters cover only part of the history of anxiety and depression. While this is partly attributable to the introductory nature of this review, the main reason is that this discussion has been limited to concepts alone. Behind these concepts lies individual suffering, the often ineffective concern of those around the patient, and the numerous therapeutic efforts of physicians and paramedics. Although not covered here, this history is at least as worthy of our consideration as the one described above.

In the Introduction, it was stated that the history of anxiety and depression should be interpreted as the interplay of cultural changes and of changes not only in psychopathological symptoms per se, but also in their scientific interpretation. This interplay has, to some extent, been reflected in the preceding chapters.

Viewed in the context of the microcosm–macrocosm theme, which was current both in antiquity and in the renaissance, the mentally ill appeared as the personification of a

disruption in the subtle balance of cosmic forces. Such people were different, but not so different as to warrant either ostracism or confinement.

The moralizing and allegorizing of humoral pathology in the Middle Ages raised the question of where disease ended and sin began. But it was difficult to know where to draw the line, as the 19th-century theory of degeneration made clear. According to this theory, a transgression (due to alcoholism, for example) in one generation could lead to increasingly serious forms of psychopathology in succeeding generations.

The idea that melancholics show traits of the exceptional and of genius is a common thread running throughout history. Starting with Aristotle, it can be traced via Marsilio Ficino, through Burton and the poets of the Elizabethan Era, and onward into the present century in the work of William James. The fact that depressives' weaker perceptual defenses allow them a more adequate perception of reality may, perhaps, be a pale reflection of this very idea. The rationalization and mechanization of the world view in the post-Cartesian era finally resulted in a view of the mentally ill in which the irrational and uncontrollable elements of their behavior received special emphasis. The bestial side of human nature revealed itself in such people. Right up to the present day, domination and control have been key words in understanding the motivation behind medical concern for the psychiatric patient. It is this very contrast with earlier treatment that illustrates the extent to which the medical domination of previously uncontrollable emotions has become both the motive and guiding principle for current theoretical and therapeutic activity.

The current fascination of clinicians and researchers with the biological approach therefore comes as no surprise. This approach seems to bring the promise of control and of tangible results, in contrast to the unpredictable and much less concrete results obtained by psychological and social intervention. However, it is appropriate to issue a warning at this point, since history has shown us how intractable psychopathological reality can be. It is not without reason that clinicians repeatedly demonstrated an astonishing eclecticism concerning the theoretical explanation of various insights. Clinicians showed reticence when it came to reasoning from preconceived theoretical points of view. I would remind you of the reservations expressed against the James-Lange theory of emotions. This reticence cannot simply be attributed to an antiscientific attitude. It also stems from healthy clinical skepticism. The history of the classification of anxiety and depression serves to emphasize the fact that such skepticism was often quite appropriate. Whenever attempts were made to refine a given theory, or combination of theories, clinical reality always proved to possess an overabundance of elusiveness and unpredictability. Broadly speaking, longitudinal clinical observation advanced the classification of anxiety disorders, and especially that of mood disorders, more than did any classification based on preconceived theoretical assumptions. In view of the controversy between the unitarians and the separatists, a combination of longitudinal and interdisciplinary (bio-psycho-social) research would seem to hold out particular promise for the future.

Finally, what has been said above should also be emphasized on epistemological grounds. As scientific disciplines, neurobiology and pharmacology tackle problems abstractly and objectively. This implies that there is, by definition, a gap between the research findings in these disciplines and clinical reality. Furthermore, scientific constructs never relate to this reality in its entirety, but merely to aspects of the whole. Reification (i.e., the identification of these constructs with reality) can only lead to distortion. The conceptual history of anxiety and depression illustrates the repeated recurrence of forgotten ideas. These were eliminated in the process of abstraction, only to return via the back door.

Scientific interpretations only bear fruit when the tension-filled gap separating them from clinical reality, rather than being short-circuited, is kept open.

NOTES

1. Klibansky, Panofsky, and Saxl (1964, pp. 64–66 and 98–102) point out an irregularity in the classical interpretation of melancholia; this irregularity occurs in the theory of temperaments. In the strict humoralist tradition, temperaments are reduced to disturbances in the balance between the bodily fluids. These disturbances initially appear to fall within the normal range, like the usual variations of character dispositions. However, changes in interpretation over the centuries become obvious: only blood is now considered to be an integral part of the body, with the other three fluids regarded as degeneration products. The significance of these degeneration products (yellow bile, black bile, and phlegm) would, especially in the Middle Ages, take on a negative connotation, as a result of which they would be considered to be responsible for all kinds of character abnormalities and immoral behavior.

The Galenic and Neo-Galenic theories of temperament, on the other hand, were originally oriented toward the primary qualities (heat, cold, dryness, and wetness), and not so much toward the humors. The Galenic temperaments are not in fact real temperaments, that is to say temperaments in a biological (humoral) sense, but rather disturbances in the balance between the primary qualities. Health was considered by Galen to be an ideal state; every relative excess of a particular bodily fluid was, to his mind, a disturbance and therefore also a disease. Thus the Galenic tradition had little influence on the development of a proper theory of temperament: it could not deal with normal variation of character dispositions.

Behind this irregularity in the theory of temperaments lies a difference of opinion about disease: the strict humoralistic tradition took a concrete and natural view of normality; whereas the Galenic tradition established itself on more abstract ideas, such as the primary qualities.

2. In scholastic medicine, the emotions of anxiety and rage are diametrically opposed. In rage, heat is generated and floats to the periphery, the arms and legs display movement, the face becomes red, the pulse strengthens, and the brain also comes to life again. In anxiety, the heat drains inward, peripheral parts become cold and pale, the pulse rate decreases and the patient feels cold.

3. Plato distinguished four forms of godlike mania, namely, mania as the art of fortune telling (cf. the etymological relation between mania and mantikè, i.e., fortune telling); mania as ritual purification and consecration leading to the relief of disease and grief; mania in the sense of ecstasy inspired by the Muses; and finally mania in the sense of being emotionally moved by memories when looking at beautiful things.

4. Cf. Descartes, 1647, article 52: ". . . qui nous importent . . ."; and Guéroult 1953, p. 253.

5. Kraepelin remained a dedicated experimental psychologist throughout his life. During his years in Heidelberg (1891–1903), the work which he carried out included experiments on the function of will (task performance, level of fatigue), cognitive capacities (distractibility, attention, memory, ideational association), and expressive functions (motor activity, handwriting, and language performance) (Kraepelin 1983, p. 71). During his Münich years (1903–1922), much to his regret, he was no longer able to find the time for experimental psychology, although he did give refresher courses for colleagues (ibidem, pp. 144, 145, 149).

6. The distinction between objectless anxiety and object-linked fear, which already had been formulated by the philosopher Kierkegaard (1844), was to be introduced by Karl Jaspers in 1946, in the fourth edition of his Allgemeine Psychopathologie (General Psychopathology). In the ensuing years, it was developed as a theme in the anthropological school, which was oriented toward existential phenomenology.

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2

Epidemiology of Depression and Anxiety

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I. INTRODUCTION

Epidemiological studies in psychiatry may help in assessing the importance of a certain disorder in order to develop treatment strategies and in planning special health prevention programs. They may provide information on the use of health services and the economic impact of psychiatric disorders on the health-care system. Epidemiological research may also help us to better understand the etiology of psychiatric diseases. For example, when it is found that the prevalence rates of the anxiety disorders are more or less the same in many different countries, despite different cultural and social environments, it seems less probable that these disorders can be attributed mainly to cultural or psychosocial causes. If this is the case, neurobiological determinants that are distributed statistically among all people, regardless of their sociocultural surroundings, must also be seen as a relevant etiological factor.

A substantial underrecognition and undertreatment of anxiety disorders and depression have been shown. According to a WHO study, only approximately half of the cases of depression and anxiety have been recognized and only one-third were offered drug treatment [1].

II. METHODOLOGY OF EPIDEMIOLOGICAL STUDIES

A. Prevalence Rates

In epidemiological studies, different kinds of prevalence rates are determined, and all have their advantages and disadvantages. The *lifetime prevalence* is the proportion of the sample

who ever experienced a disorder in their life. Lifetime prevalence rates of disorders with a high mortality rate may be underestimated. The *annual prevalence* is the proportion of probands who experienced the disorder at some time in the 12 months prior to the interview. Disorders of longer duration are likely to be overrepresented in annual prevalence rates compared to those of short duration. The more chronic a disease, the more similarities between lifetime and 12-month prevalence rates should be found. The *point prevalence* is the prevalence of a disorder on a certain effective day.

B. Representativity

To determine the prevalence of mental disorders, large-scale, expensive, and complex surveys have to be undertaken.

One relatively simple way to find out how many people suffer from certain psychiatric disorders would be to make a survey among all patients who attend a number of different mental health services. However, by simply counting the individuals suffering from panic disorder or major depression who consult a psychiatrist in a private practice or a mental clinic, one would obtain prevalence rates that could be significantly distorted, as they could be influenced by various factors such as specialty of the physician. Moreover, prevalence rates could be distorted because patients with certain psychiatric diseases tend to have a high medical care utilization, such as somatization disorder patients, and others may only rarely seek psychiatric help, such as patients with social phobia or severe cognitive dysfunctions. Finally, some patients in some countries can just simply not afford to see a doctor, resulting in an underestimation of the prevalence of certain disorders in this population.

The only way to obtain reliable prevalence rates is a so-called "knock-door" survey, in which representative samples are collected by using methods known from population polls. From a listing of all residential addresses, systematic samples are selected, and one household member is addressed using a special procedure. Then, interviewers knock at the door of these households and interrogate the selected member using a structured interview.

To obtain a complete overview, representative surveys should also include patients currently hospitalized or in long-term facilities. However, not all studies have incorporated this population.

Samples should be taken from different regions, including urban and rural sites. The sample sizes of these surveys should be very large in order to obtain reliable and generalizable results, not only for frequent disorders but also for rare illnesses. The largest of these studies, the ECA study (see below), comprised 24,371 respondents.

When these large samples are investigated in population surveys, it is too expensive to have the work done by experienced psychiatrists. Thus, these studies are usually conducted by professional interviewers who go through a specific training program for psychiatric interviews.

However, studies conducted in psychiatric outpatient services or in primary care setting may also yield valuable information. Recently, a worldwide survey conducted by the World Health Organization (WHO) explored the frequency of psychological problems in primary care or general health settings [2].

In statistical investigations conducted with psychiatric inpatients, psychiatric disorders like depression, schizophrenia, or personality disorders are usually overrepresented because certain features of these disorders require inpatient treatment, including suicid-

ality, hostility, or reduced social integration. In these surveys, patients with anxiety disorders are generally underrepresented, as anxiety disorders rarely require inpatient treatment.

C. Diagnosis and Interview Technique

In order to obtain reliable diagnoses, interviews are usually based on the standard diagnostic tools, *Diagnostic and Statistical Manual for Mental Disorders* (DSM) [3] or *International Classification of Diseases* (ICD-10) [4]. In order to structure the diagnostic process and to obtain objective results, special interview manuals have been developed. These include:

The Structured Interview for DSM (SCID), which is a semistructured interview for making the major axis I DSM diagnoses. It is administered by a clinician [5,6].

The Mini-International Neuropsychiatric Interview (M.I.N.I.), which is a short, structured diagnostic tool for DSM-IV and ICD-10 psychiatric disorders. With an administration time of approximately 15 min, it was designed to meet the need for a short, but accurate, structured psychiatric interview for multicenter clinical trials and epidemiological studies [7].

The Diagnostic Interview Schedule (DIS), which is an interview schedule that allows lay interviewers or clinicians to make psychiatric diagnoses according to DSM criteria [8].

The Composite International Diagnostic Interview (CIDI) for DSM [9] or its modified version for ICD-10 [10], which combines questions from the DIS with Present State Examination questions and is fully structured to allow administration by lay interviewers and scoring of diagnoses by computer.

Some representative surveys have been conducted in recent years, using complex sampling methods, well-defined diagnostic criteria, elaborate questionnaires, and sophisticated statistical methods (see Table 1). The largest ones were the following:

In the Epidemiologic Catchment Area Program [11], a project conducted in the early 1980s, a probability sample of households was selected and one adult residing

Table 1 Lifetime and 6- or 12-Month Prevalence Rates for Major Depression, Sorted by Lifetime Prevalence

Authors	Site	N	Lifetime	12 months	6 months
Karam [21]	Beirut, Lebanon	526	19.0%	—	—
Kessler et al. [12]	U.S. (NCS)	8098	17.1%	10.3%	—
Lépine et al. [86]	Paris, France	1746	16.4%	4.5%	
Joyce et al. [87]	New Zealand	1498	12.6%	5.3%	—
Wittchen et al. [14]	West Germany	657	9.0%	—	3.0%
Bland et al. [17]	Edmonton, Canada	3258	8.6%	—	3.2%
Robins et al. [22]	U.S. (ECA)	18,571	5.2%	3.0%	—
Canino et al. [88]	Puerto Rico	1551	4.6%	—	3.0%
Hwu et al. [13]	Taiwan	11,004	1.5%	0.8%	—
Faravelli et al. [60]	Florence, Italy	1110	—	5.2%	—
Lee et al. [89]	Korea	5100	2.9	2.3%	

in this household was interviewed in five U.S. communities; the DSM-III and the DIS were used; it included 18,571 persons.

The National Comorbidity Survey (NCS) [12] was based on a stratified, multistage probability sample of persons aged 15 to 54 years in 48 U.S. states and was conducted from 1990 to 1992. DSM-III-R diagnoses were made with the CIDI; 8098 persons were interviewed. The interviewers were not clinicians, had an average of 5 years of prior interviewing and went through a 7-day study-specific training program for this survey.

The Taiwan Psychiatric Epidemiological Project was a representative survey and used the DSM-III and the DIS. It was conducted by trained lay interviewers in seven urban and rural regions in Taiwan [13]; 11,004 persons were interviewed from 1982 to 1986.

Nonpsychiatrists sometimes express the opinion that prevalence rates for some mental disorders obtained in these studies are grossly exaggerated. For example, according to the NCS study [12], every third woman suffers from an anxiety disorder once in her life. It is a problem that the classification systems DSM and ICD cannot reliably differentiate between subthreshold cases and clinically significant cases, which definitely need treatment. Some of the DSM and ICD criteria were decided by committee rather than being empirically derived from field studies. If a survey is carried out by lay interviewers, there is the possibility that prevalence rates of some less well-defined disorders may be inflated. If a survey is conducted by psychiatrists (e.g., Ref. 14) or uses a general psychiatric outpatient sample (e.g., Ref. 15), the clinical cases will probably be identified more reliably.

Recently, a worldwide survey conducted by the WHO has explored the frequency of psychiatric disorders in primary care [2]. In this kind of study, persons who are consulting health-care services are screened for psychological problems and psychiatric disorders, regardless of their reason for attending that service. This also means that persons who consulted the doctor for a cold are included. These studies are not appropriate for obtaining representative prevalence rates for the reasons given above. However, they may yield valuable information on the use of health services and the social and financial impact of psychiatric disorders.

Even the representative population surveys show substantial discrepancies in prevalence rates. This may be attributed to various factors, including methodological differences that could distort the actual prevalence rates, for example:

Variation in the use of the diagnostic criteria (e.g., DSM-III or DSM-III-R).
Variation in the use of the interview manual (e.g., CIDI or DIS).
Type of interviewer (trained lay or psychiatrist/psychologist).
Interviewer instructions [16].
Language differences or translating problems.
Cultural differences in conveying psychiatric symptoms.
Differences in age range of the sample investigated [12].
Standardization of prevalence rates to the census population of each site instead of to an identical population [17].

However, actual differences between the investigated populations may exist also and may be due to:

Biological differences across races and ethnic groups.

Culturally determined psychosocial differences (e.g., a different role of women in society).
Traumatic stressors that influence whole nations or ethnic groups (e.g., war, poverty, natural disasters, or suppression of minorities).

III. DEPRESSION

A. Prevalence

Lifetime and 6- or 12-month prevalence rates in different countries are listed in Table 1. There was a wide variability in the rates of major depression across nations [18]. There is no simple explanation for this finding. The highest rates were found in war-shaken Lebanon, but rates in the U.S. (NCS study) or Paris were almost the same. The lowest rates were found in the Taiwan study. In this study, very low rates were also found for most other psychiatric disorders, the reasons for which are unknown. It would be premature to attribute these low rates to actual differences rather than to methodological biases (see Refs. 19,20 for a discussion).

Women have higher depression rates than men across all countries. The female-to-male ratio is 2:1 on average, ranging from 1.6:1 in Taiwan and Lebanon [13,21] to 2.6 in the U.S. [22]. A number of reasons for the higher rates in women have been proposed without a clear resolution. The higher occurrence of anxiety disorders in females than males beginning early in life might explain the higher female risk for major depression, as anxiety rates are also higher in women [23].

B. Course

The mean age at onset is very similar in most studies, ranging from 24.8 to 29.5 in the majority of surveys—with the exception of Italy, where a mean age of 34.8 years was found [18]. Depression rates are highest in midlife. The highest 1-month prevalence rate was found in the age group 25 to 44 (3.9%), followed by 18 to 24 (2.9%), 45 to 64 (2.6%), and 0.9% in persons aged 65 and older [11]. However, these prevalence rates may have been influenced by high rates of suicidality for depressed patients.

IV. BIPOLAR DISORDER

In contrast to the high variability found in major depression, the lifetime rates for bipolar disorder were rather consistent across the nations and varied between 0.3 and 0.9%, with the exception of New Zealand, where a higher rate of 1.5% was found.

Although women have higher rates for major depression, for bipolar disorder the opposite was found in some countries. In Canada, Puerto Rico, Korea, and New Zealand, female-to-male ratios between 0.3:1 and 0.7:1 were registered. In the U.S. (ECA), the rate was 1.2:1, and in Taiwan rates were equal for men and women [18]. The mean age at onset ranged between 17.1 in Canada to 29.0 in West Germany.

V. DYSTHYMIA

A. Prevalence

The lifetime prevalence rates of dysthymia in different countries are listed in Table 2. The average female-to-male ratio in these studies was 1.5 to 2.5. The highest 1-month

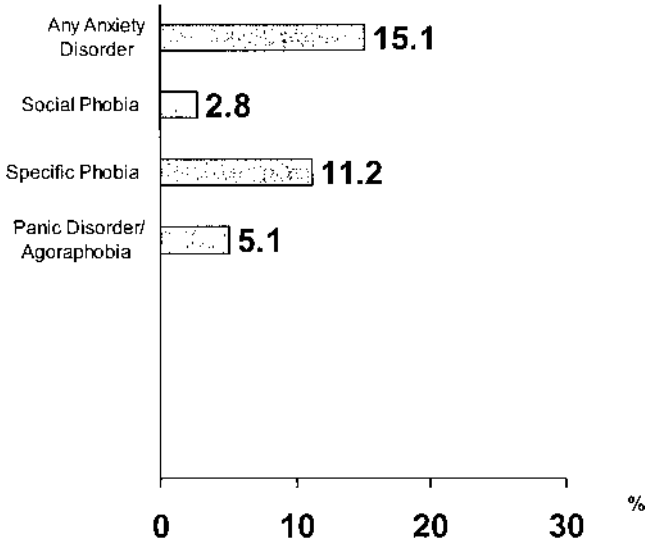

Figure 1 Lifetime prevalence rates for anxiety disorders (Ref. 22).

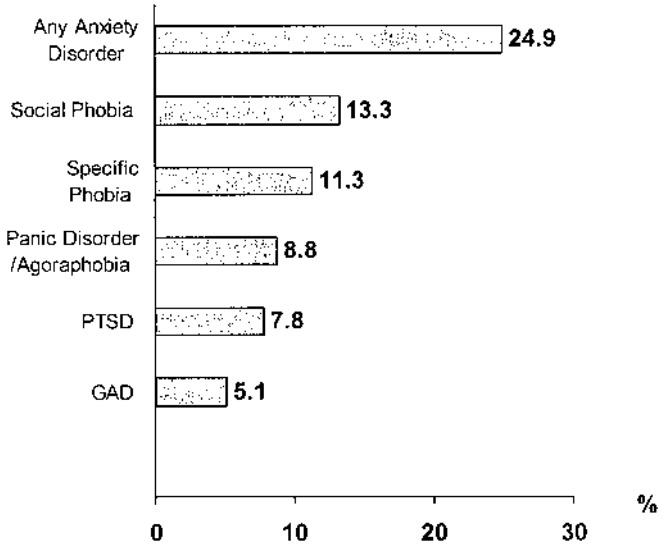

Figure 2 Lifetime prevalence rates for anxiety disorders (Ref. 12). OCD: obsessive-compulsive disorder; GAD generalized anxiety disorder; PTSD: post-traumatic stress disorder.

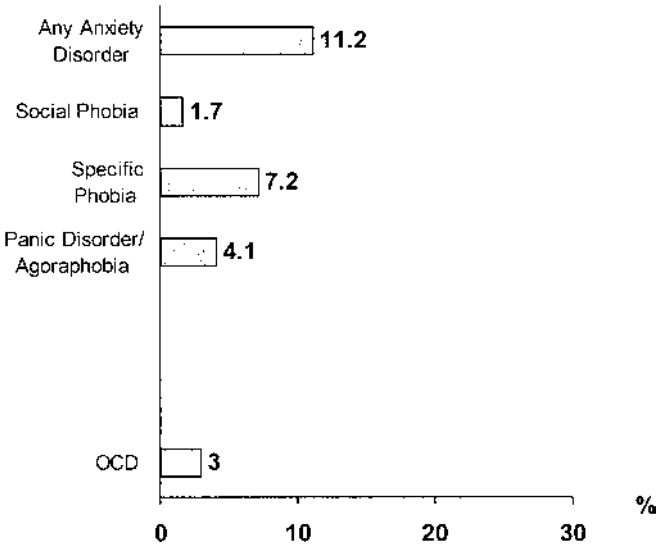

Figure 3 Lifetime prevalence rates for anxiety disorders (Ref. 17). OCD: obsessive-compulsive disorder.

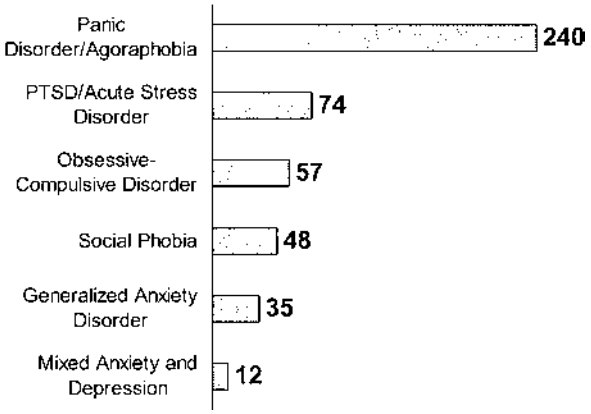

Figure 4 Number of patients attending an anxiety disorders unit at the University of Goettingen, Germany in 6 months (May–Oct 1999). Diagnoses according to ICD-10 (Bandelow, unpublished).

Table 2 Lifetime Prevalence Rates of Dysthymia

Refs.	Site	Lifetime Prevalence
Kessler et al. [12]	U.S. (NCS)	6.4%
Canino et al. [88]	Puerto Rico	4.7%
Wittchen et al. [14]	West Germany	4.0%
Bland et al. [17]	Edmonton, Canada	3.7%
Robins et al. [22]	U.S. (ECA)	3.0%
Hwu et al. [13]	Taiwan	0.9–1.5%

prevalence rates were found in the age group 45 to 64 (5.4%), followed by 25 to 44 (5.1%) in persons aged 65 and older (2.3%) and the age group 18 to 24 (2.2%) [11].

VI. ANXIETY DISORDERS

A. Prevalence

Among all psychiatric disorders, the anxiety disorders are the most frequent [12]. Because patients with anxiety disorders are mostly treated as outpatients, they probably receive less attention from clinical psychiatrists than patients with other disorders that require inpatient treatment but are much less frequent, such as mania or schizophrenia.

The prevalence rates of the anxiety disorders in three epidemiological studies are presented in Figures 1 to 3.

In a special anxiety disorders unit, the numbers of patients seeking help differed substantially from the actual prevalence rates in the population (Fig. 4). Panic disorder with or without agoraphobia seems to be by far the most frequent reason for seeing an anxiety specialist psychiatrist, whereas patients with GAD were rare, and specific phobia patients did not appear at all.

B. Is There an Increase in the Prevalence of Anxiety Disorders?

Many newspaper articles start with the line "More and more people are suffering from anxiety," suggesting there has been a relative increase in anxiety disorders over the past years. This tendency was already observed before the terrorist attacks in the U.S., which shocked the world and may have made many people feel insecure. However, although the question is of great interest and a general increase in media reports on anxiety disorders has surely been observed in recent years, it is difficult to prove a change in anxiety rates over the years. Epidemiological data obtained before the introduction of classification systems such as the DSM-III are too imprecise to be compared with modern studies. To verify the hypothesis that there is an increase or decrease in certain psychiatric disorders, one would have to repeat large epidemiological surveys such as the ECA or NCS programs—in the same population using the same methodological setting—after an interval of, for example, 30 years.

C. Panic Disorder with or Without Agoraphobia

The prevalence of panic disorder is presented in Figures 1 to 3. For better clarity, the rates for panic disorder and agoraphobia have been added up in the figures, although they were reported separately in the surveys. In the majority of cases, the disorder starts with spontaneous panic attacks, with patients later developing agoraphobia as they learn to avoid situations in which they might have spontaneous attacks, such as crowds, public transport, or enclosed spaces. In a survey of outpatients [24] it was found that 60.4% of the patients had panic disorder with agoraphobia, 28.9% panic disorder without agoraphobia, and 10.7% had agoraphobia without panic disorder. In a cross-national survey, 22.5 to 58.2% of panic patients had agoraphobia [19].

In a primary care setting, 0.9–1.7% of the patients attending a general practitioner had panic disorder and 1.5 to 1.6% had agoraphobia [25]. It has consistently been found that more women than men suffer from panic disorder. Weissman et al. [19] found female-

to-male ratios between 1.3 : 1 and 5.8 : 1 in different countries. Relatively few studies have investigated whether women with anxiety disorders have characteristics that are distinct from those of men with the same disorders. The cause of the enhanced vulnerability to anxiety for women remains largely undetermined. Some data suggest that female reproductive hormones and related cycles may plan an important role [26]. There is some evidence that the vulnerability to anxiety disorders may be genetically determined [27]. This genetic vulnerability may manifest in neurobiological differences. For example, increased monoamine oxidase A activity in women has been found in female patients with panic disorder [28]. The role of women in society may also play a substantial role. Furthermore, it has been discussed that prevalence rates for men are underestimated because it may be less socially acceptable for men to admit suffering from anxiety.

1. Course

According to the Cross-National Epidemiologic Study, the majority of studies found an age at onset between 23.2 and 27.9 years, with two exceptions (32.1 years in Korea and 35.5 in Germany) [19]. In a clinical sample of 322 patients with panic disorder from 6 countries, subjects indicated that they had a mean age at onset of panic disorder of 28.9 years. The average onset of agoraphobia was 6 months later [29].

Panic disorder seems to be rare in children before puberty. In a retrospective study, 343 adults with panic disorder were interviewed whether or not they fulfilled the criteria for panic disorder before age 13. This was condirmed by only 1% of the panic patients [30]. During adolescence, the risk of developing panic disorder steadily increases. A representative survey among 388 high-school attendants revealed a lifetime prevalence of 4.7% for the age period 12 to 19 [31].

Panic disorder has a waxing and waning course. As a whole, it follows a chronic course if left untreated. However, "chronic" is a relative term, as spontaneous remission is generally observed beginning after the age of 40 [11]. Mental health providers rarely see typical panic patients after the age of 60. Bland et al. [32] reported a 6-month prevalence rate of 0.0% for the age of 65 and older. The age distribution of patients referred to an anxiety disorders clinic shows that most patients are between 30 and 45 when they are referred for treatment (Fig. 5). Only persons aged 16 and older were included in this study.

2. Cross-Cultural Differences

Epidemiological data on panic disorder from community studies from 10 countries around the world have been compared [19,33]. These studies were conducted in countries that differ quite a lot in terms of culture, socioeconomic status, religion, and other aspects, and included Canada, Germany, Italy, Korea, Lebanon, New Zealand, Puerto Rico, the U.S., and Taiwan. Although the various surveys differed in design, they were comparable, as DSM-III/DMS-III-R and the interview manuals DIS or CIDI were used for all of them. Some of the studies had very small samples sizes (e.g., 234 in Lebanon and 481 in Germany). Despite some limitations, there seemed to be high agreement in prevalence rates of panic disorder across the majority of countries. Most studies found a lifetime prevalence rate of 1.4 to 2.9%.

Only in the NCS, a high prevalence rate of 3.5 was found, probably because DSM-III-R was used, which has a broader definition of panic disorder. The other studies used DSM-III. In the DSM-III-R criteria, patients are included if they have had only one attack

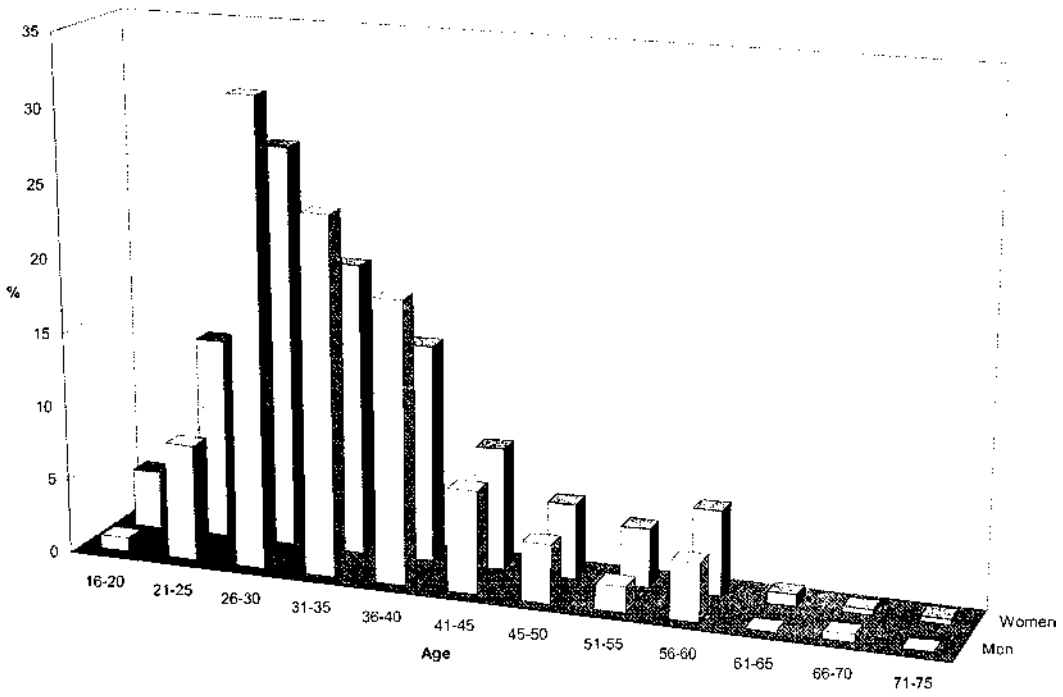

Figure 5 Age distribution of 512 patients with panic disorder with or without agoraphobia referred to an anxiety disorders clinic (Ref. 92). Percentage of all men or all women, respectively. Mean age of all patients: 34.9 years.

in their life and then had "persistent worry about having a panic attack" for at least 1 month. In contrast, DSM-III requires at least 3 panic attacks in 3 weeks.

In Taiwan, very low rates were found for panic disorder, as was found also for many other psychiatric disorders in the Taiwan study. This has been widely discussed (see above). It was hypothesized that culturally determined response bias might have lowered the rates for panic disorder [19,20]. However, as rates for generalized anxiety were relatively high in Taiwan (3.7–10.5%) [13], it may also be speculated that differences in wording or lack of clinical experience of the lay interviewers led to an overestimation of generalized anxiety and an underestimation of panic disorder. When the interview technique is imprecise, it might happen that panic patients are misdiagnosed as having generalized anxiety disorder because most panic patients roughly fulfill GAD criteria. On the other hand, panic disorder criteria are more exact so that it is less probable that GAD patients are misdiagnosed as panic patients.

Still, it seems that the prevalence of panic disorder is relatively consistent in different cultures. This may have consequences for assumptions on the etiology of panic disorder. Neurobiological hypotheses on the etiology of anxiety disorder are getting some support by these findings, whereas cultural influences may play a less important role.

D. Specific Phobia

According to most studies, specific phobia is one of the most frequent anxiety disorders (Figs. 1 to 3). However, patients with isolated fears rarely seek professional help, probably

because they do not have the same restrictions in quality of life as do patients with the other anxiety disorders (Fig. 4). Fear of animals, heights, and blood are most common. The most prevalent specific fears were of animals (among women) and heights (among men) [34]. A female-to-male ratio of 2.3 : 1 was found [12]. Specific phobia seems to have a chronic course. Despite treatment, 64% of specific phobia patients still had symptoms after 10 to 16 years [35].

E. Social Phobia (Social Anxiety Disorder)

Estimates for the lifetime prevalence of social anxiety disorder differ substantially (Figs. 1 to 3), ranging from 0.5% in Taiwan [13] to 14.4% in France [36]. Methodological reasons for different prevalence rates found in various countries have been discussed above. Additionally, the DSM and ICD definitions for social phobia may not be appropriate for reliably differentiating between pathological fear of social situations and simple "shyness" or "stage fright." For example, according to these definitions, someone who is afraid of having a single presentation at a university commission, which significantly interferes with his academic functioning, would receive a diagnosis of social anxiety disorder.

There is a high rate of underrecognition in social anxiety disorder. In a study exploring the prevalence of social phobia in general health care, the disorder was diagnosed in only 24.2% of the patients [36]. However, patients may also contribute to underrecognition, as one of the typical features of the disorder is being easily embarrassed when talking about one's psychiatric problems. Thus, only a minority of patients with social phobia actively seek treatment (Fig. 6).

A female-to-male ratio of 1.4 : 1 was found in the Edmonton [17] and NCS study [37]. Although more women suffer from social anxiety disorder, it appears that men are more likely to seek treatment [38].

1. Course

Unlike other anxiety disorders, which tend to have an onset at the end of the 20s, the mean age at onset for social anxiety disorder is in midadolescence (between age 15.2 and 16.2) [39–41]. Onset after the age of 25 was uncommon [40]. Because of its early onset, development of psychosocial behavior is impaired at an early stage, later leading to reduced social interaction, poor school performance, and poor employment status [42]. The natural course of the disorder seems to be chronic and unremitting.

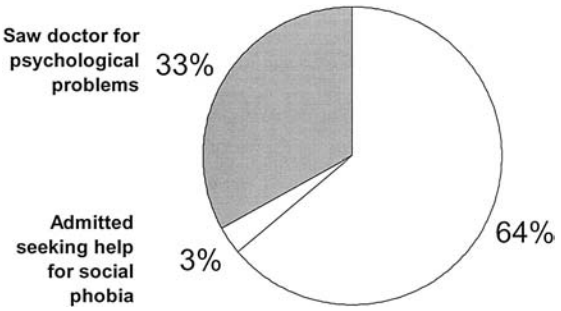

Figure 6 Out of 98 sufferers of social phobia, only a minority saw a doctor for psychological problems or admitted seeking help for social phobia (Ref. 42).

F. Obsessive-Compulsive Disorder

Obsessive-compulsive disorder (OCD) has previously been underestimated because of a number of factors, including patients' reluctance to spontaneously admit obsessions and compulsions because of fear or shame [43]. Annual prevalence rates ranged between 1.1% in Korea and New Zealand to 1.8% in Puerto Rico, according to a cross-national comparison. Again, Taiwan, where only 0.4% was found, was the exception [44]. The NCS study did not address OCD. The female-to-male ratio ranges between 1.2 and 3.8 [11,44].

Consistent evidence shows the average age at onset to be in adolescence or early adulthood [45,46]. OCD follows a chronic course. In patients with an illness duration of at least 10 years, 27% were episodic and 73% chronic [47]. Steketee et al. [48] found that only approximately 20% of patients had full remission and 50% had partial remission during a follow-up of up to 5 years. In a 40-year follow-up, Skoog et al. [49] found improvement in 83%, including recovery in 48% (complete recovery, 20%; recovery with subclinical symptoms, 28%). Approximately one-half of the patients had obsessive-compulsive disorder for more than 30 years.

OCD in children is often underrecognized, as children often keep their OCD secret and do not easily request treatment [50]. Fifty percent of the childhood OCD cases have their onset by age 15 [51]. In a 9- to 14-year follow-up of patients with OCD onset during adolescence, 6 of 14 patients retained their diagnosis [52].

G. Post-Traumatic Stress Disorder

In two sites of the ECA study, lifetime prevalence rates of 1.0 and 1.3%, respectively, were found [53,54] for post-traumatic stress disorder (PTSD). The 6-month prevalence was found to be 0.44% [54]. In the NCS study, a lifetime rate of 7.8% was found (Fig. 2).

Notably, only a moderate percentage of persons exposed to trauma develop PTSD. Risk factors, such as prior psychiatric history, a family history of psychiatric illness, and neurobiological factors influence vulnerability to the onset of the disorder [55]. In a study by Norris [56], respondents in a representative sample were asked if they had ever experienced traumatic events. Rates of current PTSD varied by type of exposure and were highest for sexual assault (14%), physical assault (13%), and motor vehicle accidents (12%). Some epidemiological studies have assessed survivors of specific types of traumas. Of men who served in the Vietnam war, 15.2% met the criteria for PTSD. The rate for women was 8.5% [57]. Among women with a history of sexual assault, the PTSD rate was estimated to be 3.7% compared to less than 1% for those with no such history [58]. Of respondents who were exposed to hurricane Andrew, approximately 3% of males and 9% of females met the criteria for PTSD [59].

H. Generalized Anxiety Disorder

Prevalence rates for generalized anxiety disorder (GAD) found in the ECA and NCS studies are shown in Figures 1 and 2. In different countries, the following lifetime prevalence rates were found: 4.0% in West Germany [14], 5.4% in Florence [60], 10.0% in Paris [15], and in 3.7 to 10.5% in different regions in Taiwan [13].

Of patients consulting a general practitioner for any kind of illness in different countries in Europe, 8.5% were identified as suffering from GAD [61]. However, it is surprising that patients with GAD were relatively rare as compared to panic patients in a special

anxiety disorders outpatient department (Fig. 4). Possible explanations for this discrepancy to epidemiological studies may be that the differentiation between GAD and anxious depression may be very difficult in some cases because of the high comorbidity of these conditions. The differential diagnosis may require all the experience of a well-trained psychiatrist and may be asking too much of lay interviewers. Also, lay interviewers may mistake panic disorder for GAD (see above).

Because of the 90.4% comorbidity of GAD with a wide spectrum of other mental disorders, including depression and other anxiety disorders [62], it has been discussed whether GAD is an independent diagnostic entity at all.

In most cases, GAD has its onset after the age of 25 years. For women, a substantial increase in the incidence was observed after the age of 35, whereas in men an increase occurred after the age of 45 [63]. According to all available studies, it follows a chronic course.

VII. COMORBIDITY

Most studies show a high overlap between depression and anxiety syndromes. Relevant data come from representative studies. In clinical settings, the relative proportion of comorbid cases is even higher than that found in representative population surveys because individuals with two concomitant disorders, suffering from a high overall burden, are more likely to seek treatment than individuals with only one disorder (Berkson's paradox).

Both depression and anxiety syndromes also co-occur with other psychiatric conditions such as substance abuse or personality disorders.

A. Depression and Panic Disorder

> Patients with anxiety of long standing are subject to melancholia
>
> *Aristotle, Epidemics, III*

Of all anxiety disorders, panic disorder has been investigated most thoroughly with regard to its association with depression. Frequently, both panic disorder and depression occur simultaneously. Consistently, high estimates of the lifetime prevalence of major depression in panic disorder between 22.5 and 68.2 have been reported [19,64–66]. Point prevalence rates vary between 30 and 38% [67–70]. Conversely, high lifetime rates of panic disorder among subjects with bipolar disorder or unipolar depression have been found. They vary from 10–59% [69,71–73]. Looking at different depression types, a lifetime prevalence rate of panic disorder of 20.8% was observed among subjects with bipolar disorder, compared to 10.0% among subjects with unipolar depression and 0.8% among reference subjects [74].

Subjects with both panic and depression usually have worse symptoms than those who had only one disorder [64,75–77]. The disorders begin earlier in life when they are comorbid than when they occur singly [77,78].

Models explaining co-occurrence of panic disorder and depression include the hypotheses that the co-occurrence of panic attacks and major depression

1. Results from a common underlying pathogenic process.
2. Is a third disorder separate from panic disorder and depression.
3. Is a coincidence of two common psychiatric disorders by chance.

4. May be explained in some cases by secondary depression due to demoralization by panic attacks.
5. In other cases may be explained by major depression with secondary anxiety symptoms.

From the available epidemiological, family, and neurobiological studies it is difficult to decide which model is most appropriate to explain the high frequency of comorbidity of panic disorder and major depression (for details, see Refs. 75, 76, 79–82).

B. Depression and Other Anxiety Disorders

In patients with major depression, comorbidity with simple phobia was found in 24.3%, with social phobia in 27.1%, with post-traumatic stress disorder in 19.5%, and with obsessive-compulsive disorder in 5.4 to 10.9% [18,83]. Conversely, patients with these disorders have a higher than expected rate for major depression. Of all anxiety disorders, generalized anxiety disorder shows the highest comorbidity with major depression (e.g., 62.4%) [62]. It can be assumed that the same comorbidity models apply for these anxiety disorders as for panic disorder (see above).

C. Is There a "General Neurotic Syndrome"?

It has been discussed that anxiety disorders (and probably "neurotic depression") are not diagnosed entities but are just different manifestations of a "general neurotic syndrome" [84] because there is such a high overlap between these disorders. Moreover, no biological markers have been found that are able to differentiate between these disorders and antidepressants seem to be effective in all of these conditions. However, it still seems to make sense to demarcate different diagnostic entities. Reasons for this include:

In most comorbid cases, one disorder can be identified which is in the foreground (see below).
Some features, such as symptomatology and age of onset (see above) seem to differ quite substantially between these disorders.
There is some evidence for specificity in the genetic transmission.

D. Comorbidity of Anxiety Disorders

Although comorbidity rates are very high (Table 3), the majority of patients still do not have another anxiety disorder, underlining the fact that anxiety disorders should not be easily lumped together.

Table 3 Overlap Between Anxiety Disorders (Lifetime Prevalence)

	Comorbidity with			
First diagnosis	Panic disorder	GAD	Specific phobia	Social phobia
Panic disorder	—	23.5%	14.8%	10.9%
GAD	23.5%	—	16.0–35.1%	13.3–34.4%
Specific phobia		35.1%	—	37.6%
Social phobia	10.9%	13.3%	37.6–44.5%	—

Source: Refs. 19, 37, 62, 90, 91.

VIII. HEALTH-CARE UTILIZATION

Anxiety disorders show different patterns in health-care utilization, explaining why prevalence rates found in representative epidemiological surveys differ from statistical studies in clinical settings. As shown in Figure 7, panic disorder has a relatively high proportion of individuals seeking professional help. Patients with panic disorder often assume that they have a medical rather than a psychiatric condition and tend to have themselves rechecked again and again in internal medicine or emergency wards.

In contrast, patients with social phobia tend to hide their problem. As shyness and shame are typical features of social anxiety, it is not surprising that patients are hesitant to see a physician and to discuss their problem (Fig. 6).

Patients with specific phobia mostly can cope with their problem. Without major restrictions in quality of life they can avoid having contact with the objects or situations they fear, such as dogs, heights, or insects. Thus, these persons very rarely seek professional help.

These considerations may explain why psychiatrists or special anxiety disorders units mostly see patients with panic disorder (Fig. 4), although specific and social phobia are more frequent according to epidemiological studies. GAD patients were also underrepresented in the clinical setting, perhaps because they are overrepresented in representative epidemiological studies (see above). The high health-care utilization of panic patients also explains why more clinical studies have been conducted with panic disorder than with any other anxiety disorder, due to the easy access to high patient numbers needed for scientific studies. For example, a MEDLINE query found 2151 references for "panic disorder" and only 334 for "generalized anxiety disorder."

IX. CONCLUSIONS AND FUTURE PERSPECTIVES

The anxiety disorders and depression are among the most prevalent psychiatric disorders. They are associated with a considerable degree of impairment, high health-care utilization,

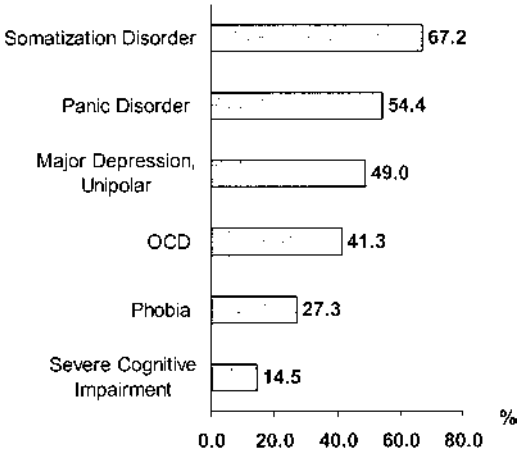

Figure 7 Health-care utilization. Promotion of individuals with specific mental disorders treated in any professional health-care service in 1 year (Ref. 93). For a comparison, the numbers for somatization disorder and severe cognitive impairment are also given.

and an enormous economic burden for society. Epidemiological studies may help in planning treatment and prevention programs and they also may help us to better understand the etiology of these disorders.

Future large epidemiological initiatives to investigate the prevalence of depression and anxiety among different cultures are currently ongoing. They include the European Study of Epidemiology on Mental Disorders (ESEMeD), which will involve 25,000 individuals, and the WHO World Mental Health 2000 study, which will comprise 100,000 interviews conducted in 23 countries around the world [85].

For more accurate projections of treatment need and further explication of rate discrepancies, data on clinical significance (e.g., responses to questions on life interference from, telling a professional about, or using medication for symptoms) should be taken into account in mental disorder prevalence estimates [94].

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3

Comorbidity of Depression and Anxiety

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I. INTRODUCTION: GENERAL CONCEPTS ON COMORBIDITY AND DEFINITIONS

Patients with anxiety or mood disorders often have features of multiple mental disorders, but the extent and significance of this comorbidity has received surprisingly little attention in clinical practice or research in the past [1]. However, the comorbidity of psychiatric disorders is emerging as a recent topic of major practical and theoretical significance. As more systematic attention has been devoted to psychiatric diagnosis in general, psychiatric comorbidity has commanded increasing consideration, and the high frequency of multiple diagnoses has discredited the previously popular assumption that a particular patient is unlikely to have more than one disorder. Parallel, the success of DSM-III [2] concept is largely the result of meeting the clinical research community's need for a better diagnostic system, addressing a confluence of dissatisfaction with the DSM-I [3] and DSM-II [4], and meeting the need increasingly recognized in the late 1960s and the 1970s for an empirical knowledge base (e.g., Ref. 5). However, even after its fine tuning through successive editions, the current edition, DSM-IV [6], represents only a fraction of clinical reality. In DSM-IV and DSM-IV-TR [7], clinicians find categories defined appropriately by descriptive, observable definitions; they also find that the boundaries of any given category are an inadequate match with the patients they treat. Comorbidity and the frequent presentation of atypical and subclinical symptoms are the primary reasons for failure to match patients with the DSM-IV's discrete, categorical, prototypes of mental illness [8].

Given the short history of the term comorbidity, there are a surprisingly large number of definitions. Feinstein [9] coined the term comorbidity to mean "any distinct additional clinical entity that has existed or that may occur during the clinical course of a patient who has the index disease under study." Strictly speaking, use of the term is restricted to diseases or disorders, not symptoms. Symptoms can associate or co-occur, but they are not comorbid with disorders or with each other. In psychiatric epidemiology, the term comorbid is used somewhat differently, the emphasis being on relative risk. When a patient has a particular index disorder, there may be a relatively greater or lesser risk of other disorders being diagnosed or other symptoms observed.

Clinical studies also use the concept of comorbidity in the sense that more than one disorder can be diagnosed in the same individual. In addition, any individual who meets the full diagnostic criteria for only one disorder may still have an increased frequency of symptoms from other categories, but to an extent that is insufficient to diagnose another disorder. Diagnostic studies may identify symptoms or relationships between syndromes that improve diagnostic precision by increasing the discriminant power of diagnostic criteria.

Kaplan and Feinstein [10] also introduced a number of distinctions about types of comorbidity to clarify the concepts of comorbidity that arise in medicine in general and, possibly, in psychiatry. They distinguished between pathogenic, diagnostic, and prognostic comorbidity. Pathogenic comorbidity arises when a particular disease leads to certain other complications or diseases, which are therefore considered to be etiologically related. For an example of two diseases that are diagnostically comorbid, Kaplan and Feinstein cited a patient with polyuria, which is caused by diabetes and a coexisting renal ailment. Diagnostic comorbidity is likely whenever diagnostic criteria are based on patterns of symptoms that are individually nonspecific. Disorders that predispose the patient to develop other disorders have prognostic comorbidity.

It is often difficult to distinguish these subtypes of comorbidity, however, unless the pathogenesis of the disorder is well understood, which rarely happens with psychiatric disorders. The proper terminology—comorbidity versus some other word or phrase—is not unanimously accepted. Winokur [11], for example, prefers cosyndromal or use of the primary–secondary distinction over comorbid. The multiple uses of the primary–secondary distinction have been discussed in Maser et al. [12], and Winokur refers only to the chronological meaning of the primary–secondary distinction. In medical terms, comorbidity conveys, at least in part, the notion of a disease process. Disease is produced by pathogens, but despite the suspicion of many, as said before, there are very few pathogens known to underlie the mental disorders described in DSM-IV. Cosyndromal is a more technically accurate term, and the temporal definition of the primary–secondary distinction has value; but in line with current usage, we shall continue with the term comorbid in relation to mental illnesses, even when there is no known pathogen.

In psychiatry, comorbidity appears to be the rule rather than the exception. Numerous studies of clinical samples of inpatients and outpatients [13–17] have demonstrated the large proportion of patients who simultaneously meet diagnostic criteria for more than a single disorder, both within axis I and between axes I and II of the DSM-III-R [18]. Similarly, multiple diagnoses within individual subjects appear to be quite frequent in epidemiological surveys conducted in the general population [19–21].

Two major approaches have been employed to classify multiple diagnoses within a single individual: (1) assignment of a primary and secondary diagnosis based on order of onset; and (2) application of hierarchical diagnostic systems in which one condition is

inferred to supercede the other. The former approach is preferable because no preconceived etiological assumptions regarding the relationships between disorders are necessary. However, the primary–secondary distinction may be difficult to apply to the assignment of retrospectively ascertained lifetime diagnoses, which require accurate determination of the age of onset of disorders that often emerge in an insidious manner. The latter approach has not been applied consistently across studies because of difference in the hierarchical structure of the diagnostic systems employed. Moreover, hierarchical relationships may often belie clinical data. The elimination of hierarchical relationships between many of the disorders in the DSM-III-R [18] criteria facilitated the assessment of relationships between two or more disorders.

II. BASIC CONCEPTS: SYNDROMAL AND SUBSYNDROMAL COMORBIDITY

During the last two decades, a large number of epidemiological studies conducted in the community [20–23], general health-care sector [13,24], and clinical settings [14–17] have documented that comorbidity between different psychiatric disorders is a frequent phenomenon. Most of these studies focused on "threshold" comorbidities; that is, the coexistence of two or more mental disorders in the same individual in a defined period of time (lifetime, 6 months, 1 month). Each disorder meets the diagnostic criteria for disorders found in the DSM-IV [6] or the ICD-10 [25].

Whether the co-occurrence of two or more mental disorders in the same person reflects the presence of pathophysiologically independent entities is far from clear. High levels of comorbidity raise questions about the specificity and the boundaries of certain diagnostic categories and provide important clues to the etiology, pathophysiology, and phenomenology of both the index and comorbid disorders. Klein and Riso [26] argued that there are at least four theoretical models of comorbidity that may explain the simultaneous co-occurrence of two or more mental disorders in the same individual: comorbidity due to sampling bias, artifacts of diagnostic criteria, drawing boundaries in the wrong place, and common etiological relationships (see Table 1). The concept of comorbidity

Table 1 Relationships Between Depression and Anxiety

Both depression and anxiety are reflection of the same phenomenon
1. Both conditions are reflections of the same phenomenon.
2. One of the two conditions is a mere reflection of the other.
3. One of the two induces changes that leads to the other.

Common factor for both anxiety and depression
1. Vulnerability hypothesis.

Artifact of diagnostic criteria
1. Comorbidity due to overlapping criteria.
2. Comorbidity due to one disorder encompassing the other.

Anxiety and depression are two separate entities
1. They can be either depression or anxiety.
2. They may appear together (comorbidity viewpoint).
3. Each can appear at threshold or subthreshold level. Any combination is possible (mixture subsyndromal viewpoint).
4. Comorbidity is a common final pathway of two distinct conditions.

Source: Adapted from Refs. 26 and 65.

is a valid and important clinical construct to capture and depict different components of psychopathology.

The amount of comorbidity may be influenced in different ways. First, the exclusion of hierarchical rules in the classification of mental disorders and the separation of a more pervasive condition into more specific conditions may increase comorbidity rates. Second, the period of time through an individual's lifespan when a disorder is present can affect comorbidity. Third, the definition of a threshold for a diagnosis may also sensitively affect levels of comorbidity as low threshold tends to increase prevalence rates, while high threshold tends to decrease prevalence rates.

Comorbidity and the classifications found in DSM-IV [6], the ICD-10 [25], and similar categorical systems are inextricably related. Without a categorical classification scheme, there is no reason to discuss comorbidity, and diagnostician would then quantify attributes; syndrome categories, defined by clusters of symptoms, would not exist, and therefore, could not coexist. The DSM-IV disputes the claim that "each category of mental disorder is a completely discrete entity with absolute boundaries" (p. xxii). However, although its designers can make this statement with every honest intention, resarchers and insurance companies act as if each category is a discrete entity. Moreover, DSM-IV does not provide any standardization information about the frequency of co-occurrence of disorders, further suggesting that each category is discrete. The claim is also made that "there is no assumption that all individuals described as having the same mental disorder are alike in all important ways" (p. xxii). They may differ in severity, impairment, and symptom expression, and we will focus attention on the last of these possible differences. The DSM-IV uses polythetic criteria sets, which means that a given individual may present with a subset of diagnostic criteria from a larger set. Thus, a person diagnosed as depressed must have at least five symptoms out of the longer list of nine diagnostic criteria. Polythetic criteria sets, however, speak to heterogenous clinical presentations only within a diagnostic category. Consider adjustment disorders (DSM-IV, pp. 623–627) as a relatively understudied but common example seen in clinicians' offices. Adjustment disorders are not usually considered a serious mental disorder (compared to schizophrenia, bipolar disorder, or panic disorder). But, as we shall see, they can be serious enough when comorbidity, substance use, hospitalization, and suicide are taken into account.

The issue becomes even more problematic when considering "subthreshold" comorbidities. Clinical correlates of subthreshold forms of anxiety and their relationship with other mental disorders have not, to our knowledge, been investigated systematically. Epidemiological studies suggested that brief recurrent hypomania and mixed states, arising within the setting of an attenuated bipolar spectrum, in some cases, may be detectable in the context of co-occurring symptoms that fall below the threshold for a diagnosis of panic-agoraphobia, generalized anxiety, social phobia, and obsessive-compulsive disorders [20]. However, differences in the outcome and treatment response are still not clear [27–29].

It has also been argued that in the majority of patients with a "neurotic syndrome," symptoms drawn from two or more diagnostic categories on the basis of predominant features, would often be found. In such cases, the diagnostic groups may overlap or fade into another. Therefore, neurotic disorders have been hypothesized to occur generally among individuals who show deviations along a number of independent dimensions, which may predispose them to either anxiety, obsessive symptoms, or depressive disorders, as well as other emotional disorders. In many cases, it may be difficult to disentangle the specific components of such neurotic syndromes [30,31].

The DSM-IV [6] has tentatively recognized a disorder composed of symptoms from two subclinical disorders: depression and anxiety. Mixed anxiety–depressive disorder, however, has been placed in an appendix to the main text. It is one of a number of new categories that were suggested by consultants for possible inclusion in the DSM-IV [6] or a future edition, but lacked sufficient empirical backing to be included in the current nomenclature. The term "mixed anxiety–depression states" has been also proposed and incorporated into the ICD-10 [25].

According to DSM-IV, persons with this disorder present with persistent or recurrent dysphoric mood and a minimum of four of the following symptoms for at least 1 month: concentration or memory difficulties, sleep disturbance, fatigue or low energy, irritability, worry, being easily moved to tears, hypervigilance, anticipating the worst, hopelessness or pessimism about the future, and low self-esteem or feeling of worthlessness. If the critiera for mood or anxiety disorders have ever met, then mixed anxiety–depressive disorder cannot be diagnosed. Both DSM-III-R and DSM-IV also mention "limited-symptom" panic attacks, which have fewer than four somatic or cognitive symptoms.

The phenomenology, course, and the treatment outcome of less severe forms of depression have received greater attention than that of less severe forms of anxiety because of their high prevalence in the general population and in general health-care settings [32,33]. Patients with subthreshold depression were nevertheless found to have a family psychiatric history and level of medical and psychiatric comorbidity similar to those of patients with depressive disorders [34].

A spectrum model of psychopathology is more adept at recognizing the subclinical or threshold symptomatology that may occur concomitantly with core psychiatric disorders. The term spectrum has been traditionally used to underlie relationships among clusters of symptoms or to place defined syndromes in relation to one another. In a broader way the spectrum model can bring coherence to complex psychiatric symptoms, and include: (1) core, atypical, and subclinical symptoms of the primary axis I disorder; (2) signs, isolated symptoms, symptom cluster, and behavioral patterns related to the core symptoms that may be prodromal, may represent a precursor of a not-yet fully expressed condition, or may be sequelae of a previously full-fledged disorder; and (3) temperamental and/or personality traits. The spectrum model of psychiatric disorders evolved (initially with the "panic-agoraphobic spectrum") at the University of Pisa, and has been further developed in collaboration with researchers from the University of Pittsburgh and elsewhere in the United States [35]. This approach has the potential to answer to various problems that arise by splitting disorders into narrow, distinct, nonoverlapping diagnostic entities, like: (1) failure to encompass subthreshold and atypical symptomatology; (2) artificial enhancement of comorbid diagnosis; and (3) failure to replicate genetic markers of narrow, restrictive phenotypes. It gives clinical weight to low-severity and isolated symptoms that either appear alone or occur concomitantly with a major disorder. Such symptoms, even in the absence of syndromal illness, may lead to considerable suffering and disability. In the presence of a syndromal axis I condition of another type (e.g., a small number of, or even a single, panic–agoraphobic spectrum symptom in the presence of syndromal major depression), they may have as significant an impact on functioning and treatment outcome as the comorbidity of two fully syndromal conditions [35,36]. Therefore, the identification of these "spectrum phenomena" should be included as a routine part of clinical assessment because exclusive use of the categorical system and the virtual exclusion of other broader concepts of psychiatric classification have led to stereo-

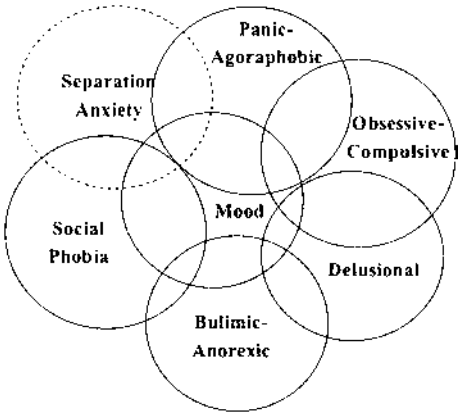

Figure 1 Possible associations among spectrum disorders.

typical portraits of patients and have limited the available descriptive clinical information, resulting in oversimplification of multifaceted clinical profiles.

For example, a patient who presents with major depression, but with no complaints of somatic manifestations of anxiety or phobias, differs significantly from a patient who exhibits precisely the same criterion symptoms of depression along with palpitations, gastrointestinal distress, and multiple phobias related to feelings of suffocation or entrapment (e.g., fears of elevators or highway driving). However, limiting the diagnostic workup to DSM or ICD criteria would result in identical descriptions of these patients.

Spectrum symptomatology may be viewed as the part of the iceberg that is hidden beneath the surface of the water, while the core, diagnostic criteria symptoms represent the obvious, visible portion. Of course, the various conditions may evidence substantial overlap in terms of individual symptoms, and symptoms of mood spectrum may overlap with symptoms of panic spectrum, obsessive-compulsive spectrum, social phobia spectrum, bulimic-anorexic spectrum, as well as with what we have termed the separation anxiety spectrum (Fig. 1).

Potential uses for the spectrum approach may include the improvement of treatment selection, development of better strategies for outcomes measurement, monitoring the course of illnesses, strengthening of therapeutic alliances, and improvement in subtyping of patients for clinical, biological, and genetic research.

III. CLINICAL DESCRIPTIONS AND SYMPTOM CLUSTERS: COMORBIDITY AS A SPECIFIER FOR TREATMENT

The World Health Organization (WHO) Collaborative Study on Psychological Problems in General Health Care indicated that anxiety and depressive disorders are the most common co-occurrence of psychiatric disorders [13]; of those patients with a current anxiety disorder, about 45% also had a current depressive disorder, and 40% of those with a current depressive disorder had a current anxiety disorder [19].

Given the presence of multiple disorders, which disorder appeared first? What implications does this knowledge have for treatment? These questions are the basis of an important debate, one that will ultimately be resolved by longitudinal data revealing the natural

course of these disorders and the long-term outcome of treatment. Some believe, for example, that childhood separation anxiety is a prodrome (i.e., precursor or predictor) for adult panic disorder. If we knew that separation anxiety disorder or an extreme sensitivity to separation in adulthood preceded the onset of panic disorder, we could screen for that disorder, intervene when appropriate, and (theoretically) prevent the onset of panic. Similarly, if we knew that clinical or subclinical social phobia preceded the onset of depression, we could screen for the anxiety disorder and take similar steps to those described for separation/panic disorder. Knowledge about the course of illness will impact prevention and treatment, and it will enhance our understanding of the origins of mental disorders. Because longitudinal research is complex, tends to become dated, and requires stability of investigators, subjects, and funding, course of illness is not easily studied. In spite of these difficulties, such studies have been done and data exist. In this regard, consider three data sets related to depression and anxiety: the National Institute of Mental Health (NIMH) Collaborative Depression Study (CDS), the Munich Follow-up Study, and the Zurich Cohort Study of Young Adults. The CDS is a naturalistic clinical investigation of consecutive patients who sought treatment at five major teaching hospitals in the United States. Patients were periodically tracked and, at their 5-year follow-up, depression was found to precede their anxiety disorders [16]. In a community epidemiological survey, Wittchen and Essau [37] followed patients in the German city of Munich to report that after 7 years, the majority of their subjects with both disorders experienced anxiety before depression. This is the opposite finding of Coryell and associates [16]. Subjects in the Munich Follow-up Study came from both the community and a cohort of former psychiatric inpatients. In Switzerland, Angst and colleagues [38] found that persons diagnosed 7 years earlier with pure anxiety tended to develop additional depressive disorders, but persons originally diagnosed with pure depression tended to retain that single diagnosis over the follow-up period. That is, a diagnosis of pure depression remained relatively stable, whereas one of pure anxiety exhibited substantial instability as reflected in additional major or recurrent brief depressions. There are several implications of these three sets of data, two of which (i.e., Munich and Zurich) agree that anxiety tends to precede depression. The Munich and Zurich surveys, as well as Epidemiologic Catchment Area (ECA) study [39], are more likely to reflect true rates of pure and comorbid disorders. A second implication is that the DSM-IV is most relevant when its individual diagnostic categories are applied to pure depression found in community surveys. Pure depression subjects are less likely to seek treatment and less likely to have additional mental illnesses during the course of their depression. Conversely, clinicians seeing a patient with a pure anxiety disorder (i.e., no depression or substance abuse) may expect additional disorders to appear if effective treatment for the person is not promptly instituted. The degree to which treatments are effective in preventing the appearance of these other disorders or in reducing their symptomatology when they already exist concurrently is represented sparsely in the literature. A third implication is that mixed cases seem to have a worse outcome. Wittchen and Essau [37], found that subjects with mixed anxiety/depression were more severely impaired in psychosocial functioning and evidenced more management problems in social roles than subjects with a pure disorder. In terms of remission rates, Wittchen and Essau's mixed group evidenced lower rates than those individuals with pure depression. An unfavorable, chronic course was found in 44.1% of the mixed anxiety/depression group but in only 26.3% of the pure depression group. The clinical study found depressive symptoms to be more severe, persistent, and recurrent when the patients had a coexisting anxiety syndrome than when depression occurred among patients with anxiety [16,40]. Moreover, mood-congruent de-

lusions and the delusional subtype of depression are more frequent among patients with both depression and panic disorder than in patients with depression only [40]. Obsessions and compulsions clearly had the gravest prognosis when they were present in a depressed patient, but the presence of any anxiety syndrome predicted a worse outcome than was projected for patients without a comorbid anxiety syndrome. A common limitation of these three studies is the lack of any assessment of temperament as a maker of mood vulnerability. Savino and colleagues [27], for example, studied 140 panic patients and showed that high rates of temperamental dysregulation often co-occur (over a lifetime) with anxiety disorders and may represent the only manifestation of a mood lability preceding the onset of an anxiety disorder. In this perspective, any data lacking information on temperamental features appear incomplete for characterizing the temporal relationship between anxiety and mood disorders. A limitation of the report by Coryell and colleagues [16] is that it is a clinical, not a community, study. As such, it may not be a valid representation of the natural course of illness. Berkson [41] demonstrated an often replicated rule in epidemiological research: persons seeking treatment for a medical problem usually have an additional medical diagnosis, and prevalences recorded in clinical settings do not reflect the base rate for either diagnosis in the community. Most of the subjects entered the NIMH-CDS seeking treatment for existing comorbidities. Perhaps this particular set of patients was unusual in having their depression precede anxiety. Perhaps this progression is the more severe and drives people into a clinic. The answers are not yet clear, but the patients who entered the proband group of the Collaborative Depression Study and who were reported on in the Coryell study probably reflect what practicing clinicians see.

A. Bipolar Depression

Lifetime diagnoses of anxiety disorders are very common in bipolar patients, but research is still limited in this field for two main reasons: the relative underdiagnosis of bipolar II disorders, often misdiagnosed as unipolar or personality disorders [42], and the lack of utilization of structured interviews for the diagnosis of anxiety disorders in hypomanic patients [43], or bipolarity in anxious patients [44].

Recent data from the Stanley Foundation Bipolar Treatment Outcome Network [45] report a lifetime comorbid anxiety disorder in 42% of 288 bipolar patients, while 30% of them have a current comorbid anxiety disorder. In the National Comorbidity Survey (NCS), patients who had a bipolar disorder in the past 12 months had a 95% frequency of lifetime anxiety disorders, with a reported risk of comorbid panic disorder and social phobia higher in bipolar disorder (odds ratios of 11.0 vs. 4.6) compared to unipolar disorder (odds ratios of 7.0 vs. 3.6) [21]. Chen and Dilsaver [14], using the Epidemiological Catchment Area (ECA) data, found that the prevalence of panic disorder among bipolar patients was higher than in unipolars (20.8% and 10.0%, respectively) and that the risk of having panic disorder was 2.1 times higher for a bipolar patient than for patients with unipolar depression. Furthermore, panic disorder was frequently found to be associated with obsessive-compulsive disorder comorbidity in bipolar and unipolar patients [15]. Savino et al. [27], in a clinical sample of panic patients, found a prevalence of 47.8% of bipolar spectrum disorders and 22.9% of unipolar depression.

Comorbidity rates of obsessive-compulsive disorder with bipolar disorder have been found to range between 7.3 and 21.1% [28, 46, 47]. A reanalysis of the ECA data showed a higher prevalence of obsessive-compulsive disorder comorbidity in bipolar patients than in unipolar depressives (21.0% and 12.2%, respectively), with a risk of having obsessive-

compulsive disorder two times greater for bipolar than for unipolar depressives [15]. By contrast, Krueger et al. [48] found a similar lifetime prevalence of obsessive-compulsive disorder in bipolar (35.1%) and unipolar depressives (35.2%). These two studies suggest that the incidence of this disorder in bipolar disorder was higher than had previously been thought.

Wittchen et al. [49] reported a rate of comorbidity of generalized anxiety disorder (GAD) with bipolar disorder of 10.5%, lower than with major depression (62.4%). In a recent clinical trial, Pini et al. [28] reported higher lifetime rates of generalized anxiety disorder in bipolar subjects (31.6%) even if still lower than in unipolar depressives (37.1%). These findings support the hypothesis that bipolar disorder has a strong connection to panic and obsessive-compulsive disorders, while GAD may be related to affective disorder.

B. Major Depression

A high proportion of patients receiving a diagnosis of depression also manifest a variety of anxiety symptoms that fulfill the criteria for one or more concomitant anxiety disorders. Several epidemiological and clinical studies indicate that comorbidity of major depression and anxiety disorders range from 21% to 91% [20, 22, 28, 50] (Table 2). The concomitance of anxiety disorders with depression has important prognostic and therapeutic implications for clinical practice. Although there has been controversy in recent years as to whether anxiety and depressive disorders are continuous and merged insensibly with each other or whether they constitute distinct entities, it is clinically evident that the co-occurrence of the two conditions has been found to be associated with greater overall psychopathology, high risk of chronicity of illness, greater social and occupational impairment, high risk of suicide, and poor prognosis [48, 51, 52].

1. Enquiries into the Relationship Between the Anxiety Disorders and Depressive Disorders in Clinical Samples

Panic Disorder. The rate of panic disorder in the general population is estimated to be between 1.4 to 2.9%. Individuals with panic disorder have high rates of comorbidity with mood and other disorders. Epidemiological studies estimate that 74 to 90% of subjects with a history of panic also meet criteria for at least one other mental disorder and 56 to 73% have had a lifetime history of major depression [22,53]. Similar rates have been reported in clinical samples. In approximately one-third of cases, the onset of panic precedes the onset of major depression; in one-third, major depression has a primary onset; and in the remaining one-third, the two conditions occur in close temporal relationship [23,54]. Predictors of subsequent episodes of major depression in patients with panic disorder include prior history of major depression, presence of generalized anxiety disorder, and the severity of agoraphobia, with the latter two factors significant after controlling for lifetime history of depression [55].

Social Phobia. The prevalence of social phobia in the general population is reported to be around 1.7 to 3.8% [56,57]. Social phobia is estimated to precede the onset of major depression in two-thirds of cases [57,58]. Furthermore, percentage ranging from 67 to 92% of individuals with a lifetime history of social phobia meet criteria for at least one other psychiatric disorder and, of these, 15 to 20% had a lifetime comorbid major depressive disorder. Higher rates of comorbidity between major depression and social

Table 2 Comorbidity Between Depression and Anxiety: Prevalence and Chronology of Onset

Diagnosis	Prevalence	Anxiety disorder comorbid with any psychiatric disorder	Anxiety disorder comorbid with MDD	MDD comorbid with anxiety disorder	Onset of anxiety disorder precedes MDD
Panic disorder	1.4%–2.9%	74%–90%	56%–73%	10%	29%
Social phobia	1.7%–3.8%	67%–92%	15%–21%	27%	65%
GAD	1.9%–6.6%	80%–90%	62%–67%	17%	63%
PTSD	1%–13.8%	73%–83%	37%–48%	20%	53%–78%

Source: Adapted from Ref. 66.

phobia have been reported by Cassano et al. [52] in clinical samples, including cohorts of subjects with psychotic depression. Results of genetic epidemiological studies suggest that genetic factors may contribute to the development of social phobia, with heritability estimated at approximately 30% [59].

2. The Relevance of Treatment Response for Relationship Between Depression and Anxiety Disorders

There is an extensive literature that testifies that both tricyclic antidepressants and MAOI compounds are effecting in alleviating the symptoms of panic-agoraphobic and general anxiety disorder. More recently, the SSRIs have also been demonstrated to be effective in the treatment of agoraphobia and related panic attacks. On the basis of a meta-analytic review of the literature, it has been reported that these drugs had proven superior to imipramine and to alprazolam in treating panic disorder [60]. However, the interpretation of these results testifying to the unity of anxiety and depressive disorders is far from clear. The way in which patients with panic-agoraphobic spectrum conditions respond to each of these groups of drugs differs from the response of depressive patients. In the initial phases of treatment of patients with anxiety disorders, anxiety and tension are initially exacerbated. It is plausible that effects in anxiety disorders are probably achieved along a different pharmacodynamic route than along which changes are induced in patients with depressive illness. Further, the similarity of treatment response of two or more groups of disorders with distinct profiles does not signify an identical etiology or pathogenesis. The wide range of efficacy of corticoid compounds and of antibiotics are illustrative of this point. Any classifications of disease based on similarity of therapeutic response to either of these compounds, would prove chaotic and meaningless.

Differences in treatment response are more informative. Most clinical trials in depression have not evaluated the efficacy of antidepressant treatment for comorbid anxiety disorders and symptoms. Only a handful of studies have examined this issue but none have sought to evaluate the relative efficacy of one treatment over another. In an open study, response to fluoxetine for the treatment of depression was investigated in a large cohort of depressed patients with, versus without, comorbid anxiety disorders [61]. The results indicated that patients with comorbid anxiety disorders were more likely to be nonresponders. In a naturalistic study of 30 outpatients with major depression, who also met the criteria for at least one current comorbid anxiety disorder, fluvoxamine was found to be effective in reducing both depressive and anxiety symptoms [62]. A limitation of these studies was that neither a placebo nor an alternative treatment was utilized, making it difficult to know if there was a specific antianxiety effect and whether fluvoxamine was superior to other antidepressants in treating depression with comorbid anxiety disorders. More recently, fluoxetine, sertraline, and paroxetine were found to be equally effective in depressed patients with high levels of anxiety [63]. However, in this comparative study, only anxiety symptoms as measured by several items on the Hamilton Rating Scale for Depression were used, making it unclear if a broader range of anxiety symptoms would also respond to these treatments. Similarly, because no comorbid anxiety diagnoses were made, we cannot know whether these treatments would be effective in depressed patients with anxiety disorders.

We conducted a 4-month multicenter, open, controlled clinical trial to compare the efficacy and safety of paroxetine and moclobemide in patients with major depression with current comorbid anxiety disorders. In this study, we addressed several limitations of the previous research: that is, comorbid anxiety diagnoses were made, a more comprehensive

assessment of anxiety symptoms was used, and two antidepressant medications were studied.

Both moclobemide and paroxetine produced significant improvement in symptoms of depression and anxiety. However, we found that compared to moclobemide, paroxetine showed greater efficacy in patients with depressive disorders and comorbid panic disorder in reducing depressive and anxiety symptomatology; furthermore, paroxetine was more rapid than moclobemide in improving anxiety symptoms. Interestingly, this effect of paroxetine was not found in the subgroup of depressed patients with comorbid generalized anxiety disorder.

Although not directly comparable with our data, Fava et al. [63] found no differences in efficacy and tolerability of fluoxetine, sertraline, and paroxetine in the treatment of depressed patients with high levels of baseline anxiety, as assessed by the six items of the anxiety factor on the 17-item HDRS. These results suggest that for at least some anxious symptoms, there may be no difference between the three antidepressants studied. However, in light of the results reported here, it is plausible that a diagnostic characterization of comorbid anxiety diagnoses (e.g., using the DSM system) could have revealed differences in the efficacy of these three drugs. Given the high rate of anxiety comorbidity in patients requiring treatment for depression, the most appropriate drug will be the one that shows the highest and earliest efficacy in both conditions. This property will enable clinicians to limit the use of drug combination or of benzodiazepines in those cases of depression associated with anxiety conditions. In the future, a better clinical profile characterization of new drugs should be obtained through protocols specifically designed for patients with comorbid mood and anxiety disorder, as well as the adoption of multiple disorder outcome measures either in their full-fledged or subthreshold manifestations [36]. Our study suggests that two drugs of proven efficacy in depression and anxiety, moclobemide and paroxetine, perform differentially depending on specific comorbid anxiety diagnosis. In light of the fact that panic disorder, GAD, and obsessive-compulsive disorder can co-occur in patients with depression [22,28,64,65], and not all antidepressants may be equally effective, it is important that clinicians choose a treatment that would be most effective in treating both types of disorders.

C. Dysthymic Disorder

There is considerable evidence that patients with a principal diagnosis of dysthymic disorder manifest a variety of nondepressive symptoms typically associated with anxiety [17,66,67]. Studies conducted in community samples [68] and in clinical settings [17,28,69] show rates of anxiety comorbidity ranging from 20% to 40% in patients with dysthymia. Generalized anxiety disorder, panic disorder, and social phobia are the most frequently associated conditions [28]. Recently, Klein et al. [70] conducted a prospective, longitudinal follow-up study of 86 outpatients with early-onset of dysthymic disorder and 39 outpatients with episodic major depressive disorder. They found that 36% of dysthymic patients had a concurrent anxiety disorder compared to 17.9% of counterparts. During the course of the follow-up, patients with dysthymic disorder exhibited significantly greater levels of symptoms and lower functioning than patient with episodic major depressive disorder. Haykal and Akiskal [71] have suggested that anxious features may often complicate the course of dysthymia and may contribute to make dysthymia less responsive to treatment interventions.

Tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), reversible inhibitors of monoamine oxidase (RIMA), and benzamides have all proven efficacy in dysthymia [72]. In a double-blind study, moclobemide (300 mg/day) was found to be superior compared to fluoxetine (20 mg/day) in treating dysthymia with superimposed depression [73]. On the other hand, Haykal and Akiskal [71] reported more satisfactory results with fluoxetine than with imipramine in patients categorized as pure dysthymic, the difference being much more pronounced in females than in males. In these studies, the impact of associated anxiety component on treatment outcome was not investigated. Indeed, specific patterns of anxiety comorbidity may play a role in rendering partially inconsistent the results of different clinical trials in patients with dysthymia. In Haykal and Akistal's study, for example, dysthymic subjects with axis I comorbid anxiety disorders that dominated the clinical picture were excluded from the trial. On the contrary, in Duarte et al.'s study [73], a substantial number of subjects with dysthymia had comorbid anxiety syndromes.

Comorbidity of dysthymia with various discrete anxiety disorders was rarely taken into account in comparative clinical trials, despite being commonly encountered in psychiatric practice and being suggested to be more prone to develop chronicity [74]. Recently, we compared moclobemide (effective in dysthymia [72,73]) and paroxetine (effective in a broad spectrum of anxiety disorders [75,76]), in a population of dysthymic patients with anxiety comorbidity and without superimposed major depression (unpublished data). Our analyses showed that paroxetine and moclobemide were in general effective both on depressive and anxious symptoms in patients affected by primary dysthymia comorbid with anxiety disorders. However, paroxetine was found to be significantly superior to moclobemide in reducing depressive and anxious symptomatology in patients with dysthymia with comorbid panic disorder, suggesting that specific pattern of anxiety comorbidity may substantially affect treatment-response of patients with depressive disorders [24,36,77].

In conclusion, our data suggest that specific anxiety comorbidity patterns such as panic disorder may affect treatment response, predict different antidepressant efficacy, and indicate best treatments in dysthymic patients.

D. Spectrum Comorbidity: Diagnostic and Therapeutic Implications

The difficulty, if not the impossibility, of classifying many patients with multiple disorders into one of the DSM categories has spawned a variety of other procedures to cope with clinical reality [12]. These include use of the primary–secondary distinction, multiple diagnoses, use of both axes I and II, associated features of a disorder, and the spectrum of a disorder concept [78]. In this section we describe the concept of spectrum as an additional perspective for the clinical evaluation of concomitant psychiatric disorders. The necessity of this additional conceptual tool to describe mental disorder phenomenology derived from similar observations that gave rise to other coping mechanisms: sharply divided categorical systems of classification do not capture the complex and variable psychopathology seen in most patients. Usually this complex phenomenology is simplistically attributed to premorbid personality traits, to personality disorder, or to residual symptoms and/or maladaptive behavior residual to a previous axis I disorder. The definition of spectrum includes a range of subclinical and/or atypical symptoms and isolated behavioral features that are partial or subclinical expressions of a categorical disorder. These are usually overlooked by clinicians. The spectrum itself is not a diagnostic category. A good

example of this concept was given by Cassano and Savino [78], who described the panic-agoraphobic spectrum as an expansion of panic disorder phenomenology described in DSM-III-R. It included symptoms and behavioral features belonging to the following areas: panic attacks; anxious expectation; polyphobic features; avoidant behavior; reassurance sensitivity; help-seeking behavior; maladaptive behavior; predisposing or prodromal factors; and physiological sensitivity to chemical substances. When adhering to a DSM-IV diagnosis, co-occurring symptoms and subclinical or atypical manifestations of other disorders that cut across different diagnostic categories are often seen as associated features of the primary disorder. However, when even a few isolated or subclinical symptoms are present, there may be significant impairment in the patient's social functioning [79], and the phenomenology of the axis I disorder may be significantly modified, thereby delaying and misleading the diagnosis. For example, symptoms of an overlooked comorbid anxiety and bipolar II disorder can often be observed within the categories of borderline personality disorder and atypical depression. In these cases, feelings of insecurity and agitation and episodes of dyscontrol brought about by atypical panic symptoms co-occurring with depressive episode in a bipolar patient may be easily confused with the impulsivity and aggressivity of borderline patients. In the same way, unrecognized, subclinical, or isolated panic symptoms may bring atypical features such as mood reactivity to a depressive episode. The high rates of comorbidity between panic disorder and bipolar disorders found by Savino and associates [27] and Chen and Dilsaver [14] support these observations. Higher rates of comorbidity could be reasonably expected if subjects were selected using a symptomatic extension of DSM-IV criteria. The impact of a mild, subtle, and long-lasting psychopathology on parents' quality of life was very clear to Sir Aubrey Lewis, who wrote in 1936 [80]: ''It may be said, simply, that severe emotional upsets ordinarily tend to subside, but that mild emotional states, when often provoked or long maintained, tend to persist, as it were, autonomously. Hence the paradox that a gross blatant psychosis may do less damage in the long run than some meager neurotic incubus: a dramatic attack of mania or melancholia, with delusions, wasting, hallucinations, wild excitement and other alarms, may have far less effect on the course of a man's life than some deceptively mild affective illness which goes on so long that it becomes inveterate. The former comes as a catastrophe, and when it has passed the patient takes up his life again, while with the latter he may never get rid of his burden (p. 998)."

Detection of subtle, long-lasting, underlying psychopathology is more difficult when it is concomitant with an axis I disorder. Description of a clinical reality that includes a complex array of symptoms belonging to different disorders and their proper treatment led us to speak in terms of subclinical and atypical spectrum comorbidity. Early recognition of spectrum comorbidity (observed over a lifetime) leads to a significant improvement in diagnostic accuracy, choice of a more appropriate treatment strategy, management of treatment, control over the potential for substance abuse, and prediction of outcome [35]. Moreover, clinical experience shows that the adoption of a proper treatment strategy covering both the axis I disorder and the lifetime occurrence of spectrum symptoms often produces dramatic changes in the lifestyle of the patient, who feels free from psychopathological features that the clinician and even the patient believed were stable personality traits. Unrecognized spectrum symptoms occurring at one time in the patient's life may impact his or her personality and the presentation of any psychopathology at some future time. An example might be of a young man who, at the age of 20, experiences one or two mild panic attacks. He seeks no treatment, but the attacks change his life in certain ways. Prior to the mild panic attacks, he was described as energetic, open to new experi-

ences, and socially outgoing with a hyperthymic temperament. Following the onset of panic symptoms, he becomes increasingly pavid, dependent, socially avoidant, and worried about his health. These features become stable and traitlike. When this patient is seen by a clinician at age 40 for a depressive episode, his panic symptoms have long been forgotten, and he presents as a fearful person, somewhat hypochondriacal, and dependent on others to such an extent that he tends to avoid remaining alone. Now the recognition of distinct symptoms of the panic spectrum and the consequent adoption of a well-targeted treatment strategy become fundamental. If the clinician attempts to treat only the depressive episode, he or she will miss the main reason for the patient's 20 years of fearfulness, dependency, avoidness, and hypochondriasis, which were initiated and maintained by a partial expression of panic disorder.

It is not clear how a psychosocial treatment would deal therapeutically with the clinical case described above, but in terms of medications, a drug such as imipramine (or paroxetine) is much more likely to treat both the depression and residual symptoms of the panic attacks than compounds such as nortriptyline, which are targeted at depression alone [81]. Also, the clinician–patient relationship, the cornerstone of any treatment strategy, can be significantly improved by adopting the spectrum approach. Providing clinicians with new keys with which to interpret the patient's behavior, this approach enables an easy identification of individualized psychopathological profiles, capturing mild symptoms and details of patients' lifestyles. In some profiles, patients often recognize themselves. Receiving credible, practical, and clear interpretation of their long-lasting problems helps patients establish a positive and reassuring relationship with their therapist. With the quick establishment of preferential channels of communication, patients usually feel syntonic with the therapist and, in return, they comply more completely with the therapeutic plan. There are diagnostic and therapeutic implications of a lifetime presence of spectrum symptoms in a patient with an axis I disorder. Referring to the sample above, missing the spectrum comorbidity means overlooking potential complications related to the concomitance of panic disorder and depression—that is, greater severity of illness [40,82], poorer prognosis [37,83], higher rates of chronicity [84], decreased patient compliance and responsiveness to treatment [85,86], poorer psychosocial functioning [87], increased family loading [88], higher risk for substance abuse [88,89], and elevated odds ratios for suicide attempts [90,91]. From a therapeutic perspective, this patient will benefit mainly from a drug or other treatment modality acting on both the axis I disorder and the spectrum symptoms. A common example is that of two patients presenting with the same severity of depressive episode. Presume that the clinician prescribes the same antidepressant (e.g., clomipramine) to both patients. After 10 to 15 days, one patient improves as expected, whereas the other now evidences panic attacks as well as depression. Without asking about the spectrum of symptoms, even those that may have occurred much earlier in the patient's lifetime, a therapist will have difficulty understanding these dramatically different outcomes. With the spectrum approach used as a magnifying lens on the patient's psychopathology, it is possible to solve the problem. The patient who improved may have chronic, mild obsessive traits, such as perfectionism, which makes clomipramine the drug of first chice. On the other hand, the patient who developed panic attacks may reveal a previous trait of separation and loss sensitivity, which exlains his or her adverse reaction to the clomipramine. The patient who worsened had an overlooked panic vulnerability. When the spectrum reveals such a panic profile, the patient's hypersensitivity to antidepressants and to benzodiazepine withdrawal should be taken into account. Finally, the patient will probably need reassurance and cognitive support, as do most panic patients. A similar

example is that of a depressed, grieving patient who does not recover unless the clinician gives an antidepressant with antipanic action. An abnormal grief reaction reflects a particular vulnerability to separation, which in most cases belongs to the panic-agoraphobic spectrum. Besides the same panic habitus is often expressed by extremely intense and tight interpersonal relationships and dependency on others, characteristics typical of panic patients.

IV. IMPLICATIONS FOR TREATMENT AND SUGGESTED GUIDELINES FOR CLINICIANS

Comorbidity is clearly important in patient management and treatment. Feinstein [9] noted that researchers try to exclude patients with associated disease from therapeutic trials for a given disease. However, findings in such presumably homogeneous samples may not apply to routine clinical practice. Failure to classify and analyze comorbid disease can create misleading medical statistics and may cause spurious comparisons during the planning and evaluation of treatment for patients. Comorbidity can alter the clinical course of patients with the same diagnosis by affecting the time of detection, prognostic anticipations, therapeutic selection, and post-therapeutic outcome of an index diagnosis [10]. Also, the presence of soft signs and symptoms belonging to the spectrum of other disorders (observed over a lifetime) is systematically overlooked, leading to an opposite bias of a heterogeneous sample, which may raise doubts and questions about the reliability of such studies in the light of a more descriptive approach. For example, in a hypothetical trial for an antipanic medication, a panic patient, who has obsessive symptoms but lacks one symptom to fulfill the criteria for OCD, is included in the trials as well as a patient without obsessive traits. Practical consequences may include poor knowledge about the spectrum of action of the treatment, difficulty in predicting response to the treatment, and/or atypical outcomes.

The DSM-IV does not suggest specific treatments for each disorder category and subcategory. However, modern treatment researchers—psychosocial and psychopharmacological—have attempted to design treatments tailored to specific DSM categories. The strategy links treatment to diagnosis, and we may expect this strategy to succeed to the extent that the targeted DSM-IV classification is valid. It is possible that treatment researchers will successfully design treatments that fit DSM-IV categories but fail to treat their patients successfully because the categories do not completely represent the patients. To the extent that comorbidity presents a challenge to the official nomenclature, it presents a similar challenge to treatments designed and targeted for DSM categories.

If not treated properly, patients with comorbid anxiety and depression have a worse outcome than patients with either illness alone [92], and have more severe and a greater number of symptoms than those diagnosed with depression. Furthermore, they are more likely to have low mood, panic attacks, and suicidal ideation, with greater disruption to social, work and family life [93,94]. Thus, the most effective treatment requires attention to symptoms of both anxiety and depression. Usually, diagnostic theories inform treatment protocol, but since the newer antidepresssants are successful for treating not only depression but also anxiety disorders, there may be less need to employ different medications for each disorder. After identifying the patient's specific disorder(s) and/or subdiagnostic symptoms, the ideal treatment for depression and comorbid anxiety should take the form of a single drug that is efficacious in the treatment of both disorders. Drugs that achieve an effective dosage through once-daily administration and with a good tolerability should

be preferred to aid patient compliance. Safety in overdose is also important because of the higher risk of suicide in depressed patient with associated anxiety than in those with depression alone [94–96].

As both depression and anxiety disorders tend to be chronic and recurring, treatment should be effective in the long term and should prevent relapse of the condition. Patients then need to be educated about the time it takes to heal, the potential side effects of treatment, the fact that most side effects will resolve with time, and the collaborative approach to treatment, which will lead to significant relief of symptoms and recovery.

V. CONCLUSIONS

Clinicians who seek only the diagnostic criteria for a specific disorder, with rigid adherence to the DSM-IV diagnostic criteria, and by extension, the DSM-IV categories, will probably miss a more global perspective of the entire pathology. Such a narrow perspective is mainly justified in research, but is unacceptable in clinical practice. The DSM-IV was conceived, at least in part, as a research tool, allowing common, standardized, and atheoretical communication among clinical investigators. But the DSM-IV is also used as a clinical manual when the practitioner is face to face with the client/patient. In such a nonstandardized, unstructured setting, the clinician more often than not sees the DSM-IV as a means to collect payment from a third party and focuses on the predominant symptom picture as the diagnosis. A more integrative approach that takes comorbidity into account should not only reflect a more valid psychiatric classification, but should also improve treatment and treatment outcome.

Evidence for the dramatic intrusion of comorbidity phenomena (axis I and spectrum comorbidity) in psychiatry has been derived from several sources, including epidemiological, pharmacological, clinical, and genetic studies. Despite this broad body of evidence, proponents of the categorical approach mostly conceive subclinical symptomatology that coexists with the disorder as background noise, overlooking the complex degree of overlap, both phenomenological and neurobiological, among the different symptoms. In fact, specific patterns of comorbidity appear to be stable, as if there were common neuronal networks that probably represent functional *loci minoris resistentiae*. We have discussed the diagnostic improvement brought about by the punctual and refined recognition of subclinical and atypical comorbidity. Another relevant consequence involves therapeutic strategy, as comorbid syndromes often require different acute, continuation, and maintenance doses as well as a distinct timing of administration and suspension of the treatment. It is not difficult to believe that drug targets in the brain are different in different patients; for example, a patient with a pure disorder compared to a patient with the same disorder plus spectrum symptoms of panic, obsessive-compulsive disorder, and related symptomatology. However, official guidelines for the therapy of depression and bipolar disorders still do not provide clinicians and researchers with any treatment-specific indications, devoting relatively little attention to the clinical importance of comorbidity, treatment strategies, and outcome. Also, the reliability of clinical trials can be questioned in light of a more descriptive approach as pharmacological trials are usually conducted with patients whose symptomatology fits a particular diagnosis coded by standardized criteria.

Diagnostic procedures should attempt to combine descriptive, categorical, and dimensional approaches, devoting more attention to the cross-sectional and longitudinal analysis of nuclear, subclinical, and atypical symptoms that may represent a pattern of full-blown and partially expressed comorbidity. Within this conceptual framework, psycho-

pathology can be more specifically approached in clinical terms of either individualized treatment or prevention. Further, some traditional points of weakness of clinical psychiatry and psychology, such as the chronic forms of illness and the treatment-resistant disorders, may be contrasted more successfully.

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4

Anxiety, Depression, and Personality

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I. INTRODUCTION

Biological variations, social influences, psychological and cognitive factors, childhood experience, individual goals and choices, and other aspects of personality have been described by various researchers as components of personality [1]. People differ markedly from one another in multiple quantifiable dimensions of personality—the dynamic organization of the psychobiological system that moderates adaptation to experience [2]. For a long time scientists such as Freud, Adler, Jung, Rogers, Catell, Allport, Bandura, and Michel, explored personality and developed various theories about it. But there is no consensus in the field about which model should guide personality research [1].

Rapid progress is being made in understanding the neurobiology of human personality. This has been facilitated by using questionnaires that assess multiple dimensions of personality quantitatively and reliably. Hypothesis-driven research has mapped models with five to seven dimensions of personality to results from recent work on the biology of these personality dimensions. These reliable personality inventories permit tests of specific hypotheses about regional brain activity, neurophysiology, neurochemistry, and neurogenetics [2]. People's responses to such questionnaires are remarkably stable, even over several decades. Human personality is currently approached by a number of dimensional personality traits. The underlying assumption is that describing the individual's personality profile (i.e., his or her score on each of the selected traits) is enough to provide a relevant description of that individual's personality. We can accept that the thousands of adjectives with which we characterize people can be reduced to a few dimensions. But it should be noted that mathematical or theoretical analyses do not provide only one unique solution—an infinite number of different solutions seem possible [3].

II. PERSONALITY

A. Personality Models

Interest in the study of personality has existed for a long time, but theories differ and are innumerable. Jung [4] described the personality dimensions of introversion and extroversion, Kretschmer [5] defined the dimensions of schizothymia and cyclothymia. Biological mechanisms are proposed as the basis of Gray's [6] two-dimensional model of personality, with reward and punishment mechanism traits. Eysenck [7,8] has proposed three major factors of personality: neuroticism versus emotional stability, extroversion versus introversion, and psychoticism versus superego control. He argues that these three dimensions are biologically determined. They exist in animals and in people from many countries with vastly diverse cultures, which supports the idea that they are caused by a fundamental biological variability.

Cloninger's psychobiological theory proposed seven basic dimensions of personality that are based on inherited biological differences—the dimensions of temperament (novelty seeking, harm avoidance, reward dependence, and persistence) and character (self-directedness, cooperativeness, and self-transcendence). Temperament refers to automatic emotional responses to experience that are moderately heritable and stable throughout life. In contrast, character refers to self-concepts and individual differences in goals and values, which influence voluntary choices, intentions, and the meaning of what is experienced in life. Differences in character are moderately influenced by sociocultural learning and mature in progressive steps throughout life. Each of these aspects of personality interacts with one another to motivate adaptation to life experiences and to influence susceptibility to emotional and behavioral disorders. There are weak correlations within the domains of temperament and character and moderate, nonlinear correlations between temperament and character. In other words, each possible temperament profile is associated with two or more possible character profiles and vice-versa [2]. According to Cloninger, "each of the temperament dimensions corresponds to a specific neurotransmitter: dopamine (in low levels, for novelty seeking), serotonin (in high levels, for harm avoidance), and norepinephrine (in low levels, for reward dependence)." Accumulating clinical and biological research findings from laboratories throughout the world are now confirming the cross-cultural validity of this seven-factor model for both normal and abnormal personality. The drugs that psychiatrists use to treat various disorders have biological effects that fit the model [1]. Harm avoidance is the inhibition of approach behaviors, and the enhancement of escape behaviors, and these are principally served by the serotonin system. The trait has resemblance to neuroticism and perhaps depression. Novelty seeking, the increased tendency to respond with approach to novel and promising situations, is principally served by the dopamine system. Rewarding activities increase domapine release or inhibit its uptake. Reward dependence reflects the persistence of behavior that is likely to be intermittently rewarded, so that a key subscale of reward dependence is persistence [9].

In contrast to Cloninger, Fiske [10] introduced by linear factor analysis five uncorrelated dimensions, a model he called the "Big Five" personality factors model. Studies were conducted to determine what dimensions people use when they describe themselves and one another. The data showed that extroversion, agreeableness, conscientiousness, neuroticism, and openness were the personality dimensions most used. Most of the variability in normal adult personality can be described by these terms, but probably more than five are needed to be comprehensive [2].

B. Biological Aspects of Personality

Relationships between personality and biology have been suggested since ancient Greek times when Hippocrates proposed his humoral theory. Hippocrates claimed that each person's temperament reflects the balance of the body fluids: blood—sanguine (optimistic, hopeful); black bile—melancholic (depressed, sad); yellow bile—choleric (angry, irascible); phlegm—phlegmatic (unemotional, apathetic) [11]. Psychologists such as Eysenck have documented psychometric dimensional factors in personality; these factors have been hypothesized to reflect a genetic and biological basis [12]. Current theories emphasize the importance of the brain and neurotransmitter systems. Neurotransmitters have specific functions and, depending upon how these various systems are balanced, the result may correspond to personality variations, such as impulsiveness, easy arousal, and so on [13,14]. From what we know about the functioning of various parts of the brain, it is tempting to speculate that neural differences predispose people to develop differences in personality.

In recent research, factor-analytical-derived personality dimensions are shown to be more biologically heterogeneous than the seven dimensions proposed by Cloninger, simply because interactive biological systems are unlikely to be uncorrelated. For example, high neuroticism scores can result from either high harm avoidance or low self-directedness. However, the biological correlates of harm avoidance differ from those of self-directedness, just as anxiety disorders (which are high in harm avoidance) differ from personality disorders (which are low in self-directedness) [2].

1. Biology, Genetics, and Environment in Personality

Many twin- and adoption studies confirm that hereditary factors influence the development of personality. The field of behavioral genetics studies the impact of heredity on personality and other behavior. It assesses the heritability of traits; that is, the extent to which they are genetically determined. Heritabilities of 25 to 50% for personality traits are commonly reported. Many twin studies have been conducted [15–17]. Loehlin [16] showed the heritability of the "Big Five" personality traits (extroversion, agreeableness, conscientiousness, neuroticism, and openness). Genetics accounted for approximately 28 to 46% of the variability of these traits. Other scientists try to confirm the heritability with different personality models in twin studies. Tellegen et al. [17] report heritabilities of 0.39 to 0.58 using the Multidimensional Personality Questionnaire (MPQ).

Several findings seem to be consistent: 25% of variation is transmissible from parent to child, another 25% of variation is dependent on nonlinear interaction among multiple genes. Consequently, the correlation between sibs for a specific personality trait is usually about 0.15 to 0.30. Multiple genes contribute to each dimension so individual genes are unlikely to explain more than 3 to 10% of the total variance in a trait. Therefore, there are often inconsistencies about the importance of a particular candidate gene when comparisons are made between different populations, particularly when the samples studied are small [2].

However, all these heredity studies permit an estimation of environmental effects. The crucial point to research in this area is to distinguish genetic and environmental effects. However, environment is, after all, more difficult to quantify than genetic relationship. For example, anxiety might develop from having an anxious parent, but it might also result because the parent creates a frightening world. Adoption studies are useful to esti-

mate these effects. There is some evidence that the shared family environment influences some areas more than others. Loehlin [18] found strong influences for conservatism and religiosity. Hoffman [19] reported that family environment also influences whether siblings develop common interests, such as an interest in music. But there is also evidence that siblings who grow up together tend to have greater differences in specific personality characteristics. "Dependency is one such characteristic," according to Cloninger; "a variety of factors, including birth order and illness, work to make one sibling the dependent one and to prevent the expression of dependency in the others [19]." Scarr [15] notes that heredity and environment can combine in various ways. Sometimes heredity influences people to select or pay attention to particular facets of environment.

In molecular genetics, many have studied human personality (linkage disequilibrium in families, association in case-control designs, systematic genome scan). The first genome-wide scan of personality traits detected significant linkage between the trait harm avoidance and a locus on chromosome 8p21-23 that explained 38% of the trait variance. There was significant evidence of epistatis between the locus on 8p and others on chromosomes 18p, 20p, and 21q. These oligogenic interactions explained most of the variance in harm avoidance [20].

Extensive work has been carried out with particular candidate genes, including polymorphisms related to the serotonin transporter, serotonin 1b receptor, serotonin 2c receptor, dopamine transporter, dopamine D3 receptor, dopamine D4 receptor, and catechol-O-methyltransferase [2]. For example, the role of the serotonin transporter in temperament and character has been studied in detail in many laboratories since the initial report by Lesch et al. [21] of association and linkage with anxiety-related personality traits like harm avoidance. Lesch et al. demonstrated an association between the short form (containing a 44 bp deletion) of the serotonin transporter promotor region polymorphism (5-HTTLPR) and anxiety-related traits such as neuroticism and harm avoidance. Heritabilities in the 30 to 50% range are typical [22]. The finding has been replicated in some studies, but not in others. Subsequent studies analyzed the association between 5-HTTLPR and the TCI dimensions of cooperativeness and self-directedness in a group of healthy volunteers [23]. Contrary to Cloninger's theory Hamer et al. [23] found a strong association between 5-HTTLPR and the character traits of self-directedness and cooperativeness. In that study, the short allele was associated with lower scores in self-directedness as well as in cooperativeness compared to the long allele. On the other side, the linkage and association are even stronger with the character traits of self-directedness and cooperativeness than with harm avoidance.

Association studies between novelty seeking and the dopamine D4 receptor have shown for the first time the importance of interactions among multiple genes [3,24]. It was shown to be positive in most large studies, but there are some negative findings [2]. The dopamine D4 receptor (DRD4) was the first common genetic polymorphism (the seven repeat of the dopamine D4 receptor) providing evidence of an association with a normal human personality dimension [9,25]. It is clear now that novelty seeking is a behavior that depends on the epistatic interaction of multiple gene loci, not just the average effect of a single gene. In addition to the dopamine D4 receptor locus, variation in novelty seeking has also been found to be influenced by variation at the dopamine transporter gene and at the dopamine D3 receptor locus. The same gene may interact with multiple other genes, creating partly overlapping sets of epistatic systems. For example, dopamine D4 receptor variation influences novelty seeking, but it also interacts with polymorphisms

in the serotonin 2c receptor locus to influence the level of reward dependence and persistence [9].

Another dopamine receptor that has been examined for a role in personality traits is the dopamine 3 receptor (DRD3), which was associated in a study with high scores in neuroticism and behavioral inhibition [26]. Another investigation showed an association of DRD3 and novelty seeking in bipolar patients [27]. A particularly exciting development is the possibility that the role of genes in human temperament could be recognized very early in development at a time when environmental influences are minimum [22].

In brain imaging studies it has been shown that individual differences in specific human temperament traits are associated with specific differences in regional cerebral blood flow. Novelty seeking was shown to be positively correlated with activity of the paralimbic cortex; in contrast, harm avoidance and reward dependence were negatively correlated with different regions [2]. Another study using the distinction between extroversion and introversion (novelty seeking is moderately correlated with introversion; high extroversion corresponds primarily to low harm avoidance) came out with highly convergent findings [2]. But brain systems are necessarily interactive with one another and therefore partially overlapping.

Another approach is to correlate the regional brain distribution of specific ligands (dopamine transporter, dopamine D2 receptor binding in positron emission tomography) with measures of personality. Studies found a negative correlation of detachment scores (Karolinska scales of personality) with striatal dopamine transporter binding [2]. Detachment scores resemble low novelty seeking and reward dependence. But such studies in resting states could be more fully evaluated in activation.

In neurophysiology studying event-related potentials, low P300 amplitude in an auditory task was associated with high harm avoidance and low novelty seeking [2]. But the functional significance of P300 as an indicator of executive functions and behavioral inhibition, as well as the interplay of temperament and character in these cognitive functions, must further be evaluated. Further studies focused on the positive correlation of pupil reactivity to the noradrenergic alpha-1 agonist challenge and fear of dangerous or risky situations (resembles harm avoidance).

Pharmacological challenges were also tested in relation to personality traits (e.g., high harm avoidance has been associated with differences in serotonergic function: low plasma tryptophan availability, blunted prolactin response to d-fenfluramine, low platelet $5HT_2$ receptor sensitivity). Novelty seeking is related to both dopaminergic and noradrenergic processes (amphetamine and norepinephrine-dependent testosterone and prolactin levels). Personality profiles show consistent relationships with risk for cardiovascular disease (blood pressure, epinephrine levels, lipids, cholesterol). Blood lipid levels, diet, activity and personality appear to interact in a complex interdependent manner [2].

Progress is being made in understanding the biology of human personality at every level of observation, brain imaging, neurophysiology, neurochemistry, and neurogenetics. Multidimensional models of human personality help to facilitate investigation of the architecture of relationships at each of these levels. When we get to the level of molecular genetics, we find extensive epistatic interaction among multiple loci influencing variation in each trait and also partial overlap. There is some specificity in relationships, but there is universal interrelatedness at every observable level. This interactive relatedness means that we will need to shift away from linear causal thinking about one or two variables at a time and certainly give up exclusive reliance on categorical labeling of personality disor-

ders [2]. Impressive progress is being made in understanding the organization of the psychobiological system that regulates our adaptation to experience.

C. Psychosocial Aspects of Personality

Scientists agree that personality is a dynamic process that develops over time [1]. Environment, society, culture, etc., influence the development of personality. Longitudinal studies illustrate continuity of personality and change over time. Cloninger states: "Continuity across life span has been found for various characteristics, including ego control, shyness, dependency, and ill temper. Change occurs, too, especially when life roles change or when education breaks maladaptive patterns" [1]. In this context, Erikson [28] proposed that eight developmental stages from infancy, early childhood to adulthood, and old age. Cloninger reports that this "psychological theory" describes the relationship between the psychological development of the individual and the social context in which this development occurs: "At each stage, the developing ego strength is in conflict with its opposite: trust with mistrust, and so on. We achieve a healthy balance if our strengths prevail over their opposites. It is best not to extinguish mistrust, shame and other negative characteristics, however, because we need them to keep a realistic equilibrium: the person who trusts everyone or who never feels ashamed cannot function in the real world" [1]. Various stages of life (infancy, childhood, adolescence, adulthood, old age) can influence the development of personality. For example, during infancy secure attachment to the parent becomes the basis for later social relationship. Acklin et al. [29] reported that psychiatric outpatients with inadequate relationships in early memories showed a higher degree of pathology measured with various clinical scales. In recent studies [30], researchers found that attachment was associated with higher performance on commonly used intelligence tests for the next decade. Furthermore, the findings from Acklin et al. [31] indicate that undergraduates who experienced lack of control or difficulties in relationships in early childhood showed higher scores on a questionnaire measure of depression.

D. Assessment of Personality

There are many instruments to assess the personality differences among individuals. Measurements are sometimes categorical and called qualitative measurements. More often, personality is expressed as quantitative measurements. But qualitative measurements as well as quantitative measurements must be reliable (the measurement is dependable) and valid (the test measures what the rater is trying to measure). Some often-used instruments are the Tridimensional Personality Questionnaire (TPQ), Temperament and Character Inventory (TCI), Minnesota Multiphasic Personality Inventory (MMPI), the Myers-Briggs Type Indicator (MBTI), and the Rorschach inkblot test [1]. A widely used questionnaire based on the consensual "Big Five" model, is the Revised NEO Personality Inventory (NEO-PI-R). Although reliance on self-report may seem unsound, the self-report version of the NEO is stable over time, and the factor structure replicates in different cultures around the world [9].

Some personality inventories take a different approach, basing themselves on specific psychological or biological theories rather than the neutral mathematical technique of factor analysis. A recent example that has proved of heuristic value in psychiatry is the Tridimensional Personality Questionnaire (TPQ). The TPQ was developed by Cloninger to measure the dimensions of his psychobiological theory. It draws on human and animal work to suggest that behavior is mediated by certain neurotransmitters that underlie three

basic and largely heritable dimensions (novelty seeking, harm avoidance, and reward dependence). TPQ harm avoidance is highly correlated with NEO neuroticism, and TPQ novelty seeking is positively correlated with extroversion and negatively correlated with conscientiousness. Reward dependence is the TPQ term for attachment to others, sentimentality, and warmth; its opposites are pragmatism and tough-mindedness [9]. A key subscale of reward dependence is persistence. The Temperament and Character Inventory (TCI) was further developed by Cloninger after the TPQ for assessment in clinical settings and to test specific predictions based on a seven-dimensional model of psychobiology of personality. The TCI measures seven dimensions of personality, including three character dimensions (self-directedness, cooperativeness, and self-transcendence) in addition to the four dimensions of temperament measured by the TPQ (harm avoidance, novelty seeking, reward dependence, persistence) [2].

III. PERSONALITY DISORDERS

Pinel [32] was the first to distinguish personality disorders from mental illness. With the term "manie sans délire" he described people who had no delusions but were susceptible to unexplainable, sudden violent behaviors. Other classifications of personality disorders were gradually developed during the 19th century. In Germany, in the late 1920s, Kraepelin [33] finally endorsed the term "psychopathic personality" and described seven different types of personality disorders. The main importance of the psychopathology for the conception of personality disorders was indicated by Schneider [34]. He regarded abnormal personalities as "constitutional variants that are highly influenced by personal experiences" and identified 10 specific classes of "psychopathic personality." The classification system influenced the DSM-IV [35] and ICD-10 classification system [36]. A comparison [37] shows that some types of personality disorders identified by Schneider are similar to types of personality disorders described in the DSM-IV and ICD-10 classification systems.

In general, definition of personality disorders, according to the ICD-10 [36] and DSM-IV [35] diagnostic guidelines include three key concepts:

1. A main disorder of characteristic constitution and behavior that concerns different fields of personality.
2. An onset in childhood or adolescence. (A diagnosis of personality disorder before age of 16 or 17 is inappropriate.)
3. Mostly related with personal and social impairments.

Table 1 lists the specific personality disorders as classified in DSM-IV.

A. Prevalence of Personality Disorders

There is evidence that up to 10% of the population shows an abnormal personality [38]. In the international WHO-study (716 psychiatric patients from 12 different countries) Loranger et al. [39] diagnosed in 39.5% of patients a personality disorder according to ICD-10 diagnostic criteria. Table 2 [40] shows the prevalence rates in populations, ranging from 0.1%, found by Lin et al. [41] to 9.8%, found by Langner and Michael [42]. Few studies provide information about temporal trends in the prevalence of personality disorders [43]. In a 15-year follow-up study, Lin et al. [41] reported that there was no increase in the prevalence of personality disorders over the years using an unstructured psychiatric interview. Two other studies [39,44] showed a marked increase of personality disorders

Table 1 Personality Disorders According to DSM-IV

Cluster A	301.0: Paranoid Personality Disorder
	301.20 Schizoid Personality Disorder
	301.22 Schizotypal Personality Disorder
Cluster B	301.50 Histrionic Personality Disorder
	301.7 Antisocial Personality Disorder
	301.81 Narcissistic Personality Disorder
	301.83 Borderline Personality Disorder
Cluster C	301.4 Obsessive-Compulsive Personality Disorder
	301.6 Dependent Personality Disorder
	301.82 Avoidant Personality Disorder
	301.9 Personality Disorder, not otherwise specified.

Source: American Psychiatric Association, 1994/1996.

in psychiatric inpatients over 10 years and 15 years, respectively [43]. However, further studies are needed to evaluate temporal trends in the prevalence of personality disorders.

B. Psychopharmacological Drugs and Personality Disorders

In principal, treatment with psychopharmacological agents in patients with personality disorders can be focused on specific symptoms or syndromes (e.g., cognitive dysfunctions, mood disorders, impulsive behavior, or anxiety) but only partly on personality disorders as a whole [45]. On the other hand, treatment can be directed against complications of personality disorders (suicide, aggression, or a lack of social adjustment) [45]. Finally, comorbid axis I disorders (depression, anxiety, etc.) in patients with personality disorders can be treated with psychopharmacotherapy. Psychopathological symptoms of axis I disorders will be reduced, but personality disorders can be treated in addition with interventions of different psychotherapeutic techniques. Tables 3 and 4 give an overview of studies in which patients with personality disorders received pharmacological interventions [45].

We will present in more detail a few studies selected at random using antidepressants from the group of selective serotonin reuptake inhibitors (SSRIs). Fava et al. [46], for example, treated patients with depressive disorder and personality disorders with fluoxe-

Table 2 Prevalence of Personality Disorders in Epidemiological Surveys

Author(s)	Country	Date of survey	Size of sample	Method of assessment	Prevalence (%)
Allebeck et al. (1988)	Sweden	1969–70	50465[a]	Clinical interview using ICD-8 criteria	2.7
Bash and Bash-Liechti (1987)	Islamic Republic of Iran	1963–70	1468	Clinical interview using ICD-8 criteria[b]	1.0
Bremer (1951)	Norway	1939–44	1080	Clinical interview using Scandinavian criteria	9.4
Dilling et al. (1989)	Germany	1975–79	1536	Clinical interview	6.4
		1980–84	1666	CIS using ICD-8 criteria (1975–79), and ICD-9 and DSM-III-R criteria (1980–84)	2.8
Essen-Moller (1956)	Sweden		2550[c]	Clinical interview using ICD-8 criteria	6.4
Helgason (1981)	Iceland	1957–77	5395	Clinical interview using Scandinavian criteria; informants	4.6
Langner and Michael (1963)	USA	1954	1660	Clinical interview[d] using American criteria	9.8
Leighton (1959)	Canada	1952	1010	Specific interview schedule[e] using DSM-I criteria	0.1
Lin et al. (1989)	China (Province of Taiwan)	1946–48	19931	Clinical interview using European criteria; informants	0.1
		1961–63	39024		0.1
Sethi et al. (1972)	India		2691	Clinical interview	0.1

Source: Adapted from Ref. 40.

[a] Refers to a survey of male military conscripts aged 18–20 years.

[b] Diagnoses were recoded using ICD-9 criteria.

[c] The Lundby study: a community survey of an entire population from Lund.

[d] The clinical interview was based on symptom questionnaires such as the Minnesota Multiphasic Inventory, the Cornell Medical Index, and the World War II Screening Neuropsychiatric Adjunct.

[e] The interview schedule was partly based on the Health and Opinion Survey.

Table 3 Double-Blind, Placebo-Controlled Treatment Studies with Neuroleptics in Various Personality Disorders

Author	*n*	Medication	Result
Montgomery and Montgomery (1982)	42	Flupenthixol 20 mg i.m. every 4 weeks	Significant reduction of suicide attempts after 6 weeks
Goldberg et al. (1986)	50	Thioridazine 5–40 mg	Significant drug–placebo differences were found on "illusions," "psychoticism," "obsessive-compulsive symptoms" but not on "depression"
Cowdry and Gardner (1988)	16	Trifluoperazine 7.8 mg ($n = 7$ for 3 weeks)	Improvement of behavioral control, anxiety, and depression
Soloff et al. (1993)	36 38 34	Haloperidol 4 mg/d Phenelzine 60 mg/d Placebo	Superior efficacy for phenelzine, followed by placebo and haloperidol on measures of depression, anxiety, anger, and hostility
Frankenburg and Zanarini (1993)	15 (open study)	Clozapine 253.3 +/− 163.7 mg/d for 2–9 months	Twelve of 18 symptoms as rated by the brief psychiatric rating scale (BPRS) decreased significantly; global assessment score increased
Chengappa et al. (1995)	1 (casuistic)	Clozapine 300 mg/d	Impulsivity and self-injurious behavior significantly decreased
Khouzam and Donnelly (1997)	1 (casuistic)	Risperidone 4 mg/d	Impulsivity in combination with self-injurious behavior decreased
McDougle et al. (1997)	18 (12 weeks)	Risperidone 1.8 +/− 1.0 mg/d	Reduction of aggressive and impulsive behavior, improvement in social behavior

Source: Adapted from Ref. 45.

Table 4 Double-Blind, Placebo-Controlled Treatment Studies with Antidepressants in Various Personality Disorders

Author	*n*	Medication	Result
Soloff et al. (1986)	60	Amitriptyline 147 mg, Haloperidol 4.8 mg	Significant improvement in depression with haloperidol in comparison to amitriptyline
Cowdry and Gardner (1988)	12	Tranylcypromine 40 mg/d on average	In comparison to trifluoperazine, carbamazepine, and placebo, improvement on mood
Liebowitz et al. (1984)	60	Phenelzine, imipramine	Response rate with phenelzine was 67%, significant higher rates on dysphoric features than imipramine and placebo
Parsons et al. (1989)	?	Phenelzine, imipramine	Improvement with phenelzine in comparison to imipramine
Soloff et al. (1993)	38 36 34	Phenelzine 60 mg/d Haloperidol 4 mg/d Placebo	Superior efficacy for phenelzine, on measure of depression, anxiety, anger, and hostility in comparison to haloperidol and placebo
Markovitz (1995)	22	Fluoxetine 80 mg/d	Significant improvement in anxiety and depression
Simeon et al. (1997)	17	Fluoxetine 55 mg/d	Fluoxetine was significantly superior to placebo in the treatment of skin picking
Coccaro and Kavoussi (1997)	40	Fluoxetine 20–60 mg/d	Significant reduction on irritability, aggressive and impulsive behavior during week 6 and 12; no influence on the self-report.

Source: Adapted from Ref. 45.

tine for 8 weeks. The presence of a cluster B diagnosis before treatment predicted positive outcome for depression. Following treatment, they found reductions in the frequency of most individuals' personality disorder diagnoses and total Personality Disorder Questionnaire (PDQ-R) scores. Patients with cluster B personality disorders may experience changes in measures of personality function in response to treatment, regardless of changes in depression or symptoms associated with depression.

In a placebo-controlled study, Salzman et al. [47] treated borderline personality disorder volunteers with fluoxetine for 13 weeks. The most striking finding from this study was a clinically and statistically significant decrease in anger among fluoxetine recipients, independent of changes in depression. But possibly this result cannot be duplicated in severely ill patients.

Coccaro et al. [48] performed a double-blind, placebo-controlled trial of fluoxetine in personality-disordered patients with current histories of impulsive aggressive behaviors and irritability. Over 3 months of treatment, fluoxetine demonstrated an antiaggressive effect on these individuals, suggesting a therapeutic role for fluoxetine or other SSRIs in personality disorders.

Eskelius et al. [49] reported a trial with sertraline and citalopram in patients with major depressive disorder in whom a personality disorder measure was performed. Following treatment, reductions in the frequency of paranoid, borderline, avoidant, and dependent personality disorder diagnoses were seen in both treatment groups. Reduction of depression scores accounted for 0 to 8.4% of the observed variance. Therefore, they conclude that in depressed patients SSRIs significantly affect personality traits.

Studies from the 1980s found that patients with both depressive disorder and personality disorder were less likely to receive medication [50] and received antidepressants for a shorter time period [51]. More recent studies [52] indicate that patients with both an affective disorder and a personality disorder were as likely as patients without a personality disorder to receive pharmacotherapy. Regarding psychotherapy, individuals with depression and a comorbid personality disorder were significantly more likely than those without a personality disorder to have received psychotherapy in the past.

Phillips et al. [53] in a longitudinal study (1991 to 1996) were the first to examine the relationship between comorbid personality disorders and treatment received in patients with anxiety disorders. The results of this study showed that anxiety disorder patients with a personality disorder were just as likely to receive medication, and they received a higher number of drugs compared to patients without a personality disorder. Individuals with a borderline personality disorder were more likely to receive heterocyclic antidepressants and interventions characteristic of psychodynamic psychotherapy and cognitive therapy. Notable is that in this study the percentage of personality-disordered patients who received medication, and the number of medications received, did not increase over a span of 5 years. According to Phillips et al., "the reason for this stability is unclear, but it may reflect clinicians' impressions that while medications are often beneficial for personality disorders, their effects are often modest" [53].

IV. PERSONALITY DISORDERS AND DEPRESSION

It is well established that there is a relationship between personality disorders and comorbid axis I disorders [54]. Cluster A personality disorders associate with schizophrenia; cluster B personality disorders with substance abuse, eating disorders; and somatization disorders; and cluster C personality disorders are related with anxiety and mood disorders.

Sanderson et al. [55] investigated whether the type and prevalence of personality

disorders differed among outpatients with dysthymia ($n = 63$), major depression ($n = 197$), and dysthymia plus major depression ($n = 32$) diagnosed by the Structured Clinical Interview for DSM-III-R (SCID-I and SCID-II). Fifty percent of patients with major depression, 52% of patients with dysthymia, and 69% of patients with a major depression and dysthymia received a clinical diagnosis of at least one personality disorder. Sanderson et al. [55] also reported that patients with a personality disorder had significantly higher scores on the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) in comparison to patients without a personality disorder. The most frequently diagnosed personality disorders were from DSM-III-R cluster C (avoidant, dependent, obsessive-compulsive, and passive-aggressive personality disorders); personality disorders from cluster B (histrionic, narcissistic, antisocial, and borderline personality disorders) were less frequent; and personality disorders from cluster A (paranoid, schizoid, and schizotypal personality disorders) were rare. Similar results found Kool et al. [56]. The authors investigated the incidence of personality disorders among individuals with both major depression and dysthymia and patients with major depression without dysthymia. Approximately 60% of patients with depressive disorders ($n = 211$) suffered from one or more personality disorders (diagnosed by the personality questionnaire, VKP) in comparison to a control group. The most common personality disorders are those in cluster C (anxious/fearful). The literature suggests higher incidence of personality disorders in patients with seasonal affective disorder compared to normal populations [57,58]. These studies found up to 23% of the depressed patients with seasonal disorder to meet DSM-IV criteria for personality disorder, mostly cluster C personality disorders. But the prevalence of personality disorders seems to be lower than in patients with nonseasonal depressive disorder [59,60]. Notable in this context is that patients with personality disorders have significantly more symptoms (assessed with the Symptom Check List SCL-90) than patients without these disorders. Regarding predictive value of personality disorders and the development and relapses of major depression, borderline personality disorders and dependency personality traits predicted relapses of major depression in a 6-year follow-up study [61]. Fava et al. [62] reported that patients with early onset of major depression in comparison to those with late onset of depression had a significantly higher prevalence of avoidant, histrionic, narcissistic, and borderline personality disorders.

Hirschfeld [63] reviews the literature with respect to comorbidity of personality disorders and depression. Personality disorders may precede the development of depression, depression may precede the personality disorder, or there may be an interface between both. Contrary to expectation, with respect to treatment, the presence of the personality disorders did not affect the rate of response to pharmacotherapy for chronic depression (dysthymia, chronic major depression, double depression). For acute depressive states, personality disorders are prevalent and are likely to adversely affect response to treatment. Furthermore, data strongly support the hypothesis that successful treatment for depression is associated with improvement of the personality disorder as well. The results of O'Leary et al. [64] support the view that the presence of a personality disorder and high neuroticism scores modify the short-term course to remission onset in depression: longer time to remission onset is associated with a high neuroticism score.

V. PERSONALITY DISORDERS AND ANXIETY

Over the years, there has been an increasing interest in the prevalence of patients with anxiety disorders and personality disorders [65]. Several studies have found high rates of personality disorder diagnoses among patients with anxiety disorders such as panic disor-

der and agoraphobia [66,67], obsessive-compulsive disorder [68], generalized anxiety disorder [69], and social phobia [70]. But is there a relationship between certain personality disorders and anxiety disorders in general, or between certain personality disorders and certain anxiety disorders? In this context, Sanderson et al. [71] investigated the prevalence of personality disorders of patients with anxiety disorders and compared the prevalence within each specific anxiety disorder. The findings indicate that 35% of patients with anxiety disorders were diagnosed with at least one personality disorder. The prevalence rate ranged from 12% (simple phobia) to 49% (generalized anxiety disorder) and 61% (social phobia) among the different anxiety disorders. It is not surprising that 71% of all patients with a personality disorder were from the "anxious/fearful" cluster C of personality disorders. Dyck et al. [72] found particularly consistent results in their study. Twenty-four percent of 622 individuals with at least one past or current episode of one or more anxiety disorders had one or more personality disorders. The most commonly diagnosed personality disorders were avoidant, obsessive-compulsive, dependent, and borderline personality disorders. Patients with social phobia and generalized anxiety disorders had the highest rates of one or more personality disorders. These findings suggest that the development of social phobia and generalized anxiety disorder is probably more closely linked to personality disorders than panic disorder and agoraphobia [72]. But there are divergent findings for specific associations between particular anxiety disorders and personality disorders, although each of these studies used structured interviews (SCID II) as the basis for diagnosis. For example, Brooks et al. [65] and Nesdadt et al. [73] associated obsessive-compulsive personality disorders with general anxiety disorders. In contrast, Mauri et al. [74] found a connection between dependent personality disorder and generalized anxiety disorder. However, understanding comorbidity patterns of anxiety and personality disorders is complicated further by the frequent co-occurrence of depressive disorders in anxiety-disordered patients.

VI. PERSONALITY AND DEPRESSION

Tellenbach [75] described the melancholic type ("typus melancholicus") of personality as a premorbid personality structure predisposing to episodes of melancholia. von Zerssen [76] conceived the manic type as its counterpart (i.e., a premorbid marker of predisposition to the manic component of a bipolar disorder). Hecht et al. [77] investigated in this context the personality features in patients with affective disorder (unipolar depression, bipolar I, and bipolar II), in a high-risk group (first-degree relatives) and a control group. Features of the melancholic personality type are more pronounced in patients with unipolar depression in comparison to controls. In the bipolar I group with mainly manic episodes, personality features do not differ from those of controls. Regarding personality in healthy relatives, melancholic personality features tend to be somewhat more pronounced in high-risk subjects. It was concluded that premorbid typus manicus and typus melancholicus predicted, respectively, a predominant manic and a predominant depressive course of an affective disorder. Other investigators addressed the same questions using different measures and found that high neuroticism predicted increases in depressive symptoms across time. Additionally, high conscientiousness predicted increases in manic symptoms across time. Therefore, they concluded that specific personality traits may assist in predicting bipolar symptoms [78].

Several reports have shown that anxiety-related personality traits are related to depression [9]. A large female twin study showed that neuroticism (and not self-esteem)

was a predictor of risk for major depression. Hansenne et al. [79] studied the role played by the TCI character dimensions in depression. Depressed patients exhibited higher harm avoidance and self-transcendence scores as well as lower self-directedness and cooperativeness scores as compared to healthy controls. Among the depressed group, harm avoidance, self-directedness, and cooperativeness dimensions are related to the severity of depression.

Furthermore, Bagby et al. [80] found higher scores in the openness dimension in seasonal affective disorder (SAD) patients compared to both nonseasonal depressive patients and healthy controls, using the NEO-PI-R [81]. In a twin study, Jang et al. [82] found moderate, but significant, associations between seasonality and neuroticism in healthy volunteers, using the Five-Factor Inventory [81], and these associations seem to be attributable to common genetic factors rather than being influenced by environmental factors [83]. A recent study [84] failed to show a correlation between the severity of neuroticism and seasonality, using the NEO-PI-R in a sample of SAD patients. Sher et al. [85] found the 5-HTTLPR polymorphism to be independent in its effect on both seasonality and neuroticism in a sample of healthy patients [85].

Poor response rate to bright light therapy has shown to be associated with cluster C personality disorders and high scores in the harm avoidance scale in the Tridimensional Personality Questionnaire [58].

SAD patients showed a decrease in neuroticism scores after successful bright light therapy, correlating with changes in the total score in the Hamilton Depression Rating Scale [60]. Lilie et al. found personality abnormalities in 60% of a small number of SAD patients during winter, but only in 35% during summer [59]. The character dimension self-directedness was shown to be susceptible to the state effects of depression in major depressive disorder [86]. Similarly, recent findings suggest that SAD patients exhibit significantly lower scores in the factors of agreeableness, surgency, and emotional stability, and significantly higher in conscientiousness, during the nondepressed state when compared to healthy controls using the Five-Factor model of personality [87].

TPQ scales were also used to predict response to treatments with antidepressants. Scores on the novelty seeking and reward dependence dimensions were not affected by depressed state or treatment response status. However, scores on the harm avoidance dimension were significantly lower in antidepressant responders and were altered by depressed state [88]. TPQ scales also accounted for 35% of the variance in treatment response compared to less than 5% predicted by clinical variables in a study by Joyce et al. [89]. They treated depressed patients with double-blind clomipramine or desipramine (tricyclic antidepressants) for 6 weeks.

Curiously, although harm avoidance or neuroticism has been associated in some studies with the short 5-HTTLPR allele, no firm association between this polymorphism and major depression could be reported. Serretti et al. [90] showed that 5-HTTLPR has not a major influence on the depressive symptomatology in mood disorder subjects. Only the short 5-HTTLPR variant was marginally associated with higher psychic anxiety scores. A study conducted by Rosenthal [91] showed an association between seasonal affective disorder and the 5-HTTLPR short allele. However, these findings could not be replicated in more recent studies [92,93]. The study by Willeit et al. [92] resulted in an association between the polymorphism in the serotonin transporter and the melancholic type of seasonal affective disorder [92]. A meta-analysis, however, showed an association between this polymorphism and bipolar disorder [9].

In suicide victims, impulsivity (prominent in the novelty-seeking personality trait)

is an important personality profile. Association was observed between suicidal ideation and COMT gene locus (catechol-O-methyltransferase). COMT contributes to novelty seeking along with the DRD4 and 5-HTTLPR polymorphisms. The polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) was shown to be associated with violent suicide behavior [92]. A polymorphism in the tryptophan hydroxylase gene (important in the serotonergic pathway) has also been linked to suicide and other impulsivity disorders such as violence associated with alcoholism [9].

VII. PERSONALITY AND ANXIETY

It has been shown that subjects with higher harm avoidance scores have a greater probability of being affected by cluster C personality disorders and comorbid mood and anxiety disorders [9]. However, no association was observed between panic disorder and the 5-HTTLPR short allele, the so-called "anxiety gene."

We studied a sample of detoxified alcohol dependents with TPQ and found that harm avoidance was the only dimension of TPQ influenced by anxiety. Depressive symptoms did not change TPQ scores. High anxiety measures were associated with high harm avoidance [94]. Keeping in mind that harm avoidance is theoretically associated with the serotonergic neurotransmitter system, this could explain the positive therapeutic effects of serotonergic drugs on anxiety disorders.

A substantially increased score was found on the harm avoidance dimension in both panic disorder patients and patients with general anxiety disorder, but it did not differ with regard to TPQ scores [95]. This latter finding is in agreement with the findings that panic disorder and general anxiety disorder do not differ significantly with respect to the associated personality disorders.

In general, individuals with various anxiety disorders are all expected to be high in harm avoidance. They are usually about average or low in novelty seeking. Social phobics are also reported to be low in reward dependence. Panic disorder patients were found to be lower in persistence than controls [96]. Persistence was average in obsessionals compared to the general population. The stability of harm avoidance has been examined in a longitudinal study of panic disorder patients undergoing treatment with drugs and/or behavior therapy. Despite reductions in panic attacks, harm avoidance remained stable during a 6-month follow-up. This suggested that harm avoidance may reflect susceptibility to generalized anxiety independent of panic states.

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5

Is There a Common Etiology for Depression and Anxiety?

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I. INTRODUCTION

Depression and anxiety coexist so often in patients that it is natural to question whether they have a common etiology. Distinct clinical syndromes may have the same etiology (e.g., the clinical syndrome caused by pneuomococcal infection depends on whether it occurs in the lung, meninges, or elsewhere). At the same time, two disorders that occur together might have distinct etiologies. For example, lung cancer and alcoholic cirrhosis might frequently occur together because drinkers tend to smoke. The debate over whether pathological anxiety and depression constitute different aspects of the same disorder [1] or distinct, although often overlapping, conditions [2] is not new, but remains unresolved. In this chapter we review evidence pertinent to the question at hand from methodologies of epidemiology, neuroscience, pharmacology, and psychophysiology. While many of the results of these studies pertain more to the question of common pathophysiology than of common etiology of anxiety and depression, a thorough understanding of the former may be necessary to present an informed response as to the latter.

II. ANXIETY AND DEPRESSION: NORMAL AND ABNORMAL

In discussing the etiologies of anxiety and depression, one must distinguish normal affective responses from exaggerated affective responses as seen in emotionally labile persons, and from categorically defined anxiety and depressive disorders. In the most common sense of the term, "etiology" implies a pathological cause. Normal emotional responses,

therefore, do not have an etiology, but it may be enlightening to examine shared mechanisms of normal anxiety and sadness as a way to explain pathological emotions. Exaggerated emotional responses are pathological in function, but not form; the pathology exists not in the emotional response, but in the vulnerability to exaggerated response. The fundamental question posed by the common coexistence of anxiety and depression in patients is whether pathological forms of anxiety and depression have the same pathological cause.

As affects, anxiety and depression are experienced in everyday life and serve important biological functions. Normal anxiety improves motivation and performance on tasks. Anxious feelings act as a warning to focus on potentially dangerous situations and to formulate plans to deal with them. Moreover, since anxiety is an unpleasant emotion from which we like to escape, it motivates us to master or avoid anxiety-provoking situations. Normal depression, akin to grief, occurs after a loss, either real or perceived. The biological usefulness of depression may be thus to promote affiliative behavior. Some aspects of depression may be useful as a means of energy conservation; in this regard, symptoms of seasonal depression (lethargy, increased sleep, carbohydrate craving) may seem adaptive in a wintertime agrarian or hunter–gatherer economy.

In normal as well as pathological states, depression and anxiety may intermingle. The perception of imminent, but potentially correctable, danger often coexists or alternates with the perception of hopelessness. Distressed persons often experience mixed feelings of anxiety and depression, whether or not they have a psychiatric disease. In the old nomenclature, patients with exaggerated, but not necessarily pathological depression or anxiety were seen to exhibit different forms of neurosis. Thus, the psychological etiology or trigger of anxiety and depression may be the same because the same source of stress may lead to both kinds of symptoms.

The difference between normal and abnormal anxiety and depression is in part a matter of degree but is mainly a categorical question. Some emotional expressions are so intense as to interfere with a patient's ability to cope with the stress that triggered the emotion. Cases in which the emotional response is understandable, albeit extreme, are to be differentiated from cases in which the emotional response is not related to or is out of proportion to an understandable cause and is associated with a cluster of other stereotypic symptoms defining a clinical syndrome. In the former cases, the underlying biological cause of the disorder is a patient's vulnerability to experience an exaggerated emotional response of some sort. In the latter cases, the emotional response is only one of a set of symptoms that tend to emerge simultaneously with or without an apparent source of stress.

The difficulty in sorting out whether a pathological anxiety or mood state is an extreme on a dimensional scale or a categorically defined disease entity is one of the critical dilemmas in clinical psychiatry. Frequent overlap of anxiety and depressive symptoms further confounds this dilemma. An estimated 20 to 90% of patients in surveys experience both anxiety and depression [3]. Numerous studies have shown that anxiety disorders may contain an element of depression and vice versa [4,5]. Therefore, many anxiety rating scales contain subscales of depression, and depression scales also include subscales describing psychic and somatic symptoms of anxiety (e.g., Refs. 6,7). Rating instruments that measure both mood states simultaneously have been useful in the recognition of how clinicians have separated nonpsychotic anxiety patients from nonpsychotic depressive patients. While patients with "anxiety neurosis" tend to score high mainly on scales measuring anxiety, patients with "depressive neurosis" score high both on scales measuring the degree of anxiety and depression [8]. Thus, patients with depressive neurosis were as anxious, but more depressed, than patients with anxiety neurosis.

Not all patients with anxiety and depressive disorders exhibit mixed features. Some patients with anxiety disorders are free of clinically significant depression and some patients with depressive disorders do not experience significant levels of anxiety. However, the frequent co-occurrence of symptoms of both anxiety and depression in a substantial subset of patients indicates that identification of mixed anxiety and depressive disorders is an important first step toward discovering a common etiology. Differentiation of a mixed anxiety-depressive disorder has been slow to come to the diagnostic nomenclature [9,10], possibly because of mixed data on clinical response to treatment [11,12].

III. CLINICAL AND EPIDEMIOLOGICAL STUDIES

Reliable data on the boundaries and stability of psychiatric diagnosis awaited the development of a widely accepted, categorical definition of diagnostic entities. Soon after the advent of the Diagnostic and Statistical Manual of Mental Disorders (DSM), 3rd ed. [13], however, it became clear that diagnostic reliability had a limitation. Not all patients could be cleanly assigned to a single diagnostic category according to the guidelines of the manual [2,14,15]. Later editions of the DSM have employed three strategies to account for such complex forms of disorder: hierarchies, comorbidity, and specifiers. Hierarchies in the definitions of certain disorders specify that the disorder is not diagnosed if it occurs only in the context of another disorder (e.g., generalized anxiety disorder is not diagnosed if the syndrome appears only when the patient also has a major depression syndrome). Comorbidity accounts for multiple disorders by simply listing all disorders for which a patient meets diagnostic criteria whether or not one disorder is the same as, or secondary to, the other (e.g., a patient may have both major depression and panic disorder). In older nomenclatures, specifiers such as "endogenous" versus "reactive" or "neurotic" depression implied the diagnostician had knowledge about the cause of the depression. Specifiers in DSM are used with some disorders to denote a phenomenologically distinct subtype (e.g., major depressive disorder with atypical features). However, none of the specifiers available for mood disorders define an anxious subtype or account for anxiety symptoms. Therefore, for the most part our discussion of the unity or differentiation of anxiety from depressive diagnoses will focus on comorbidity data.

Comorbidity, however, has limitations as a concept, in large part because it has a number of definitions that are used erratically across studies. In some studies of anxiety and depression, the term indicates that anxiety and depressive symptoms coexist simultaneously. In other studies, however, the term denotes that one condition frequently follows the other. Earlier studies on comorbidity were mostly descriptive; more recent studies have tried to explore biological relations between disorders that frequently show comorbidity. Some investigators have proposed that anxiety and depression are biologically separate disorders [2]. If this is true, we should find little overlap in their clinical pictures. It is also conceivable that symptoms of anxiety and depression represent different aspects of the same disorder [1]. Here, symptoms of anxiety and depression could occur simultaneously or alternate. A third possibility is that one condition (e.g., anxiety) induces changes that lead to another condition, namely depression. In this model, pathological depression is the consequence of pathological anxiety and should not lead to anxiety disorders. Finally, comorbidity may be the final common pathway of two originally independent conditions; however, without enhanced knowledge of pathophysiology this possibility is hard to support.

Longitudinal studies have suggested that anxiety is often a prodromal form of de-

pressive disorder. Whereas depression without anxiety tends to maintain diagnostic stability over time, individuals with anxiety disorders and mixed anxiety-depressive disorders at baseline often are found to have depressive disorders later in life [16–19]. While patients who had originally suffered from anxiety disorders often subsequently developed depressive disorders, patients who were diagnosed as having depressive disorders without anxiety rarely developed an anxiety disorder. Thus, depression maintained a diagnostic stability over time, but this was not true for anxiety disorders.

Studies on comorbidity with various anxiety disorders have shown an inconsistent picture as to the associated risk of depressive disorder. Anxiety disorders have been found to occur in as many as the majority of individuals in treatment for depression, with social phobia as the most common disorder [20]. Results of most studies suggest that panic disorder and depression are frequently associated or that depression emerges in the course of panic disorder [21]. Indeed, panic disorder is an extremely common, albeit not universal, condition in patients with both depressive [22] and manic depressive [23] disorders. Generalized anxiety disorder (GAD), as mentioned above, overlaps so often with depression that it is not considered a comorbid condition; indeed, it is not diagnosed at all if it occurs only with depression. Breslau and Davis [24] reported a high incidence of major depression in surveyed mothers who carried the primary diagnosis of GAD. A lifetime history of major depression can be diagnosed in anywhere from 6 to 46% of patients with GAD, depending on the methodology (reviewed in Ref. 25); however, Angst et al. [17] rarely found coexisting depression in their GAD groups. Variant findings may be explained by differences in patient population, diagnostic criteria, and assessment methods. Nevertheless, taking at face value the positive reports of a chronological priority of one disorder to another does not resolve the question as to whether the two disorders derive from the same etiology, or whether one disorder produces the other.

Family studies address directly the question of a shared etiology of anxiety and depression by examining shared familial risk for these disorders. If anxiety disorder (or depressive disorder) in a family member raises the risk for both anxiety and affective disorder in relatives, then this may be evidence for a shared etiology. If anxiety and depressive disorders "breed true" (i.e., if anxiety only raises the familial risk for anxiety and depression for depression), then this argues against a common genetic etiology. The empirical results are complex. Looking at anxiety broadly, patterns of comorbidity suggesting a specific risk in some families are not apparent. Angst et al. [17] found that parents of patients with pure anxiety or depressive disorders and mixed anxiety-depressive disorders had comparable histories of anxiety and depressive disorders. Noyes et al. [26] and Crowe [27] demonstrated that the risk of having an anxiety neurosis was six times higher in relatives of probands with that disorder than in relatives of controls. The specificity of transmission of anxiety disorders is also suggested by the work of Cloninger et al. [28] and Dealy et al. [29], whose studies showed that relatives of probands with anxiety disorders, but not depressive disorders, exhibited an increased risk of anxiety neurosis. On the other hand, twin studies support the hypothesis that pure depression and mixed anxiety–depression are etiologically similar disorders, whereas pure anxiety disorders differ etiologically from depressive and mixed disorders [30]. These discrepant results as well as others that show a lack of crossover familial risk of one anxiety disorder to another [26] suggest that it may not be possible to talk about the genetic etiology of anxiety in general, but rather about each specific type of anxiety disorder.

In a similar way, it may not be possible to speak about the etiology of depression in general, as opposed to depression as part of a mood disorder syndrome [31]. A case

in point is panic disorder. Weissman et al. [32] have used a family study approach to measure the risk of pure and comorbid panic and major depressive disorders in the relatives of probands with each disorder alone, of probands with combined panic and major depression, and of unaffected control probands. They concluded that panic and early-onset major depressive disorder were specific and independent disorders, and that combined depression and panic disorder were probably not a disorder distinct from the simple types. The relationship of panic and bipolar disorders, however, suggests a different story. MacKinnon et al. [33] found that the risk for panic combined with bipolar disorder was elevated in relatives of probands with combined panic and bipolar disorder compared to bipolar and panic disorder alone. Thus, one possible account of the etiological relationship of anxiety and mood is that familial bipolar affective disorder and panic disorder may have common etiological factors, at least in some families. Major depression and panic together do not appear to constitute a subtype of illness, but may be related at a higher level (i.e., panic may arise as a complication of depression, or vice versa).

Pursuit of a common genetic etiology of depression and anxiety shows promise, but has not yet yielded a molecular basis for comorbidity. Linkage and association studies support a complex genetic basis for many psychiatric disorders [34]. Thus, it is possible—indeed probable—that a common genetic basis for two related psychiatric disorders will exist in the form of a common genetic vulnerability of several genes involved in the pathogenesis of the various disorders. Anxiety, for example, may be the result when one has variants of two of the genes A, B, and C, while depression may be produced by variants of two of the genes C, D, and E. Combinations including a variant of gene C will produce comorbidity by a common mechanism, while other combinations produce comorbidity via distinct mechanisms. A potential crossover candidate gene is the serotonin transporter gene. Functional polymorphisms in this gene have been associated or linked with anxiety-related traits in several studies [35,36] and with depression [37]; however, results have been inconsistent [38].

Longitudinal and family studies provide mixed support for the hypothesis that depressive and anxiety disorders may have common etiological risk factors. In longitudinal studies, it appears that anxiety often may be an early manifestation of a disease process that leads later in life to clear depression. In family studies, it appears that some, but not all, of the genetic alleles related to bipolar and panic disorder may be the same; however, there is little evidence that other anxiety and depressive disorders share genetic risk factors. Without detailed knowledge of pathophysiological mechanisms, it may not be possible to say whether anxiety and depressive disorders associated epidemiologically are also associated etiologically. This knowledge may come from molecular genetics or from studies of brain structure and function.

IV. IMAGING STUDIES

Changes in cerebral blood flow and metabolism, seen in imaging studies, emphasize differences between anxiety and depressive disorders rather than features common to both types of disorders. Moreover, subtypes of anxiety disorders and subtypes of depressive disorders exhibit specific imaging patterns that indicate biological differences not only between anxiety and depression per se but also between the subtypes. For example, panic disorder patients at rest, but not other anxiety disorder patients, show increased metabolism and blood flow in the right parahippocampal region [39,40] while obsessive-compulsive patients at rest exhibit higher cerebral blood flow in right prefrontal cortex [41]. Under

symptom provocation, three different anxiety disorders, namely, obsessive-compulsive disorder, specific phobia, and post-traumatic stress disorder, showed as a common characteristic increased cerebral blood flow in the right inferior frontal cortex, the right posterior medial orbitofrontal cortex, the bilateral insular cortex, the bilateral lenticulate nuclei, and bilateral brain stem foci [42]. This finding indicates a widespread activation of the brain during heightened anxiety. In contrast to anxiety disorders, depressive disorders generally show decreased metabolism and blood flow in the dorsolateral and medial-prefrontal cortex, basal ganglia, and the cingulate, reversed with successful pharmacotherapy [43,44]. However, a group of unipolar patients with positive family history of depression exhibited increased blood flow in portions of the orbital frontal cortex and amygdala [45]. Thus, imaging studies not only fail to support the hypothesis of a common etiology of anxiety and depressive disorder but indicate a biological diversity in subgroups of disorders.

V. PSYCHOPHYSIOLOGICAL STUDIES

Similarities in patterns of autonomic and neurovegetative functioning between individuals with anxiety and depressive disorders may implicate common pathophysiological mechanisms that in turn reflect a common etiological factor for these disorders. Findings of a common psychophysiological abnormality would serve as a potentially valuable clue, but would not definitively establish a common etiology until more is understood about the linkage of etiology and pathological mechanisms. A number of studies have examined the question of whether the physiology of sleep and autonomic arousal is similar or distinct in subjects with depression and anxiety.

Sleep disturbance is a common complication of anxiety and depressive disorders; however, different disorders tend to have different sleep complaints. Whereas insomnia in anxious patients tends to occur early, reflecting hyperarousal, patients with depression tend to have middle or late insomnia, indicating a fundamental disturbance of sleep architecture and regulation. These differences are reflected in sleep encephalographic studies, which consistently show in depression abnormal sleep continuity and shortened REM latency [46] as well as microarchitectural abnormalities, including decreased delta amplitude or incidence [47]. Despite common complaints of early insomnia in panic disorder [48], reports of sleep architectural changes have been inconsistent [49,50] and distinct from EEG patterns seen in depression [51]. Similarities in sleep architecture disturbance between subjects with GAD and dysthymia support the notion that these may be variants of the same disorder [52].

Several investigators have focused on quantitative encephalographic asymmetry as a means of discriminating anxious from depressed emotional states in normal subjects. Observation of a shift to the right in brain activity [53] in dysphoric states is consistent with many other lines of evidence on cerebral asymmetry [54]. Electrodermal activity, which reflects sympathetically controlled sweat gland activity, is often decreased in depressed persons [55]. Patterns of electrodermal activity have been found as well to correlate with differential activation of right versus left brain, dependent on the degree of anxious and depressed temperament in the individual [56]. Studies of electrodermal activity and/or quantitative EEG activation in patients with mood and anxiety disorders tend to find patterns of asymmetry, however, with inconsistent patterns across studies [57–59].

Evidence that major depressive disorder and panic are distinct disorders is derived from panic provocation studies using lactate or carbon dioxide, which consistently differentiate panic from other anxiety disorders [60,61] and, generally, from major depressive

disorder [62–65]. Mechanisms for panic vulnerability have not been worked out in detail, but have been hypothesized to involve faulty regulation of respiration [66] or hypersensitivity of the amygdala-related structures involved in the generation of a fearful response [67].

Other evidence that anxiety and depression may be related pathophysiologically and thus etiologically can be sought in measures of autonomic nervous system arousal. Cardiovascular activity measures such as basal forearm blood flow, heart rate, and blood pressure are similar in patients with chronic anxiety and with agitated depression, in contrast to normal controls and patients with "nonagitated depression" [68]. Individuals with anxiety and depressive disorders, as well as other dysphoric emotional states, have been found to have diminished heart rate variability, possibly through a mechanism involving decreased vagal tone [69]. Empirically, however, no consistent abnormalities in vagal tone have been found in anxiety disorders or depression [70].

In summary, studies of sleep architecture and symptom provocation offer evidence for differential mechanisms of depressive and anxious symptomatology, while studies of quantitative electroencephalography and cardiovascular regulation suggest a greater degree of physiological overlap. Heightened specificity of physiological measures correlated with emotional symptoms may yield evidence in time of overlapping disease mechanisms for specific subtypes of anxiety and depressive disorders.

VI. NEUROENDOCRINE MECHANISMS

The hypothesis that psychiatric disorder results from stress, as mediated by the hypothalamic–pituitary–adrenal (HPA) axis, has long provenance and mixed heuristic value and empirical support. Proponents of this hypothesis connect childhood trauma to affective and anxiety psychopathology via corticotropin-releasing factor (CRF) hypersecretion induced by the early stressful conditions [71]. These findings parallel biochemical and behavioral findings in animal models [72]. It is unclear, however, how applicable this hypothesis may be to affective and anxiety disorder patients who do not have a history of trauma and/or evidence of cortisol dysregulation. It is also unclear whether this hypothesis addresses the etiology of depressive and anxiety disorders, or whether hypersecretion of stress hormones reflects a response to the stress of having psychiatric symptoms rather than a root cause of psychiatric symptoms. While cortisol elevation is seen during acute stress, it is not found in chronic anxiety disorders. On the contrary, patients in some anxiety disorders, for instance in PTSD, have cortisol levels that are lower than in nonanxious individuals [73].

Challenge studies have been used to test whether cortisol dysregulation plays a role in psychiatric disorder. For a short while, it appeared that the challenge studies would have diagnostic and prognostic value in depression and anxiety disorders. Unfortunately, these neurohormonal manifestations lack specificity. Changes in the HPA axis, leading to hypercortisolemia and nonsuppression of cortisol in the dexamethasone suppression test (DST), are found in approximately 50% of patients with major depression [31: 448]. Some evidence suggests that normalization of HPA nonsuppression is a prerequisite for antidepressant response in depression [74]. The test, however, is not specific to depression as it gives positive results in alcohol withdrawal, psychotropic drug withdrawal, acute weight loss, or physical exercise, and is frequently positive in schizophrenia and dementias [75]. It also has been found positive, although to a lesser degree, in anxiety disorders. In the studies he has surveyed, Heninger [76] reported that panic disorder patients had a

median nonsuppression rate of 18%, which places them between the rate of normal persons (approximately 10%) and that of major depression. Hospitalized panic disorder patients were more frequently nonsuppressed, which suggests that nonsuppression is linked to the severity of the disorder. Elevation of cortisol levels has been found to be elevated in the saliva of patients experiencing an acute spontaneous panic attack [77] and in the serum of subjects who demonstrate vulnerability to panic provocation with lactate [78]. Cortisol nonsuppression using the DST has also been reported in GAD and in obsessive-compulsive disorder [75]. Interestingly, in one study of GAD patients, the nonsuppression normalized after successful nondrug behavioral treatment [79].

Dysfunction in the HPA axis might reside in the mechanism by which ACTH is released by CRF. In animal models, CRF has been shown to stimulate amygdala structures putatively involved in the generation of fear and anxiety responses [80]. Amygdala CRF, in turn, has been shown to be selectively stimulated by circulating glucocorticoids [81]. Diminished ACTH response to CRF has been demonstrated in major depression and panic disorder in comparison to normal controls [76,82]. However, while CRF has been found increased in depressed patients and, in some studies, in anxiety patients [83], these findings were not consistently seen in anxiety patients [72,84]. Since CRF is under partial control of the noradrenergic system, the effect of challenges on the system are reflected in the cortisol response. The cortisol response to the α_2-noradrenergic agonist clonidine and to the α_2-noradrenergic antagonist yohimbine were similar in both disorders. Thus, overactivity of the HPA axis occurs in subsets of depressive and anxiety disorders and, therefore, lacks specificity. Twenty-four-hour monitoring of cortisol levels in psychotic versus nonpsychotic depressed patients, for example, revealed higher cortisol levels in the psychotic patients, who also had greater illness chronicity, again suggesting higher cortisol as a possible effect of prolonged stress rather than a cause of illness [85].

The serotonergic system is even more complex and less well understood than the noradrenergic and HPA systems. At least 16 receptors with different functions have been described, making interpretation difficult [86]. Challenge studies of the serotonergic system are also difficult to interpret. An infusion of the serotonin precursor tryptophan induced a normal prolactin response in panic patients, but a blunted response in major depression [87]. The same group [76:393] reported that prolactin responses to the serotonin 1c agonist m-chlorophenylpiperazine (mCPP) were normal in both conditions. Kahn et al. [88] found, however, that small doses of mCPP caused anxiety and, compared with normals, heightened cortisol secretion in panic disorder, but not in major depression. The authors interpreted their findings as evidence for postsynaptic serotonin oversensitivity in panic disorder, but not in depression. Their study did not rule out a subsensitivity of the serotonergic system in depression. mCPP also caused transitory increases in obsessive-compulsive symptoms, without a differential effect on hormonal levels in patients with obsessive-compulsive disorder [89]. Since serotonin modulates areas of the brain that are involved in anxiety and depression, one cannot conclude a common mechanism. Serotonin may affect different brain functions in each disorder.

VII. CLINICAL PHARMACOLOGY

A discussion of the common pharmacological mechanisms of antidepressant and antianxiety response must begin with the caveat that the relationship between pharmacological mechanisms and etiology is likely to be indirect. Medications in the antidepressant class that work both on anxiety and depression may begin to alter neurotransmission with the

first dose, while the therapeutic effect may not be evident for several weeks. In contrast, medications that work immediately, such as benzodiazepines that act through the γ-aminobutyric acid (GABA) receptor to lower anxiety, particularly the physiological manifestations of anxiety, are generally not effective in the treatment of depression per se, except by lowering anxiety in agitated depression [90]. Antidepressants that block the reuptake of norepinephrine and serotonin reduce depression and block panic attacks, but have no acute effect on generalized anxiety [91,92]. Prolonged administration of norepinephrine and/or serotonin reuptake inhibitors reduces panic attacks, possibly by inhibiting locus coeruleus activity. However, only antidepressants with serotonin reuptake–inhibiting properties reduce psychic symptoms, such as ruminations, in GAD [91,93], psychic symptoms in panic disorder [94,95], and obsessions and compulsions [96–98], possibly through reduction of frontal lobe activity [99]. Not all antidepressants have anxiolytic effects. For instance, buproprion, an antidepressant that may exercise its effects through the dopaminergic system, does not reduce panic attacks [100] nor has it been found useful in treatment of other anxiety disorders.

Thus, some medications reduce anxiety without affecting depression. Some, but not all, antidepressants have in addition anxiolytic effects but they differ in their effectiveness. Since antidepressants alter functions of various, but not necessarily the same, brain areas their anxiolytic effects may be coincidental and cannot be taken as evidence of brain mechanisms common to anxiety and depression.

The development and application of receptor-specific drugs will enhance our understanding of biological mechanisms of anxiety and depression. Clonidine, a fairly specific α_2-noradrenergic agonist, for instance, blocks panic attacks and has a stabilizing effect on fluctuations in anxiety. However, after an initially sedating effect, it has only a modest effect on generalized anxiety and has no effect on depression [101]. Particularly interesting are drugs that affect specific serotonergic receptors. Although a general blockade of serotonin reuptake suppresses panic attacks, relieves depression, decreases obsessive-compulsive symptoms, and, after prolonged administration, decreases psychic symptoms of generalized anxiety, specific serotonin receptor agonists and antagonists have either anxiety-inducing or anxiolytic [102] effects and may lower depression [103].

In summary, pharmacotherapeutic interventions suggest complex, but different, mechanisms for anxiety and depressive disorders. Moreover, mechanisms differ in subgroups of the disorders. Some symptoms of anxiety and depression are ameliorated by the same medication. This may indicate overlapping mechanisms in certain disorders. On the other hand, many drugs have a broad spectrum of actions and may affect several independent mechanisms. Systematic investigations of the disorders with receptor-specific drugs promise to widen our understanding of these disorders.

VIII. CONCLUSIONS

A survey of research findings linking anxiety and depression reveals some evidence of common pathophysiology and some evidence of divergent pathophysiology. The pathophysiological relationship among depression and anxiety disorders varies by the anxiety disorder. Some chronic anxiety disorders—especially phobias and generalized anxiety disorder—are difficult to differentiate from depressive disorders and thus in some instances may be variants of affective disorder. These disorders may, in a sense, converge to a common etiology. Some anxiety disorders (e.g., panic disorder and obsessive compulsive disorder) do not have significant symptomatic overlap, but do have significant comorbidity

with affective disorders. These disorders may share some, but not all, biochemical or physiological markers with affective disorders; thus they appear to be distinct disorders but related at some unknown level. If they are related etiologically, it is likely to be via a complex chain of causation with some links in common. One might conceive of this relationship as either parallel or intersecting, depending on whether the disorders arise from common etiological elements or, alternatively, one disorder arises as a complication of another.

What sort of conceptual model of brain function might account for this complex picture of depression entwined with anxiety but in many details distinct from anxiety disorders? The brain can be conceptualized as a multimodular system. These modules have distinct functions, but being closely interconnected with other modules, their functions are constantly modified. The clinical differences in anxiety and depressive disorders can be explained by differences in the degree to which certain modules are pathologically affected and influence other modules. Some modules may be activated only in anxiety disorders, others only in depression, leading to "pure" disorders. However, in many subtypes of anxiety or depressive disorders, modules activated in pure anxiety interact with those activated in pure depression, leading to various types of anxiety-depressive disorders. This interaction between modules may be caused by a genetic predisposition, as suggested by family studies. They may be caused by early environmental influence, particularly early life trauma, which affects cerebral response patterns, sometimes permanently, as seen in animal models and in patients who have experienced severe trauma. In a more subtle way, these emotional patterns may manifest in "neurotic" individuals who respond with high degrees of anxiety and depression when faced with everyday stressors. Certain severe forms of one sort of disorder seem to recruit modules of another; thus patients with agitated depression complain of anxious mood and restless energy, or patients with severe agoraphobic social avoidance may become constricted in behavior to the point of inertia resembling melancholia. Therefore, rather than search for a common etiology of anxiety and depression, we need to search for the etiology of subgroups of anxiety and depressive disorders and the effects of these disturbances on the biology of the brain with respect to the interactions between modules.

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6

Measurements of Depression and Anxiety Disorders

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The extensive use of standardized methods of evaluation and quantitative psychopathology are relatively recent phenomena in psychiatry. The main goal of quantitative psychopathology is to standardize diagnostic categories in order to improve diagnostic accuracy and screening of psychiatric cases, assessment of severity of symptoms or disorders, and evaluation of treatment effects.

Diagnostic categories are reliable to the extent that clinicians can agree with each other on whether or not a given disorder is present in a patient. There are no absolute criteria against which psychiatric diagnoses can be validated. Hence scientific progress in psychiatry has been based on establishing a consensus among clinicians about the signs and symptoms that constitute specific psychiatric disorders and operationalizing clinical practice. The need for diagnostic reliability is of particular importance in the follow-up of patients in treatment, and to assure the homogeneity of patient populations in clinical research.

The main sources of poor reliability for inter-rater evaluations are information variance (i.e., differences in the type or quantity of information obtained), interpretation variance (i.e., differences in interpretation of clinical information in terms of psychopathological significance), and criteria variance (i.e., differences in the criteria used to define mental disorders).

Structured and semistructured diagnostic instruments reduce the information variance by standardizing the information-gathering process. Interpretation variance is de-

creased by use of a detailed glossary of psychopathological terms along with the instrument, as well as extensive training of interviewers. Finally, explicit and standardized diagnostic criteria diminish the criteria variance by use of a uniform set of criteria for a given diagnosis.

There are two principal types of psychopathological evaluations: categorical and dimensional evaluations. Categorical assessments are qualitative assessments and establish a category of diagnosis based on the presence or absence of certain attributes in a patient. On the other hand, dimensional evaluations are quantitative and aimed at assessing the intensity of the disorder. The dimensional evaluation can assess several factors and is a more sensitive instrument in the evaluation of treatment effects. Categorical and dimensional assessments, although conceptually distinct, are not entirely separable. Between these two classification types, there is an ordinal classification, using ordered sets of categories such as mild, moderate, and severe. Moreover, a cut-off point can be used with any continuous scale to indicate a threshold for a corresponding category.

The psychometric properties of psychiatric instruments can be considered under the headings of reliability and validity. The reliability of an instrument refers to the reproducibility of measurements. A reliable instrument is one that reproduces the same results on repeated administrations. The validity of an instrument is defined as the extent to which an instrument measures what it claims to measure.

In the following section, we will first discuss categorical (diagnostic) instruments mainly used in adult subjects to assess depression and anxiety disorders. Next, we turn to dimensional instruments providing an assessment of the severity of anxiety disorders and depression.

I. DIAGNOSTIC INSTRUMENTS FOR DEPRESSION AND ANXIETY DISORDERS

All the instruments presented here provide diagnosis of depressive or anxiety disorders. Most of them are clinician-administered interviews, requiring trained clinicians with a good knowledge of psychopathology. However, two of the interviews (CIDI and DIS) can be administered by trained nonclinical or lay interviewers and are used in epidemiological studies.

We will also present two primary care interviews that combine both self-report and follow-up examination designed for use as screening instruments by nonpsychiatric physicians. All the instruments presented differ in the extent of their coverage of axis I disorders and the degree of structure imposed. Some of them are wedded to one set of diagnostic criteria, whereas others can generate diagnosis according to multiple types of criteria. One of the limitations of these interviews is that they are all time consuming and expensive, especially those that should be administered by a clinician.

The Schedule for Affective Disorders and Schizophrenia (SADS) [1] was initially developed in an effort to reduce information variance in both the descriptive and diagnostic evaluation of the subjects for the National Institute of Mental Health Clinical Research Branch Collaborative Program on the Psychobiology of Depression. This instrument can be used in clinical or nonclinical settings. SADS is a semistructured instrument, which should be administered by a clinician. It evaluates mental disorders according to the Research Diagnostic Criteria (RDC) [2] which is a precursor of DSM-III. The RDC covers 23 major diagnostic categories including mood disorders and anxiety disorders. The instru-

ment consists of two sections: the first covers the symptoms of current mental disorders and the second covers the lifetime history of mental disorders before the year preceding the interview. Individual symptoms and other aspects of current affective (mood) disorders, psychotic disorders, and anxiety disorders are rated in detail in scales of severity. Symptoms are rated according to their most severe level during the current episode and at the level they were experienced in the week before the interview. Clinically significant symptoms of lifetime disorders are rated as present or absent. The time required for administering the instrument is typically 1 to 3 h depending on the symptoms present. Different versions of SADS include SADS-L (lifetime), SADS-LB (specifically intended to evaluate bipolar disorders), and SADS-LA (anxiety disorders). It is important to note that this instrument does not generate diagnoses according to the DSM-IV criteria; it seems to be very useful in that it provides for (1) a detailed description of the features of the current episodes of illness when they were at their most severe; (2) a description of the level of severity of manifestations of major dimensions of psychopathology during the week preceding the evaluation, which can be used as a measure of change; (3) a progression of questions and criteria, which provides information for making diagnoses; and (4) a detailed description of past psychopathology and functioning relevant to an evaluation of diagnosis, prognosis, and overall severity of disturbance. Test–retest reliability of summary scale scores from Part I of the SADS is good [3]. Test–retest reliability of disorders shows good agreement (kappa > 0.65) for most disorders and subtypes of affective disorders. Finally, SADS item and scale scores are shown to be sensitive to change in placebo-controlled medication studies.

The Structured Clinical Interview for DSM (SCID) [4] was initially developed to provide a broad coverage of psychiatric diagnoses according to DSM. It was designed to be more efficient and simpler to use than other existing instruments and, consequently, to require less time for training and administration.

SCID-I is a semistructured instrument that needs to be administered by a clinician and generates diagnoses according to DSM-IV criteria. This instrument can be used with psychiatric patients or with nonpatient community subjects who are undergoing evaluation for psychopathology. SCID-I includes nine diagnostic modules: mood episodes, psychotic symptoms, psychotic disorders differential, mood disorders differential, substance use, anxiety, somatoform disorders, eating disorders, and adjustment disorders. Two versions of SCID are available: SCID-I (research version) [5] and SCID-CV (clinical version) [6]. The majority of diagnoses generated by this instrument are made on a lifetime (ever present) and current (meets diagnosis criteria in the past month) basis. This instrument also allows evaluation of subthreshold diagnosis. The time required to administer the instrument can range from 1 to 3 h depending on the clinical symptoms of the subject.

The Schedules for Clinical Assessment in Neuropsychiatry (SCAN) [7] were developed internationally for the study of psychopathology. It forms the basis for collaborative studies of the causes, risk factors, outcomes, and consequences of mental disorders. This instrument is an outgrowth of the Present State Examination (PSE) [8], which was first developed in the early 1960s and has undergone nine revisions. Its purpose is to assess the psychopathology and behavior associated with a broad range of major psychiatric disorders in adult life. The SCAN can be used to make psychiatric diagnoses in clinical samples according to Diagnostic Criteria for Research (ICD-10-DCR), DSM-IV, and other systems. It is important to note that this instrument can also provide dimensional ratings of symptoms and syndromes that are not wedded to any single classification or diagnostic

system. This instrument can be administered by interviewers who should be well trained in assessing the signs and symptoms of psychopathology. It provides current and lifetime diagnoses.

Several brief screens are available to help identify mental disorders in primary care. In choosing among them, an investigator must consider the clinical research setting, the sampling framework, and the strategy used to assess the reliability and validity of the instruments. We chose to describe here the PRIME-MD and the SDDS-PC that are often used in primary care practice to screen patients for psychiatric symptoms.

PRIME-MD [9] is intended for use by general practitioners and is designed for the diagnosis of mental disorders that are most commonly encountered in general practice. This instrument was specifically designed for use by nonpsychiatric primary care physicians. Because of its simplicity and efficiency, it can be used as a quick assessment tool for psychiatrists evaluating patients with anxiety, depression, or somatic symptoms. Its limitations are that it covers only a few of the psychiatric diagnoses encountered in psychiatric settings and it ignores certain aspects of the criteria for the disorders that it does cover, such as impairment and differential diagnosis criteria. It consists of two sections: a 1-page patient questionnaire, which is completed by the patient prior to seeing the physician and a 9-page evaluation guide for the clinician, which is a structured interview. The Clinical Evaluation Guide generates five types of diagnoses according to the DSM-IV criteria (mood, anxiety, alcohol use/abuse, eating disorders, and somatic disorders). The average time required for administering PRIME-MD is typically less than 20 min. Spitzer and colleagues assessed the validity and utility of PRIME-MD [9] in a sample of 1000 adult patients assessed by 31 primary care physicians. In this study there was good agreement between PRIME-MD diagnoses and those of independent mental health professionals [for the diagnosis of any PRIME-MD disorder (kappa = 0.71; overall accuracy rate = 88%)]. The sensitivity of the PRIME-MD was very good for detecting any psychiatric diagnosis (0.83) and at least satisfactory for detecting any diagnosis with a particular diagnostic module.

The Symptom-Driven Diagnostic System for Primary Care (SDDS-PC) [10] was developed as a computerized instrument to improve diagnosis of mental disorders in primary care practice. It consists of three components: (1) a 29-item patient self-report screening questionnaire; (2) a diagnostic interview guide with six diagnosis modules plus a module assessing suicide risk; and (3) a longitudinal tracking form. It provides diagnoses according to the DSM-IV. The SDDS-PC is intended for use by primary care physicians and nurse practitioners, with little or no specific training in its use. It takes approximately 5 min for patients to complete the screening form. Each diagnostic module can take between 5 and 10 min to administer.

This instrument has not been tested in mental health settings and has limited diagnostic coverage for general use in psychiatric practice. The SDDS-PC covers five disorders (major depression, panic disorder, alcohol abuse or dependence, generalized anxiety disorder, and obsessive-compulsive disorder, as well as suicidal ideation. Patients who screen positive for a disorder receive the corresponding diagnostic interview module. Patients who meet mental disorder criteria on the diagnostic interview module are then followed with the longitudinal tracking form.

A cross-validation study was carried out by the instrument developers [11] and showed an overall sensitivity for any disorder of 0.88 and a fair concordance (kappa = 0.47) between diagnoses of depressive disorders made by primary care physicians and SCID diagnoses made by trained mental health interviewers. In this study, the kappa values

ranged from 0.11 (generalized anxiety disorder) to 0.48 (suicidal ideation); the overall kappa for any diagnosis was 0.50.

The Diagnosis Interview Schedule (DIS) [12] is a structured interview developed for use by lay interviewers or clinicians in subjects in community or in clinical samples. DIS generates current as well as lifetime diagnoses according to DSM-III criteria. DIS has undergone several revisions. The fourth version, DIS-IV, generates diagnoses according to DSM-IV criteria. DIS-IV also provides information about symptom duration, remission, and associated impairment and treatment received. This instrument is being used in a set of epidemiological studies sponsored by the National Institute of Mental Health Center for Epidemiological Studies.

The DIS is the precursor to the Composite International Diagnostic Interview (CIDI) [13]. The latter was developed in order to allow evaluation of mental disorders in different cultural settings and according to the International Classification of Disease (ICD) and the DSM criteria. The DIS has been revised several times, and the latest version, DIS-IV [14] yields diagnoses on the basis of DSM-IV diagnostic criteria. DIS-IV also provides information about symptom duration, remission, and recency and about associated impairment and treatment received. The DIS begins with a demographic section, followed by 19 diagnostic modules, and concludes with an open-ended question the interviewer may not have covered and a section for rating observations of the subject's speech, affect, and behavior. Additions made to version IV of the DIS have increased administration time to approximately 90 to 120 min. This instrument has been administered to 220 psychiatric patients by lay interviewers to compare the diagnoses obtained by the DIS to clinical diagnoses [15]. Kappas for agreement between DIS and chart diagnoses ranged from 0.39 to −0.03 and averaged 0.14 for 13 diagnostic categories. Agreement was best for affective, obsessive-compulsive, and schizophrenic disorders and was poorest for phobias where patients overemphasized fears.

The Composite International Diagnostic Interview (CIDI) [13] was designed at the request of the World Health Organizations/US Alcohol, Drug Abuse, and Mental Health Administration Task Force on Psychiatric Assessment Instruments. This instrument combines questions from the DIS with questions designed to elicit PSE items.

The CIDI is a highly structured instrument, which can be administered by nonclinicians. It generates diagnoses according to the DSM-IV and ICD-10 criteria. Most of the major ICD and DSM axis I diagnostic classes are addressed by the most recent CIDI (version 2.1). The 11 diagnostic modules included in the CIDI roughly correspond to the modules that cover adult axis I disorders in the DIS, with some modest differences in terminology and content. An alternative version of the CIDI was developed for use in the National Comorbidity Survey (NCS) in the United States in the early 1990s. For both the DIS and the CIDI, interviewers proceed through a series of questions formatted according to a flowchart. When the subject answers in the affirmative a question about whether a symptom has ever been experienced, the interviewer asks whether the subject has (1) told a doctor or other professional about the symptom; (2) taken medication for it; or (3) experienced significant interference with life or activities as a result of it. The time required to administer this instrument is approximately 75 min and its most recent version is CIDI 2.1

The Mini-International Neuropsychiatric Interview [16] is a short, structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and France, for DSM-IV abd ICD-10 psychiatric disorders. With an administration time of approximately 15 min, it was designed to meet the need for a short, but accurate, structured

psychiatric interview for multicenter clinical trials and epidemiological studies and to be used as a first step in outcome tracking in nonresearch clinical settings. The diagnoses generated are lifetime and present. The disorders assessed by this instrument are mood disorders, anxiety disorders, eating disorders, and psychotic disorders.

The Newcastle Diagnostic Scales [17] for affective disorders include several short, 10-item scales useful for the assessment of depressed patients. One of the first scales published was designed to make the difference between endogenous and exogenous depression through a structured interview. The same year (1965) the authors published another scale designed to predict the response to electroconvulsive therapy. These two scales have only two items in common, which assess anxiety and weight loss. These scales are easy to administer and their reliability is good. They are designed to be completed by a psychiatrist but short training could allow family practitioners to use of these scales. Davidson et al. [18] assessed the validity and the reliability of this instrument. Good interrater reliability was obtained for diagnostic category and for item scores in 36 patients. The scale showed construct validity with reference to the Hamilton and Montgomery–Asberg rating scales. Concurrent validity was shown relative to other diagnostic scales of endogenous depression.

Several diagnostic instruments not covered in this chapter have been designed specifically for use in special patient populations. The Diagnostic Interview for Genetic Studies (DIGS) [19] is a comprehensive and lengthy instrument designed as a diagnostic assessment tool in genetic studies. For patients with anxiety disorders, the Anxiety Disorders Interview Schedule (ADIS-R) [20] provides DSM diagnosis of anxiety disorders by DSM criteria, with additional coverage of mood, somatoform, and substance abuse disorders.

II. DIMENSIONAL ASSESSMENT OF ANXIETY

There are two main types of instruments for dimensional assessment of anxiety, namely, self-evaluation scales (designed to be completed by the subject) and hetero-evaluation scales (intended for use by an interviewer).

A. Self-Assessment Scales for Anxiety

The main categories of self-assessment scales used in the evaluation of anxiety are scales assessing general psychopathology including anxiety, such as the General Health Questionnaire (GHQ) [21,22] the Symptom Check List-90 Revised (SCL-90R) [23], and the Brief Symptom Inventory (BSI) [24]; scales assessing the general anxiety (e.g., the Beck Anxiety Inventory [25], and the Hospital Anxiety and Depression (HAD) [26] and, finally, scales assessing specific dimensions of anxiety such as the Fear Questionnaire [27] and the Penn State Worry Questionnaire (PSWQ) [28].

B. General Psychopathology Scales Assessing Anxiety

In this chapter we are going to examine the most commonly used measures of general psychiatric symptoms. These scales are intended as screening instruments to identify individuals likely to have psychopathology, not as specific diagnostic measures to identify particular axis I disorders. These instruments also focus on the impact of the psychopathology on the individual's ability to function. The GHQ assesses levels of mental health in general clinical practice, the SCL-90-R goes further in assessing specific symptoms with

nine symptom constructs. Finally, the BSI is a multidimensional symptom inventory derived for brevity from the SCL-90-R.

The GHQ [21,22] was developed to evaluate the psychological components of ill health, assessing the subject's ability to carry out daily functions. This measure is not designed for psychiatric diagnosis but rather as a screening device. It may be seen as a complement to a formal psychiatric interview. This instrument is a self-administered questionnaire that takes 3 to 15 min to complete.

There are four versions of the GHQ, which include 60, 30, 28, or 12 items, respectively. The GHQ-28 is a scaled version of the GHQ designed on the basis of results from principal components analysis, indicating a total score with four subscales: somatic symptoms (scale A), anxiety/insomnia (scale B), social dysfunction (scale C), and severe depression (scale D). These subscales are dimensions of symptomatology rather than distinct diagnosis.

The GHQ is highly acceptable to the general population, as evidenced by its frequent use in large-scale surveys in clinical and nonclinical settings. This instrument has been used most commonly to estimate the prevalence, presentation, detection, and outcome of psychiatric disorders among primary care attenders.

The SCL-90-R [23] is a 90-item self-administrated questionnaire assessing the discomfort created by each symptom during the past week. This instrument contains only minor revisions from the SCL-90 that are the replacement of two items and minor changes to seven other items. The SCL-90-R is constituted by nine symptom constructs: somatization, obsessive-compulsive symptoms, interpersonal sensitivity, depression, anxiety, hostility, phobic-anxiety, paranoid ideation, and psychoticism. It takes about 12 to 20 min to complete. This instrument can be used as a screening instrument of global psychological distress and as a multidimensional measure of symptom profiles. This instrument is often used in randomized clinical trials assessing antidepressants and anxiolytics, and also in clinical studies evaluating anxiety and depressive disorders. Schmitz et al. [29] investigated the screening properties of the GHQ-12 and the SCL-90-R in a primary care setting in Germany. A randomly selected sample ($n = 408$) of adult outpatients from 18 primary care offices was screened using the German versions of the GHQ-12 and the SCL-90-R. A structured diagnostic interview (SCID) and an impairment rating (IS) were used as a gold standard to which both questionnaires were compared. There was no difference in the performance of the general scores of the two questionnaires. Both instruments were able to detect cases. Analyses confirmed that the SCL-90-R subscales "anxiety" and "depression" showed acceptable concurrent validity for the diagnostic groups anxiety and depression (according to DSM-III-R). In this study, GHQ-12 and SCL-90-R appeared to be useful tools for identifying mental disorders in primary care practice and research.

The Brief Symptom Inventory (BSI) [24] is derived from the SCL-90-R. This instrument is a multidimensional symptom inventory designed to assess the psychological symptom patterns of respondents in the community, medical, and psychiatric settings. The BSI is a 53-item form of the SCL-90-R requiring 8 to 10 min to be completed. It reflects psychopathology and psychological distress in terms of the same nine symptom dimensions of the SCL-90-R. The BSI may be used as a single, one-time assessment of the patient's clinical status. It may also be used repeatedly to document formal outcomes or quantify pre- and post-treatment responses. This instrument is therefore useful both in screening primary care patients and in planning treatment and evaluating outcome.

Collectively, the sum of studies using the BSI demonstrates the instruments to be

broadly sensitive to the manifestations of psychological distress and interventions across a wide range of contexts.

C. Scales Assessing General Anxiety

Principal instruments providing broad coverage of anxiety symptomatology are described in this part. Usually, these instruments are used in situations in which a clinician does not want to assess a specific type of anxiety disorder or in patients with clinically significant anxiety that falls below the minimum threshold set by the anxiety disorder criteria. These broader measures should not be confused, as they often are, with measures of generalized anxiety disorder, which is a specific DSM diagnosis.

HADS [26] is 14-item self-report scale originally designed to screen for the presence of a mood disorder in medically ill patients. Seven items of the instrument assess anxiety and seven items assess depression. Factor analysis of the instrument has revealed that all seven items assessing depression correspond to a single factor (depression factor), whereas the items assessing anxiety correspond to two factors—motor anxiety and psychological anxiety. This instrument is a good screening device for the presence of depressive disorder in nonpsychiatric populations.

Zigmond and Snaith [26] found item-total correlations (r) ranging from 0.30 to 0.60 in 50 patients in a general outpatient clinic. An exploratory factor analysis of the HAD was carried out in 568 cancer patients by Moorey et al. [30]. Two distinct, but correlated, factors emerged that corresponded to the questionnaire's anxiety and depression subscales. The factor structure proved stable when subsamples of the total sample were investigated. The internal consistency of the two subscales was also high. The validity of this instrument was assessed in adolescents by White et al. [31].

The HADS was given to 248 schoolchildren, to 48 psychiatric outpatients, and to 38 deliberate self-harm inpatients aged 12 to 17, and validated against ICD-10 diagnoses for the outpatients. In this study, the HADS has adequate test–retest reliability and factor structure, and discriminates between adolescents diagnosed with depressive or anxiety disorders and those without these diagnoses.

Finally, the construct validation of the Hospital Anxiety and Depression Scale was assessed with clinical populations by Johnston et al. [32]. Data from patients with breast disease, myocardial infarction, and stroke were examined using factor analytic and psychometric analyses. The HADS showed high levels of internal consistency and there was little evidence that removing items would improve it.

The Beck Anxiety Inventory (BAI) [25] assesses anxiety, with a focus on somatic symptoms. This instrument was specifically developed to discriminate between anxiety and depression. The BAI is a 21-item self-report questionnaire assessing symptoms of anxiety such as nervousness, inability to relax, dizziness or light-headedness, and heart pounding or racing. Patients record how much they have been bothered by each symptom during the past week, including the day the questionnaire was administered. It takes approximately 5 min to complete this instrument. This questionnaire is a reliable and well-validated measure of somatic anxiety symptoms found across anxiety disorders and also in depression. However, it is important to note that this instrument does not assess worry, which is an important symptom in the GAD, nor does it focus on the other DSM-IV symptoms of this specific anxiety disorder. Moreover, BAI does not discriminate well between specific anxiety disorders.

The Beck Anxiety Inventory was administered to 105 outpatients who were diag-

nosed with various types of psychiatric disorders by Steer et al. [33]. A principal factor analysis was performed, and two factors were found to represent subjective and somatic symptoms of anxiety. Results of this study supported the use of the inventory for evaluating self-reported anxiety in outpatients adolescents.

D. Instruments Assessing Specific Dimensions of Anxiety

The Fear Questionnaire [27] is a self-report instrument developed to assess the severity of common phobias (mainly agoraphobia, social phobia, and blood-injury phobia) and associated anxiety and depression. The FQ agoraphobia and social phobia subscales may be useful as brief screening instruments for symptoms of agoraphobia and social phobia.

Cox et al. [34] conducted a confirmatory factor analysis using the Fear Questionnaire responses of 122 social phobia patients. The results indicated that the proposed three-factor model of the scale (agoraphobia, social phobia, and blood/injury dimensions) provided a good fit of data. These data provided strong support for the validity of the scale.

The Penn State Worry Questionnaire (PSWQ) [28] was designed to assess trait symptoms of pathological worry. This instrument was created to assess the tendency of an individual to worry, and the tendency for the worrying to be generalized and not restricted to one or a small number of situations. The PSWQ is a 16-item self-report questionnaire assessing the frequency and intensity of worry symptoms. It takes approximately 5 min to complete this instrument. The PSWQ showed good convergence with other measures of worry in nonclinical populations. The use of this instrument is recommended as a measure of severity of pathological worry and change. It may be useful as a screening tool to detect pathological worry, but it cannot be used to diagnose generalized anxiety disorder in nonclinical populations because worry could be related to many disorders besides generalized anxiety disorder.

Brown et al. [35] assessed the psychometric properties and utility of the PSWQ in a clinical sample of 436 anxiety disorders patients and 32 normal controls. Factor analysis indicated that the PSWQ assesses a unidimensional construct. Furthermore, the PSWQ evidenced quite favorable internal consistency using GAD patients and each of the other anxiety disorder groups and normal controls. The validity of the PSWQ was supported by an analysis indicating that the measure distinguished GADs from each of the other anxiety disorder groups including those with obsessive-compulsive disorder. Collectively, the findings speak favorably to the PSWQ in research examining the nature and treatment of GAD and the processes of normal and pathological worry.

E. Hetero-Evaluation Scales Assessing Anxiety

The Hamilton Anxiety Rating Scale (HARS) [36] is a 14-item clinician-administered rating scale providing an overall measure of global anxiety, including psychic (cognitive) and somatic symptoms. The symptoms assessed are anxious mood, tension, fear, insomnia, intellectual (cognitive) symptoms, depressed mood, behavior at interview, somatic (sensory) symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, genitourinary symptoms, autonomic symptoms, and somatic (muscular) symptoms. It takes approximately 15 to 30 min to administer this instrument. The HARS can be used to assess the severity of overall anxiety in patients meeting criteria for anxiety or depressive disorders but this instrument does not distinguish symptoms of a specific anxiety disorder or distinguish an anxiety disorder from an anxious depression.

The Tyrer's Brief Scale for Anxiety [37] is a 10-item clinician-administered scale

designed to assess anxiety among mentally ill patients. This instrument is derived from the CPRS (Comprehensive Psychopathological Rating Scale) [38], which assesses general psychopathology.

This instrument includes 10 specific items for generalized anxiety; eight items correspond to reported symptoms and two to the observed ones. The cognitive symptoms of anxiety are assessed by four items, whereas the somatic symptoms of anxiety are assessed by five items. It takes approximately 15 min to complete this instrument. This instrument has been used in pharmacological trials assessing the efficacy of anxiolytic drugs.

The Association for Methodology and Documentation in Psychiatry (AMDP-System) [39] is composed of two parts: a part on history data (AMDP-1 to −3) and a part on the present psychiatric and somatic state (AMDP-4 and −5). The psychopathology scale contains 100 items + 15 "French" items (mainly on anxiety); the somatic scale, 40 + 7 items, all graded from 0 to 4. The interview and the completion of the two scales take approximately 45 to 60 min. Presently there are two systems derived from the AMDP: one for gerontopsychiatry (the AGP System) and one for forensic psychiatry (the FPDS System).

Gebhardt et al. [40] carried out a study assessing the validity of the syndrome scales in the AMDP-system. In order to assess this validity, various diagnostic groups as defined by the ICD were described by these scales and distinguished from each other by discriminant analyses. As a comparison, the same diagnostic groups were distinguished using the syndrome scales of the AMP system. The analyses using the AMDP system were performed in a sample of 659 patients of the Psychiatric Clinic of the Free University of Berlin during 1979 to 1980, the analyses with the AMP system in a sample of 2269 patients of the same clinic during the period 1971 to 1976. It could be shown that different endogenous and organic psychoses as well as neuroses can be described in their psychopathology and discriminated from each other by means of the syndrome scales of the AMDP system. The validity of the syndrome scales in relation to this criterion could be proved. Moreover, a high similarity between the results with the AMDP system and the results with the AMP system was found, which demonstrates that the two systems compare well.

III. INSTRUMENTS FOR THE EVALUATION OF SPECIFIC ANXIETY DISORDERS

Use of different scales allows better appreciation of the various clinical dimensions of a specific anxiety disorder. For panic disorders, the available instruments include the Panic-Associated Symptom Scale [41], the Panic Disorder Severity Scale (PDSS) [42], and Anxiety Sensitivity Index (ASI) [43]. For social phobia, the available instruments include Liebowitz Social Anxiety Scale (LSAS) [44], the Brief Social Phobia Scale (BSPS) [45], and the Social Phobia and Anxiety Inventory (SPAI) [46]. For obsessive-compulsive disorders, the available scales include: the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) [47], and The Leyton Obsessional Inventory [48], and the Mausley Obsessional Compulsive Inventory [49]. Finally, instruments assessing the post-traumatic stress disorder that we describe in this chapter are Impact of Event Scale (IES) [50], the Mississippi Scale (MSS) [51], the Clinician-Administered PTSD Scale (CAPS) [52], and the Posttraumatic Stress Diagnostic Scale (PDS) [53].

A. Instruments Assessing Panic Disorder and Agoraphobia

The Panic-Associated Symptom Scale (PASS) [41] is a 14-item self-rating scale with a 0 to 3 scoring. The 14 items assess several panic attack symptoms and the patient is asked

to determine the severity of each symptom during the worst moment of the panic attack. This instrument is frequently used in studies comparing patients with panic disorder in terms of clinical features and outcome.

The Panic Disorder Severity Scale (PDSS) [42] is a clinician-administered instrument developed to provide a simple way of measuring the overall severity of DSM-IV panic disorder. This instrument consists of seven items assessing panic frequency, distress during panic, panic-focused anticipatory anxiety, phobic avoidance of situations, phobic avoidance of physical sensations, impairment in work functioning, and impairment in social functioning. It takes between 5 to 10 min to complete the PDSS. This instrument is useful in assessing overall panic disorder severity at baseline, and it provides a profile of severity of the different panic disorder symptoms. It is important to note that the PDSS is not a diagnostic instrument; it could be used to monitor the outcome of panic disorder specifically.

Shear et al. [54] assessed reliability and validity of the PDSS and tried to provide an estimate of a cut-off score discriminating the presence or absence of current DSM-IV panic disorder, and to determine the factor structure of the instrument. One-hundred-four psychiatric outpatients, including 54 with current panic disorder, underwent structured diagnostic assessment and the PDSS interview. The PDSS was repeated within 3 to 17 days. The reliability and the validity of the PDSS were confirmed and a one-factor solution fit the data. A cut-off score of eight identifies patients with current panic with a sensitivity of 83.3% and a specific of 64%.

The Panic and Agoraphobia Scale [55] was developed to assess the degree of severity of these disorders after a diagnosis has been made according to the criteria of the DSM-III-R/IV or the ICD-10. This instrument was specially developed for use in clinical drug trials. Factors that impair the quality of life in panic disorder and agoraphobia patients (panic attacks, phobic avoidance, anticipatory anxiety, impairment in social relationships and work, assumption of somatic disease) were considered in the development of this scale so that the efficacy of a certain drug therapy on each of these factors can be assessed separately. It takes few minutes to complete this instrument. The PAS is a useful tool for assessing the severity of PDA.

The Anxiety Sensitivity Index (ASI) [43] assesses fear of anxiety symptoms or consequences, evaluating the degree to which individuals interpret symptoms of anxiety as threatening. This instrument is a 16-item self-report questionnaire that could be used as a measure of symptom type and severity at baseline, and a clinical outcome measure in patients with panic disorder. Although the ASI is not a diagnostic measure, this instrument could be used to distinguish patients with panic disorder from patients with other anxiety disorders.

Since the development of the 16-item *Anxiety Sensitivity Index* ASI, there has been considerable controversy in the literature about whether it should be conceptualized as an unidimensional or multidimensional measure. ASI responses were collected by Cox et al. [56] from 216 panic disorder patients and 365 undergraduate students. Confirmatory factor analysis results for both the patient and student data strongly supported the view that the ASI is a multidimensional measure.

The Mastery of Your Anxiety and Panic II [57] is a method for recording self-monitored panic attacks. Any panic attacks had to be recorded as soon as the attacks are over providing measure of frequency, intensity, and duration of panic episodes. This instrument could be used as the main outcome measure in studies of panic disorder. Panic frequency data can be collected over any specified period, although a minimum of 2 weeks is recommended to establish a baseline for the panic attack.

The *Mobility Inventory for Agoraphobia (MI)* [58] is a self-report questionnaire designed to assess the frequency of panic attacks and the severity of agoraphobic avoidance behavior both in situations where the patient is accompanied by a trusted companion and where the patient is alone. It takes about 20 min to complete this instrument. The MI seems to be the best available measure of agoraphobic avoidance to data.

B. Instruments Assessing Social Phobia

The Liebowitz Social Anxiety Scale [44] is a clinician-administered semistructured interview designed to assess the social phobia as determined by DSM-IV criteria. The LSAS includes 24 items, 13 describing performance situations and 11 describing social interactional situations. The LSAS yields four subscales: fear-social, avoidance-social, fear-performance, and avoidance-performance. It takes approximately 15 to 20 min to complete this instrument. The Liebowitz scale covers a broad range of potentially fearful situations.

Heimberg et al. [59] assessed the reliability, validity, and treatment sensitivity of the Liebowitz Social Anxiety Scale. Three hundred and eighty-two patients from several studies of the treatment of social phobia were evaluated. An independent assessor administered the LSAS to each patient prior to the initiation of treatment. Patients also completed other measures of social anxiety and avoidance, although the specific measures varied across the samples. The LSAS and its subscales were normally distributed and demonstrated excellent internal consistency. The convergent validity of the LSAS was demonstrated via significant correlations with other commonly used measures of social anxiety and avoidance. These correlations also tended to be larger than correlations with measures of depression, especially after treatment. However, the pattern of correlations of LSAS subscales with one another and with the other measures suggest that the fear subscales and the avoidance subscales may not be sufficiently distinct in clinical samples. The LSAS was also demonstrated to be sensitive to the effects of pharmacological treatments of social phobia over time. In conclusion, the LSAS appeared to be a reliable, valid, and treatment-sensitive measure of social phobia.

The Brief Social Phobia Scale (BSPS) [60] is a semistructured instrument assessing severity and treatment response in social phobia as defined by DSM criteria. The BSPS consists of 11 items. The first seven items assess the severity of avoidance for each of the following situations: public speaking, talking to people in authority, talking to strangers, being embarrassed or humiliated, being criticized, social gatherings, and doing something while being watched. The second part contains four items assessing physiological symptoms (i.e., blushing, trembling, palpitations, and sweating) experienced by the patient while being confronted with the social situations. It takes approximately 10 to 15 min to complete this instrument. The BSPS should not be used as a diagnostic instrument. It does not provide information about the duration of social phobia symptoms or the degree of distress or interference they cause. Finally, this instrument demonstrates acceptable inter-rater and test–retest reliability, internal consistency, concurrent validity against other measures of social phobia, and the ability of patients to change as a result of treatment. It can also detect differences between active treatment and placebo treatment.

Davidson et al. [45] assessed the psychometric properties of the BSPS in a sample of social phobia patients. The results yielded a high level of reliability and validity. Test–retest reliability was excellent, as was internal consistency. The fear and avoidance subscales demonstrated highly significant correlations with remaining items totals; however, the physiological subscale did not. The BSPS also demonstrated significant relationships

with other established scales that assess anxiety and disability, and it proved sensitive to treatment effects in a trial of 5-HT3 antagonist and placebo.

The Social Phobia and Anxiety Inventory [46] is a self-report questionnaire assessing specifically social phobia as defined in DSM. This instrument is composed of two subscales that are the 32-item social phobia scale and the 13-item agoraphobia scale. Twenty-one of the 32 social phobia items assess the degree of distress in different social settings. Other social phobia items assess the degree to which respondents experience somatic and cognitive symptoms in social situations. Average administration time is between 20 and 30 min.

Beidel et al. [61] examined the correlation of the SPAI with daily social behavior of a clinic sample of social phobics. The results indicated that the SPAI provides a reasonable indication of the distress experienced during daily social encounters in three dimensions: behavior, cognitions, and overall distress. They also examined the validity of the SPAI with reference to the somatic response and avoidance behavior of social phobics. The results indicated that the somatic items of the SPAI are related to the somatic response of social phobics and that performance of the SPAI is associated with avoidance behavior in an anxiety-producing task.

C. Instruments Assessing the Obsessive-Compulsive Disorders

The Yale-Brown Obsessive Compulsive Scale [47] was designed to remedy the problems of existing rating scales by providing a specific measure of the severity of symptoms of obsessive-compulsive disorder that is not influenced by the type of obsessions or compulsions present. This instrument measures the severity of obsessive-compulsive symptoms in patients with obsessive-compulsive disorder. Both obsessions and compulsions are rated in terms of time spent, interference with functioning, distress, resistance, and control. This instrument is a clinician-administered interview, divided into two subscales: the obsessions subscale and the compulsions subscale. It takes approximately 10 to 15 min to administer the Y-BOCS.

The Y-BOCS is currently a standard for the assessment of obsessive-compulsive symptoms. It provides a good measure of overall obsessive-compulsive disorder severity and it is well suited for assessing changes with treatment. It can be used as a screening instrument, but it is not a diagnostic instrument because it does not specifically assess whether the diagnostic criteria for DSM-IV obsessive-compulsive disorder are met or not.

Goodman et al. [47] assessed the validity of the Y-BOCS and its sensitivity to change. Convergent and discriminant validity were examined in baseline ratings from three cohorts of patients with obsessive-compulsive disorder ($n = 81$). The total Yale-Brown Scale score was significantly correlated with two of three independent measures of obsessive-compulsive disorder and weakly correlated with measures of depression and anxiety in patients with obsessive-compulsive disorder with minimal secondary depressive symptoms. These results indicate that this is a reliable and valid instrument for assessing obsessive-compulsive disorder symptom severity.

The Leyton Obsessional Inventory [48] is a 69-item self-administered instrument designed to assess obsessive and compulsive symptoms among patients. First, the subject must answer each item, checking the presence or absence of obsessive or compulsive symptoms. Then the patient is asked to choose answers regarding the interference of the symptoms with daily life and the degree of the patient's resistance to each symptom. This instrument is often used in clinical settings, but it cannot be used as a screening instrument or in epidemiological studies.

The Leyton Obsessional Inventory was administered to 73 obsessive-compulsive patients by Murray et al. [62] and their responses were compared with those of 100 normal subjects. The ratio of the mean patient to normal scores ranged from 2.4:1 for obsessional traits and 3.2:1 for symptoms to 6.2:1 for resistance and 12.5:1 for interference with other activities. A principal components analysis on the patients' replies produced three unitary components (household order, personal contamination, and doubting plus two bipolar components (checking/parsimony and desire for closure/unpleasant ruminations). These components appeared to be quite stable and have also been identified in normal subjects, suggesting that obsessional neurotics differ from normal subjects quantitatively rather than qualitatively.

The Maudsley Obsessional Compulsive Inventory (MOCI) [49] is a 30-yes/no-item instrument assessing obsessional and compulsive symptoms such as checking, cleaning, slowness, and doubting. The psychometric characteristics and relationship among the Leyton Obsessional Inventory, the MOCI, and the Y-BOCS were examined in a sample of 30 obsessive-compulsive patients diagnosed using a structured interview [63]. The majority of the subscales of the various measures were found to have a good internal consistency across gender and the mean scores were similar to those reported in other studies.

D. Instruments Assessing the Post-Traumatic Stress Disorder (PTSD)

The Impact of Event Scale (IES) [50] is a 15-item self-report questionnaire originally developed to measure the psychological response to specific traumatic stressors or stressful life events. More precisely, it was intended to capture symptoms of intrusion and avoidance. Clinical, field, and experimental studies of potentially stressful life events give concordant findings: there is a general human tendency to undergo episodes of intrusive thinking and periods of avoidance. The IES can be employed with any stressful life events. This instrument is composed of two scales, the 7-item intrusion subscale and the 8-item avoidance subscale. Respondents rate how frequently they experienced each of the 15 IES symptoms during the prior 7 days on a 4-point scale. It is important to notice that the IES does not assess hyperarousal symptoms (e.g., irritability and exaggerated startle response) that are part of the DSM-IV diagnostic criteria for post-traumatic stress disorder. It takes about 5 to 10 min to administer the IES. Although the IES is not a diagnostic instrument, it provides a brief, reliable, assessment of intrusion and avoidance symptoms associated with post-traumatic stress disorder.

Sundin and Horowitz [64] reviewed studies that evaluated the psychometric properties of the IES. The results indicate that the IES's two-factor structure is stable over different types of events, that it can discriminate between stress reactions at different times after the event, and that it has convergent validity with observer-diagnosed post-traumatic stress disorder. The use of IES in many psychopharmacological trials and outcome studies is supportive of the measure's clinical relevance.

The Mississippi Scale (MSS) [51] is a 35-item self-report assessing the severity of trauma-related symptoms on the basis of the DSM-III criteria for post-traumatic stress disorder. It takes 5 to 20 min to complete the MSS. This instrument seems to be a useful self-report measure of baseline post-traumatic stress disorder principal symptoms. It is important to note that the MSS is not a diagnostic measure but provides an assessment of the severity of this specific anxiety disorder.

Lauterbach et al. [65] assessed the psychometric properties of the Civilian Mississippi Posttraumatic Stress Disorder Scale. The Mississippi is internally consistent (alpha

approximately = 0.89, split-half *r* approximately = 0.80), and it can discriminate between traumatized and nontraumatized respondents. However, its relationship with measures of PTSD was weaker than its relationship with measures of depression and anxiety, suggesting that it may be more of a general measure of distress.

The Clinician-Administered PTSD Scale [52] is a structured interview assessing the type and severity of DSM-III-R Post-traumatic stress disorder symptoms. The CAPS consists of 17 interviewer-rated items that cover the principal symptoms of PTSD according to the DSM criteria, which are reexperiencing the traumatic event, presence of intrusive thoughts, flashbacks, distressing dreams, avoidance of stimuli related to the trauma, numbing of general responsiveness, efforts to avoid trauma-related thoughts or situations, and restricted affect and increased arousal with sleep problems, irritability and hypervigilance. It takes approximately 45 to 60 min to administer this instrument. This scale could be used as a diagnostic instrument, as a measure of baseline severity of post-traumatic stress disorder and as a treatment outcome measure.

The Posttraumatic Stress Diagnostic Scale (PDS) [53] is a 49-item self-report scale developed to provide a reliable DSM-IV diagnosis of post-traumatic stress disorder and for quantifying the symptom severity. The score provides a profile of diagnosis, symptom severity, symptom description, and level of impairment. It takes 10 to 15 min to complete this instrument.

IV. INSTRUMENTS ASSESSING DEPRESSION

The goal of assessing depression is to measure the severity of depressive symptoms in terms of both the severity of individual symptoms and the total number of depressive symptoms that have been present. Most of the depressive self-rating scales could be used as screens and can indicate the presence of depressive symptoms. However, unless the screens were used in a patient with an earlier determined diagnosis of depressive disorder, the clinician would still need to completely assess the patient before diagnosing depressive disorder.

A. Self-Assessment Scales

The Beck Depression Inventory (BDI) [66] is a self-administered instrument developed to assess the behavioral manifestations of depression. This instrument provides a standardized assessment of depression severity in patients with previously diagnosed depressive illness. The BDI is one of the most used self-rating scales for measuring depression. A second use of the BDI is to screen patients who may have depressive illness and may require intervention, but in this case, the BDI should be followed up with a diagnostic instrument or a clinical interview. It takes 5 to 10 min to complete the BDI. Richter et al. [67] discussed validity aspects of the BDI on the basis of meta-analyses of studies on the psychometric properties. Shortcomings of the BDI are its high item difficulty, lack of representative norms, and thus doubtful objectivity of interpretation, controversial factorial validity, instability of scores over short time intervals (over the course of 1 day), and poor discriminant validity against anxiety. Advantages of the inventory are its high internal consistency, high content validity, validity in differentiating between depressed and nondepressed subjects, sensitivity to change, and international propagation.

The Center for Epidemiologic Studies Depression Scale (CES-D) [68] is a 20-item self-report instrument developed in order to measure symptoms of depression in commu-

nity populations. This instrument has also been used in many studies as a screen for the presence of depressive illness. The items assess the depressed mood, feelings of worthlessness, feelings of hopelessness, loss of appetite, poor concentration, and sleep disturbance. The scale does not include items for increased appetite or sleep, anhedonia, psychomotor agitation or retardation, guilt or suicidal thoughts.

Weissman et al. [69] presented data from psychiatric populations and a community sample on the CES-D. Results showed that the scale is a sensitive tool for detecting depressive symptoms and change in symptoms over time in psychiatric populations, and that it agreed quite well with more lengthy self-report scales used in clinical studies and with clinician interview ratings. Although a symptom scale cannot differentiate between diagnostic groups, the CES-D has demonstrated its validity as a screening tool for detecting depressive symptoms in psychiatric populations.

The Zung Self-Rating Depressive Scale (Zung SDS) [70] is a 20-item self-report instrument providing a measure of depression severity. Items are selected to tap affective, cognitive, behavioral, and physical aspects of depression. Although there is coverage for most DSM-IV symptom criteria, there is no item assessing psychomotor retardation or symptoms that are more common in atypical depressions such as increased appetite, weight gain, or hypersomnia.

Biggs et al. [71] assessed the validity of the ZSDS. This instrument correlated well (0.69) with the treating physician's global rating in 26 depressed outpatients during the 6 weeks of treatment with a tricyclic antidepressant. In a larger sample of 41 patients, a high correlation was found between the ZSDS and the Hamilton Rating Scale. The sensitivity of the ZSDS was found to be adequate. The scale was able to differentiate, at the 0.05 level, four severity groups classified on the basis of the global rating. This study indicated that the ZSDS is a valid and sensitive measure of clinical severity in depressed patients and supported its continued use as a research instrument.

This self-administered Befindlichkeits-Skala (BFS) [72] includes 28 pair of antonymous adjectives designed to assess mood. The BFS provides an assessment of mood variations. For each adjective pair, the patient is asked to choose between three answers. This scale is easy to complete and is not time consuming. It could be easily used in clinical settings.

B. Hetero-Evaluation Scales

The Hamilton Rating Scale for Depression (Ham-D) [73] is a 17-item interviewer-administered scale providing an assessment of the severity of depressive symptoms in patients with primary depressive illness. This instrument is also useful for monitoring changes in depressive symptoms with treatment. The Ham-D is probably the most commonly used observer-rated depressive symptom rating scale. It takes about 15 to 20 min to administer the Ham-D. It is important to note that because this instrument was developed before the DSM-III and the Research Diagnostic Criteria, it does not include symptoms like anhedonia, and reverse neurovegetative symptoms (oversleeping and overeating), and thus may underestimate depressive severity in patients with atypical features. Several other versions of this instrument (21 items, 23 items, and 26 items) are also available.

Ratings of 54 English and 52 Swedish patients on a 65-item comprehensive psychopathology scale were used to identify the 17 most commonly occurring symptoms in primary depressive illness in the combined sample. Ratings on these 17 items for the patients participating in studies of four different antidepressant drugs were used to create a depres-

sion scale consisting of the 10 items that showed the largest changes with treatment and the highest correlation to overall change. This new scale was the Montgomery-Asberg Depression Ratings Scale (MADRS) [74].

MADRS is a 10-item interviewer-administered scale designed to assess the overall severity of depressive symptoms in patients with depressive disorder. The main goal of this instrument is to assess treatment changes with good sensitivity. It takes approximately 15 min to administer the MADRS. The inter-rater reliability of this scale is high. Scores on the scale correlate significantly with scores on a standard rating scale for depression, the Hamilton Rating Scale for Depression (Ham-D), indicating its validity as a general severity estimate. Its capacity to differentiate between responders and nonresponders to antidepressant treatment is better than the Ham-D, indicating greater sensitivity to change.

Davidson et al. [75] assessed the reliability and the validity of the MADRS in 44 depressed inpatients. All items on the scale occurred frequently in the sample; the scale exhibited construct validity (internal homogeneity) and concurrent validity relative to the Hamilton Depression Scale and the concepts of endogenous and nonendogenous depression. Sleep disturbance, reduced appetite, and suicidal thoughts correlated poorly with the remainder of the scale. Finally, inter-rater reliability was demonstrated between a psychiatrist and a nurse on individual item and total scores.

The Scale of Psychomotor Retardation [76] assesses only one dimension of depression, that is, psychomotor retardation, whereas most of the instruments provide an assessment of several dimensions of this mood disorder. This interviewer-rated 14-item instrument describes the motor, verbal, hedonic, and cognitive behavior of the depressed patient. This scale could be used in assessing the efficacy of antidepressant drugs.

The CORE system [77] is an 18-item clinician-rated instrument assessing psychic and motor signs in depressed patients. Retardation, agitation, and lack of interaction are the three main dimensions of the CORE System. Each item is scored 0 to 3, with a total score of 54. A single cut off score of 8 is used to allocate patients to either a "melancholic" or "nonmelancholic" class.

The Bech-Rafaelsen Melancholia Scale [78] is an 11-item interviewer-administered instrument providing an assessment of cognitive and somatic symptoms in depressed patients. This scale is derived from the Hamilton 17-item scale and from the Cronholm-Ottoson Depression Scale. This instrument should be administered at a precise time, in the morning, between 8:00 and 9:30 AM. It takes approximately 15 to 30 min to complete this instrument. The Bech-Rafaelsen Melancholia Scale seems to be a good instrument in assessing the depressive disorder, among in- or outpatients. It could also be used in pharmacological and epidemiological studies. It is important to note that even though the name of this scale refers to melancholia, this instrument could be used among patients with minor, nonmelancholic depression.

Finally, depression scales developed for use in special populations are also available. We are going to describe one scale usually used in postpartum women, one used in elderly people, and one used in schizophrenic patients.

The Edinburgh Postnatal Depression Scale [79] was designed to identify patients with postpartum depression. This instrument is a 10-item self-report questionnaire providing a probable diagnosis of depression on the basis of Research Diagnostic Criteria (RDC). It takes approximately 10 min to complete this instrument. This scale seems to be well accepted and it appears to be helpful for aiding general practitioners in detecting major and minor depressive disorder occurring in the postpartum period. It also has the advantage that many usual somatic features in postpartum women, including changes in sleep and

energy, are not scored as pathological. In addition to performing as a screening tool, the EPDS measures the severity of depression.

The Geriatric Depression Scale (GDS) [80] was developed to assess depression in geriatric populations. Most of the currently available symptom scales were developed and validated in samples of medically healthy younger adults. However, it is important to notice that sleep difficulties, decreased energy, and decreased libido are frequently found in nondepressed elderly persons. Thus, there is a need to use a different set of symptom descriptors when assessing depression in geriatric populations. In constructing the GDS, a 100-item questionnaire was administered to normal and severely depressed subjects. The 30 questions most highly correlated with the total scores were then selected and readministered to new groups of elderly subjects. These subjects were classified as normal, mildly depressed, or severely depressed on the basis of Research Diagnostic Criteria (RDC). The GDS, Ham-D, and SDS are all found to be internally consistent measures, and each of the scales was correlated with the subject's number of RDC symptoms.

The GDS was designed to provide a reliable screening test for depression that would be easy to score and simple to administer. The items reflect a variety of symptoms relevant to depression, including lowered mood, poor self-image, poor motivation, past versus future orientation, cognitive problems, obsessive traits, and agitation. This instrument is a 30-item self-report scale used to screen for depressive illness in geriatric patients and also used to assess the severity of depression for monitoring change over time or with treatment. The authors suggest that the GDS represents a reliable and valid self-rating depression screening scale for elderly populations.

Lyons et al. [81] assessed the reliability, validity, and temporal stability of the Geriatric Depression Scale (GDS) in 69 elderly patients who underwent surgery for broken hips. The GDS demonstrated internal consistency, reliability, and concurrent validity with the Hamilton Depression Rating Scale. In addition, the GDS was stable across the hospital stay and thus appeared to be less influenced by the patients' acute health status.

The Calgary Depression Scale for Schizophrenia (CDSS) [83] was developed to assess symptoms of major depressive disorder in patients with schizophrenia. Rating scales developed to assess depressive symptoms have questionable validity in schizophrenic patients. The CDSS proposes to address this problem. This instrument consists of nine items: depressed mood, hopelessness, self-deprecation, guilty ideas of reference, pathological guilt, depression worse in the morning, early wakening, suicide, and observed depression. The items on the CDSS are all typical depressive symptoms and do not appear to overlap with the negative symptoms of schizophrenia. These items are selected from the Hamilton Depression Rating Scale and the Present State Examination. The time frame is typically 2 weeks prior to the interview. It takes 15 to 20 min to administer this instrument. The CDSS is potentially useful in screening patients with schizophrenia to identify those with possible major depressive disorder. This instrument is also useful in monitoring the response of depressive symptoms to treatment.

V. CONCLUSION

The first step when choosing an instrument in psychopathology is to determine whether one intends to use the instrument for diagnosis or to evaluate the severity of a given disorder. If the main aim of the evaluation is diagnosis, a categorical instrument is suitable. On the other hand, if the severity of the disorder is being assessed, a dimensional instrument is appropriate. Next, one must consider who will administer the instruments. Certain

instruments can only be used by psychiatrists or clinical psychologists (e.g., SADS), whereas others (e.g., CIDI or DIS) can be used by trained lay interviewers.

Clinical studies, including randomized controlled trials, often rely on specialized instruments that can only be administered by psychiatrists or clinical psychologists. Epidemiological studies, however, make use of instruments that can be used by trained lay interviewers. Finally, some instruments are particularly suitable for screening of patients in the primary care setting (PRIME-MD).

In case of dimensional evaluations, certain instruments are intended for evaluation of general psychopathology, including anxiety (e.g., GHQ, SCL90R), whereas others assess only general anxiety (e.g., BAI, HAD). Another group of instruments evaluate specific dimensions of general anxiety (e.g., Fear Questionnaire, PSWQ). A final group of dimensional instruments (e.g., PASS, PDSS, Y-BOCS) assess specific anxiety disorders such as panic disorder or obsessive-compulsive disorder.

The use of standardized instruments in clinical practice can improve the collection, synthesis, and reporting of information as compared with the use of unstructured clinical examinations. Standardized assessments can ensure that relevant topics are covered in appropriate depth and decrease the likelihood of omitting important domains of information. These instruments also allow categorization and quantification of symptoms according to an agreed-upon system and provide consistency of assessments across examiners.

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7

Combining Psychotherapy and Pharmacotherapy for Depression and Anxiety

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I. INTRODUCTION

Depressive and anxiety disorders are clinically and neurobiologically heterogeneous conditions [1,2]. One consequence of heterogeneity is that it is implausible that a single type of treatment will be universally effective. Because depression and anxiety often occur at times of grief or life stress; affect how the sufferer feels about his or her self, world, past, and future; and disproportionately afflict those with histories of early trauma or personality difficulties, psychotherapy has long been considered a reasonable and useful treatment [3]. Since the 1950s, however, many drug therapies have been studied and are now commonly used in the treatment of depression and anxiety [4,5]. Moreover, during the past 20 years, greater emphasis has been placed on the theoretical and clinical development of time-limited structured psychotherapies that are specifically tailored toward core psychopathologies found in the depressive and anxiety disorders [6–8], although the specificity of different psychotherapies has been debated [9]. Given the wide variety of effective psychotherapies and drug therapies that are currently available for treating depression and anxiety, a clinically and scientifically important question is whether the combination of psychotherapy and pharmacotherapy is more effective than either treatment modality alone. In this chapter, we will review the use of combination psychotherapy and pharmacotherapy for depression and anxiety, discuss some of the clinical and methodological problems with these studies, and suggest directions for future research in this area.

II. REASONS FOR COMBINING PSYCHOTHERAPY AND PHARMACOTHERAPY

Various psychotropic drugs, especially antidepressants, have been found to be effective not only in the treatment of depression [10], but also in the treatment of different anxiety disorders, including panic disorder with or without agoraphobia [11], generalized anxiety disorder [12], obsessive-compulsive disorder [13], social anxiety disorder [14], and posttraumatic stress disorder [15]. In addition, interpersonal psychotherapy (IPT), cognitive behavioral therapy (CBT), and various forms of behavioral therapy (BT) are effective treatments for depression [6]. Modified forms of CBT and BT have been better studied than IPT for anxiety [16–18], and they have been found to be effective in the treatment of different anxiety disorders [7], including panic disorder [19], generalized anxiety disorder [20], obsessive-compulsive disorder [21], social anxiety disorder [22], and posttraumatic stress disorder [23].

Despite the effectiveness of drug therapy, patients may experience residual symptoms, may not be fully compliant in taking medication, may have deficient interpersonal and problem-solving skills, may have underlying maladaptive personality or temperamental traits, and may face ongoing life stressors [24,25]. Most patients and psychiatric practitioners also strongly prefer the use of psychotherapy to drug therapy [26–28]. Combining psychotherapy and pharmacotherapy for the treatment of depression and anxiety is commonly done in clinical practice and often is recommended by clinical practice guidelines [29–31]. Together with drug therapy, the use of psychotherapy can instill hope and reestablish patient morale, increase levels of social and physical activity, help develop appropriate coping behaviors, address medication noncompliance, mobilize psychosocial resources, and provide practical psychosocial skills development [32].

Although the majority of patients will clearly show clinical improvement with pharmacotherapy or psychotherapy alone, only a significant minority will experience a full remission with treatment [33]. Because psychotherapy and pharmacotherapy likely have different mechanisms of action, combining them might have additive or synergistic therapeutic effects that lead to a greater overall clinical outcome [6,34,35]. Drug therapy is more likely to target underlying biological abnormalities and to improve somatic symptoms [4,5]. Psychotherapy is more likely to affect psychological and cognitive processes and to improve psychosocial functioning [30,36,37]. However, successful drug therapy is associated with concomitant improvements in psychological and psychosocial functioning [38]. Successful psychotherapy has also been associated with normalization of abnormal regional brain metabolism [39,40]. Thus, pharmacotherapy and psychotherapy each may have primary and secondary effects on the underlying biological pathophysiology of depression and anxiety, as well as associated psychosocial impairment, which may reciprocally enhance and complement their therapeutic effects [41,42].

There is also some evidence that the clinical effects of pharmacotherapy may work more quickly, whereas the effects of psychotherapy may be delayed but more durable over time [43,44]. For example, antidepressants have been shown to have more rapid therapeutic benefits compared to psychotherapy [45,46]. By contrast, several studies reported that the therapeutic benefits of psychotherapy were more durable after treatment discontinuation than those observed in depressed patients withdrawn from antidepressants [47–50]. As a result, short- and long-term clinical effects of combining psychotherapy and pharmacotherapy may complement each other.

Drug therapy may facilitate the use of psychotherapy in other ways [51]. Because

the use of psychotherapy depends on adequate cognitive functioning (e.g., concentration, verbal comprehension, and memory), severe depression and anxiety states may be associated with impaired neuropsychological functioning, making it difficult for a patient to use psychotherapy effectively [52]. By improving underlying neuropsychological functioning, drug therapy may improve the cognitive skills necessary to engage in psychotherapy. Also, psychotherapy may facilitate adherence to pharmacotherapy, especially early in treatment when side effects are more likely to occur before substantial clinical improvement [53]. For example, in a recent study of chronic depression, subjects treated with a combination of psychotherapy and pharmacotherapy were less likely to drop out of treatment because of adverse drug effects compared to subjects treated with pharmacotherapy alone [46]. In addition, another recent study of bereavement-related major depression found that subjects receiving combined treatment were less likely to drop out compared to subjects receiving psychotherapy, pharmacotherapy, or placebo [54].

III. EFFICACY OF COMBINED TREATMENT FOR DEPRESSION AND ANXIETY

Previous reviews of studies comparing psychotherapy and pharmacotherapy in the treatment of depressive and anxiety disorders have concluded that the two modalities were generally comparably effective [6,7], although these studies and their conclusions have been criticized [55]. Most studies investigating the efficacy of combined treatment have been conducted in depressive rather than anxiety disorders.

A. Depressive Disorders

One of the earliest studies on depression contrasted IPT and amitriptyline, each alone and in combination, against a minimal-contact control group [56]. Not only did the effects of both IPT and amitriptyline surpass those of the minimal contact control group, but there also was a small additive effect favoring the group receiving both active therapies, suggesting that each modality has unique or complementary therapeutic effects. However, most other early studies of psychotherapy and antidepressant combinations failed to demonstrate statistically significant additive effects [57–61]. More recently, a research group studying late-life major depression found that approximately 80% of subjects showed a full response during open treatment with the combination of IPT and the antidepressant nortriptyline [62], a response rate significantly higher than is usually seen in antidepressant treatment studies. This group has also compared IPT and nortriptyline, singly and combined, in a group of older adults with grief-related major depressive episodes [54]. The investigators found a significant effect for nortriptyline (versus placebo), but not for IPT (versus placebo). They observed several trends suggesting an advantage for the IPT and nortriptyline combination versus nortriptyline alone, although these modest effects were not statistically significant because of the small sample sizes of the treatment groups.

Surprisingly, newer generation antidepressants have not been well studied in combination with psychotherapy. Recently, Ravindran et al. [63] compared group CBT and sertraline, each alone and in combination, in a placebo-controlled multicenter study of dysthymic disorder. Sertraline was significantly more effective than placebo or group CBT, whereas group CBT was not significantly more effective than placebo. Several nonsignificant trends suggested an additive effect for group CBT and sertraline, but the study was too small to determine if this modest advantage was clinically significant.

Thase et al. [64] reported the results of a pooled analysis of a series of 595 depressed outpatients treated with psychotherapy alone (using CBT or IPT) or the combination of IPT and tricyclic antidepressants. Patients receiving combined therapy had a 14% greater remission rate than those treated with psychotherapy alone. Moreover, there was a large difference (41%) in the subgroup of patients with more severe, recurrent depressive episodes. These results support the general finding that psychotherapy and pharmacotherapy have modest additive effects for a broad range of depressed patients and a large advantage for selected subgroups (e.g., more severe depressions).

A large multicenter, randomized clinical trial evaluated the cognitive behavioral analysis system of psychotherapy (CBASP), which is a modified form of CBT, and the antidepressant nefazodone, singly and in combination, in 681 patients with chronic major depression [46]. Nefazodone and CBASP were each associated with significant reductions of depressive symptoms after 12 weeks of treatment, but the combination group had significantly greater proportions of responders and fully remitted patients. Nefazodone alone and combined with CBASP was more rapidly effective than CBASP alone. Subjects treated with combination therapy appeared to benefit from both the more rapid effects of nefazodone and the slower emerging benefits of CBASP. Patients receiving combination therapy also were less likely to relapse during 16 weeks of continuation treatment compared to CBASP or nefazodone alone [65].

Two controlled studies have examined the efficacy of IPT combined with the antidepressants imipramine [66] or nortriptyline [67] for prevention of recurrent major depression. In both studies, sustained remission was initially achieved during acute treatment with the combination of IPT and medication. Following 4 months of continuation therapy, the next stage of treatment was random assignment to maintenance pharmacotherapy, psychotherapy, or a combination of both. In the first study [66], patients receiving imipramine alone or combined with IPT had significantly better outcomes compared to placebo, IPT alone, or IPT plus placebo during 3 years of maintenance treatment. There was no advantage for the patients who received combined treatment. In the second study [67], older patients receiving nortriptyline alone, IPT alone, or combination therapy had significantly better outcomes compared to placebo during maintenance treatment. This study, however, found an additive effect in the group receiving the combination of IPT and pharmacotherapy.

Several studies have examined the sequential use of combined psychotherapy and pharmacotherapy after an initial monotherapy treatment trial of depression. These studies suggested that adding CBT or IPT to pharmacotherapy after completion of acute-phase pharmacotherapy alone leads to better clinical outcomes [68–70]. A recent study in women with recurrent major depression found that adding pharmacotherapy in patients who did not fully respond to an initial course of IPT alone resulted in a greater remission rate (79%) compared to those patients who had received combined treatment initially (66%) [71]. Moreover, some studies have found that the sequential addition of psychotherapy after an initial course of pharmacotherapy not only improves residual depressive symptoms, but also will improve psychological symptoms and psychosocial functioning [70,72]. Hence, combined therapy may be especially useful for patients who have shown a partial response to psychotherapy or pharmacotherapy alone [32].

Mood-stabilizing medications are considered the mainstay of treatment for bipolar disorder, but there is developing evidence that the use of specific psychosocial interventions together with pharmacotherapy may lead to a better clinical outcome [73]. For example, family-focused psychoeducational treatment was effective in reducing relapses and

improving depressive symptoms in bipolar disorder [74]. In addition, modified forms of IPT and CBT have been shown to improve compliance, reduce depressive symptoms, and increase psychosocial functioning [75–79].

B. Anxiety Disorders

Studies comparing psychotherapy and pharmacotherapy in the treatment of anxiety disorders have generally found that the two modalities are effective [7], although there has been some debate about their relative efficacy [55]. Compared to depression, however, the use of combined treatment for various anxiety disorders has not been as well studied [7,43].

Panic disorder with or without agoraphobia can be effectively treated either with drug therapy, typically antidepressants, or with psychotherapy, usually some form of CBT or BT such as exposure therapy [30]. Earlier meta-analyses of treatments for panic disorder did not find substantial or consistent evidence that combined therapy is better than psychotherapy or pharmacotherapy alone [80–82], but various methodological problems with these studies prevent any definitive conclusions [55]. More recently, a large multicenter randomized double-blind placebo-controlled clinical trial evaluated CBT and the antidepressant imipramine, singly and in combination, in 312 patients with panic disorder [83]. This study found that imipramine and CBT alone were significantly better than placebo on most measures during acute and maintenance treatment. There was a trend for greater improvement with combined therapy compared to CBT or imipramine alone during acute treatment, but combined therapy showed a substantial advantage by the end of maintenance treatment. Studies with newer generation antidepressants such as paroxetine [84] and fluvoxamine [85] have found that combined therapy may be more effective than psychotherapy alone. Similar to studies in depression, there is developing evidence that adding psychotherapy to pharmacotherapy may be useful for treating residual symptoms [86] or preventing relapse [7,87] in panic disorder. Finally, studies of agoraphobia have found that the combination of imipramine and exposure BT is superior to either therapy alone [7].

Generalized anxiety disorder can be effectively treated with various drug therapies, including antidepressant drugs, or with psychotherapy, usually CBT [20,12]. Psychotherapy may also successfully treat comorbid conditions commonly found in generalized anxiety disorder [88]. Unfortunately, there are no systematic studies comparing the efficacy of combined therapy to psychotherapy or pharmacotherapy alone [7,89].

Obsessive-compulsive disorder can be effectively treated either with drug therapy (i.e., clomipramine or a serotonin reuptake–inhibiting antidepressant) or with some form of CBT or BT such as exposure and response prevention therapy [13,90,91]. An earlier meta-analysis of treatments for obsessive-compulsive disorder suggested that combined therapy is better than psychotherapy or pharmacotherapy alone [21], but the methodology of this analysis has been criticized [55]. Other recent studies have not found substantial or consistent evidence that combined therapy is superior [92,93], although the study by Hohagen et al. [94] suggested that obsessional and depressive symptoms may improve more with the combination of BT and the antidepressant fluvoxamine.

Social anxiety disorder can be effectively treated with various drug therapies, primarily antidepressants, or with psychotherapy, usually CBT [14,22]. Although there are no systematic studies comparing the efficacy of combined therapy to psychotherapy or pharmacotherapy alone, the differential short-term and long-term effects of pharmacotherapy and psychotherapy have suggested that they may have especially useful synergistic

effects when combined [22,95]. Also, given the substantial comorbidity found in social anxiety disorder [96], combination treatment may lead to a better overall clinical outcome.

The treatment of post-traumatic stress disorder has not been as extensively investigated as depression and other anxiety disorders, although recent studies have shown significant benefits with the use of antidepressants [15] and different forms of CBT, especially exposure therapy [23]. Because of the diverse and complex symptoms often found in post-traumatic stress disorder [97], reflecting underlying psychological and neurobiological processes, it is likely that combining psychotherapy and pharmacotherapy will be more effective than either therapy alone, but this has not been systematically studied [98,99].

C. Conclusions and Recommendations About the Use of Combined Treatment

The research studies reviewed here generally support the conclusion that combined treatment is at least as effective as psychotherapy or pharmacotherapy alone in the treatment of depression. This contradicts the notion that pharmacotherapy might interfere with the effects of psychotherapy or vice versa [51]. Additive effects have been observed in some of the studies evaluating psychotherapy and pharmacotherapy combinations, but the largest effects have been shown in patients with more severe and chronic illnesses. Surprisingly, virtually all studies of combined therapy have used older tricyclic antidepressant drugs. Additional studies investigating the use and effectiveness of combined therapy with newer generation medications are needed.

There also is some developing evidence that partial responders to psychotherapy or pharmacotherapy alone will improve by using combined treatments. These findings suggest that the sequential strategy of combining treatments only after failing an adequate monotherapy might be more efficacious and cost effective than using combination therapy for all patients at the beginning of treatment. This issue deserves further study, especially examining different sequence patterns of combined treatments (e.g., pharmacotherapy added to psychotherapy versus psychotherapy added to pharmacotherapy) as well as examining the efficacy of different treatment components (i.e., different types of medications and different forms of psychotherapy). These studies also need to specifically and prospectively investigate possible clinical and biological predictors of treatment response.

Because medications may work more quickly, whereas psychotherapy may have more delayed but sustained effects, it is possible that combination therapy might have greater long-term benefits. Combination therapy has not been as well studied during long-term treatment, however, but combined treatment can reduce the risk of recurrent depression in older patients. In addition, psychotherapy can not only target mood and anxiety symptoms, but also improve psychological and psychosocial functioning. Hence, there is a need to study further the long-term effects of combined treatment not only on illness symptoms and recurrence risk but also on psychosocial functioning. Along with this, the overall cost effectiveness of combined treatment compared to psychotherapy or pharmacotherapy alone can be assessed [9,100].

The failure to demonstrate a more significant advantage with combined treatment may be due to the relatively small sample sizes of most studies. In addition, age, gender, type of treatment, and diagnostic subgroups might also be potential confounding factors in studies comparing the effects of psychotherapy, pharmacotherapy, and their combination. For example, younger and older depressed cohorts respond differently to different types of antidepressant drugs [101,102]. Men and women also have differential response

rates to different types of antidepressants and different forms of psychotherapy [103,104]. Response rates to medications and psychotherapy may also vary according to depressive subtype [45,105–108].

Another potential problem in treatment studies of combined therapy is the adequacy of pharmacotherapy or psychotherapy. The adequacy of pharmacotherapy is relatively easy to quantify (e.g., dose, duration, and compliance). Recent efforts at quantifying the adequacy of psychotherapy have found that the quality of therapy [109], therapeutic alliance [9,110], and investigator allegiance [111] may all affect the response to psychotherapy. It is also likely that psychotherapy and pharmacotherapy engender different therapeutic expectations as well [112–114], which might affect treatment outcome.

Compared to depression, the use and effectiveness of combination therapy has not been as well studied in anxiety disorders. Studies of panic disorder have provided the strongest evidence that combination therapy may be superior to psychotherapy or pharmacotherapy alone. There is clearly a need for additional research in other anxiety disorders, as well as studies investigating different drug and psychotherapy combinations, the use of sequential treatment strategies, clinical and biological predictors of treatment response, and the long-term effects of combination therapy on symptomatic and psychosocial outcomes. Moreover, because anxiety affects the treatment response to pharmacotherapy and psychotherapy in major depression [115,116], formal combined treatment studies of comorbid depression and anxiety are needed.

Based on this review, not all patients with depression and anxiety will necessarily require or greatly benefit from combined treatment using pharmacotherapy together with a rigorous course of psychotherapy (such as IPT or CBT). Several particular subgroups of patients might be more likely to benefit from combined treatment, especially those with more severe [64], chronic [46,117], or treatment-resistant [32] depression and anxiety. The presumed benefit in reducing the high level of suffering and disability in these patients is more likely to justify the added cost and expenditure of limited therapists' time in providing combined treatment. The choice of treatment, however, will ultimately depend on patient and provider preferences as well as the availability of appropriately trained psychotherapists and pharmacotherapists.

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8

Genetics of Depression

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The hereditary nature of emotional behavior was recognized even in ancient times. In particular, specific features of temperaments were observed to run in families. Thus, immediately after the introduction of the modern concepts of affective and emotional disorders by Falret and later by Kraepelin, familial etiology was discussed and investigated: for more than 100 years it has been evident that at least some subtypes of manic depressive disorders were familial and genetic. Although the first family and twin studies were performed without standardized and criteria-based diagnoses in the absence of structured interviews, their results were clearly replicated in subsequent methodologically more refined and valid studies. Currently, the field is intensively searching for DNA variants that predispose to mood disorders with only limited success.

I. GENETIC EPIDEMIOLOGY OF MOOD DISORDERS

A. Familial Clustering

For many years, family studies dominated the research field of genetics of affective disorders. Numerous studies demonstrated the familial nature of unipolar depression and bipolar disorder (Table 1).

Overall, first-degree relatives of probands with unipolar depression are at an increased risk for unipolar depression; the mean relative risk

Table 1 Proportion of First-Degree Relatives of Bipolar (BP) and Unipolar Depressive (UP) Disorder Probands with a Lifetime History of Bipolar and Unipolar Depressive Disorder

	Sample			Rates/100 Subtype in relatives by proband subtype					
Refs.	Disorder	Probands (*n*)	Relatives (*n*)	BP–BP	BP–UP	UP–BP	UP–UP	Controls–BP	Controls–UP
	BP	54	411						
2	UP	16	113	19.6	11.5	11.0	20.1	0.2	0.5
	Controls	75	619						
	BP	130	739						
3	UP	30	166	21.9	2.6	8.0	3.0	0.4	5.7
	Controls	43	265						
4	UP	133	810	—	—	1.5	3.2	1.5	4.1
	Controls	82	591						
	BP	100	230						
5	UP	225	500	21.2	1.9	11.9	2.3	0.2	4.8
	Controls	160	543						
	BP	80	504						
6	UP	108	306	7.1	17.1	1.3	19.0	0.9	7.7
	Controls	80	221						
			Total rates/100	6.2	12.2	2.2	13.6	0.6	3.8
			Relative risk (vs. controls)	10.3	3.2	3.5	3.6		

Ratio weighted for sample size.
Source: Ref. 1.

$$\lambda = \frac{\text{risk among first-degree relatives}}{\text{risk among controls}}$$

is 1.9 [7]. The degree of familial aggregation of bipolar disorder is substantially stronger with a mean relative risk of 7.9 [8]. No gender effect was found for these relative risks. Specifically, a continuum mode of familial genetic liability to unipolar depression using gender-specific thresholds to explain the higher prevalence in females (a higher liability for males is requested for expressing the disease resulting in reduced prevalence) has been ruled out [9].

What is the familial relationship between unipolar depression and bipolar disorder? There is consistent evidence across a large number of family studies that unipolar depression is also more common among relatives of probands with bipolar disorder than expected from general population prevalence rates; relative risks for unipolar depression among relatives is very similar between probands with unipolar depression and with bipolar disorder (relative risk of 1.9). On the other hand, most family studies in probands with unipolar depression did not find elevated risks for bipolar disorder among biological relatives, although there are a minority of family studies reporting a substantially increased risk for bipolar disorder among relatives of probands with unipolar depression [5,10,11]. It is difficult to decide if these latter studies are false positives. Therefore, it is difficult to decide if

1. Unipolar depression and bipolar disorder are located on a familial genetic continuum of liability in a two-threshold model. Bipolar disorder has a stronger familial genetic determination transmitting the risk for both disorders (higher threshold in the model); however, unipolar depression, with the reduced familial risk, is also a familial condition, with a lower familial risk for bipolar disorders (with a lower threshold in the model) [12: 106]; an increased risk for bipolar disorder in families of probands with unipolar depression can be expected from this model, but is difficult to be detected because of the low prevalence rate of bipolar disorder.
2. Unipolar depression is etiologically heterogeneous with only a small minority of cases carrying an increased risk for bipolar disorder: a smaller subgroup of unipolar depression in the familial context of bipolar disorder versus a larger subgroup of unipolar depression in absence of a familial relationship to bipolar disorder.

Unfortunately, the currently available body of evidence does not prove either possibility.

B. Genetic Determination

Unipolar depression has been most widely investigated by twin studies (Table 2) in both hospital- as well as population-based samples. All published twin studies (population- or hospital-based) on unipolar depression suggest a genetic influence ranging between 30 to 50% with a mean of 37% [7]. The degree of heritability is comparable between males and females. Thus, sex-specific genetic factors cannot explain the higher prevalence of depression among females. A reduced female–male to same-sex concordance in dizygotic twins suggests that genes may exist that act differently on the risk of depression in males and females [24].

Table 2 Twin Studies in Bipolar Disorder and Unipolar Depression

Disorders	Refs.	Sample H/C—country[a]	Sample size MZ pairs	Sample size DZ pairs	Concordance MZ	Concordance DZ
Bipolar disorder	13	H-Norway	6	—	67%	—
	14	H-U.S.	5	15	20%	0%
	15	H-Denmark	34	37	62%	8%
	16	H-Norway	4	6	75%	0%
	17	H-Sweden	13	22	39%	5%
	18	H-U.K.	22	27	36%	7%
Unipolar depression	19	H-Sweden	57	157	41%	27%
	19	C-Sweden	97	169	54%	33%
	20	H-U.K.	68	109	46%	20%
	21	C-U.S.	1874	1498	23%	14%
	22	C-Australia	1323	1339	42%	35%
	23	C-U.S.	1368	2422	39%	30%

[a] Sample ascertained from Hospital (H), Community (C) population.

The majority of twin studies on unipolar depression further decomposed the nongenetic component into a component shared by both twins and an individual-specific component. Consistently across all studies the individual-specific component was substantially stronger than the shared environmental component, and exceeded the impact of the genetic component by magnitude; in most studies the shared environmental component was negligible in the model-based variance analysis [20,25,26].

As a limitation, the individual-specific environmental component also includes variance because of the measurement error that might be substantial in lifetime assessment of depressive episodes (because of memory problems). Twin studies with a follow-up component (two distant time points of clinical lifetime assessments) can estimate the relative impact of the measurement error; using this strategy, Kendler et al. [27] extracted the variance emerging from measurement error by a model-based analysis of heritability on the remaining variance in the Virginia twin study in females. As a result, the magnitude of the individual-specific environmental component decreased considerably, and the estimate for the heritability was doubled and raised to 70%.

In contrast to unipolar depression, the number of twin and adoption studies on bipolar disorder is relatively small; however, results are more equivocal and genetic factors define most of the proportion of variance. The heritability of bipolar disorder as evidenced in twin studies was estimated as 0.59% [8]. Given the overwhelming evidence of a major genetic component, bipolar disorder became the first psychiatric disorder to be submitted to linkage analysis 30 to 40 years ago.

Adoption studies are another tool used to demonstrate genetic determination of a disorder. This method received little attention during the past decade and available samples are small. The two major adoption studies of bipolar disorder found an approximately three-fold increased risk of bipolar disorder among biological compared to adoptive relatives of probands [28,29]. The results of two adoption studies conducted on unipolar depression yielded evidence for significantly increased rates of mood disorders after adoption [29,30]. In contrast, a third study in unipolar depression did not find evidence for increased

rates of mood disorders among biological compared to adoptive relatives of adopted mood disorder probands [31]; however, this study suffers from methodological insufficiencies (no personal interviews).

C. Modifiers of Familial Genetic Influences on Unipolar Depression

Severity and course of mood disorders is interindividually variable, stimulating speculations on a familial genetic influence. Thus, three study questions have been explored:

1. Familiality of and genetic influence on severity of symptoms.
2. Familiality of and genetic influence on age at onset.
3. Familiality of and genetic influence on recurrency or chronicity of the course of the disease.

Heritability to unipolar depression apparently varies along a dimension of severity indexed by number of symptoms and degree of impairment, with more severe cases carrying a stronger genetic component: A recent, population-based telephone interview twin study proposed that depression with mild or only modest degree of severity is under genetic control [21]. This conclusion is supported by prior twin studies [16], but not by another large population-based twin study [27,32]. A hospital-based twin study argues for a universal genetic influence on unipolar depression [20]; given that hospital care is delivered to more severe cases, this observation is compatible with the variation of the magnitude of the genetic component along a dimension of severity in population-based studies. On the other hand, low severity of depression is likely to be correlated with under-reporting and measurement error; thus, heritability of low-severity depression might be underestimated. A series of family studies proposed a reciprocal relationship between age at onset across the lifespan and degree of familial loading: (1) it has been demonstrated that late-onset depression goes together with a lower familial risk [33–35]; (2) however, it remains unclear if this reciprocal linear relationship between age at onset and familial loading also extends to childhood and adolescence.

Twin studies with a high proportion of young adults provide no conclusive evidence for this relationship [32:21]. Assuming the inverse linear relationship, a higher familial loading among adolescent probands with depression would be the consequence. This assumption is supported by a recent German study in adolescents using the family history method yielding the maximal relative risk in relatives (parents) for unipolar depression: the relative risk for depression in parents of depressed probands was increased by an odds ratio of 2.7 (with confidence interval 2.1–3.5) [36]. However, Klein et al. [37], using the more valid and reliable family study method reported an odds ratio of 1.8 for the risk, which is not higher than the mean relative risk found in the family studies in adults. Some prospective studies in adolescents were also unable to confirm a stronger familial genetic impact on early-onset depression [38,39].

Two reasons might contribute to the inconsistencies across studies with regard to the impact of early age at onset:

1. The concept of early-onset depression might be inappropriate: genetic influence was observed to be stronger in adolescent than in prepubertal depression, with the latter showing a pattern of familial clustering that is similar to adult-onset cases [40,41].
2. Greater heterogeneity in early-onset cases: prospective studies demonstrated a lack of longitudinal specificity of unipolar depression (in contrast to bipolar

disorder); early-onset unipolar depression is strongly intraindividually associated with anxiety disorders and often subsequently develops into pure anxiety disorder [41]; however, Klein et al. [40] did not observe greater familial risk in adolescent depression.

In sum, the concept of early-onset major depression needs further familial genetic study before early onset can be considered as an indicator of greater familial genetic risk. A relationship between increased familial genetic risk and early onset has not yet been convincingly established because of the lack of sufficient study.

Some studies proposed a linear relationship between recurrence and familial risk in unipolar depression [32,42]. Other factors predictive for the familial variant of unipolar depression are longer duration of episodes and recurrent thoughts of death and suicide [32].

In bipolar disorder, a differential familial genetic background of early versus late-onset subtypes was proposed and empirically supported [43]. In addition, intrafamilial association of the age at onset in bipolar disorder proposes direct or indirect genetic influence [44].

D. Subtypes of Affective Disorders

The search for disease genes in some complex disorders took an enormous advantage of more homogeneous clinical subgroups with greater genetic risk and Mendelian modes of transmission (e.g., Alzheimer's disease and breast cancer with early-onset cases defining those homogeneous groups).

Classic clinical concepts of unipolar depression proposed a dichotomy between two subtypes: one being biologically and genetically determined, and another being of psychosocial origin without biological etiology. According to this distinction, two different patterns of familial aggregation of depression should be observable. Andrew et al. [45] tested this hypothesis in a hospital-based twin series and were able to reject it. Maier et al. [46] and Fanous et al. [47] explored whether melancholia defines a distinct familial genetic subtype and found no support for a stronger familial determination. Thus, although there might be a quantitative variation of the magnitude of genetic determination along the various clinical and biological correlates of unipolar depression, clinical distinctions based on the melancholia/endogenous depression concept or absence/presence of psychosocial risk factors are not reflecting this putative variation.

Dysthymia is a chronic low-grade form of unipolar depression characterized by disturbed mood and vegetative functions, whereas depressive personality disorders describe persistent depressionlike cognitive sets of low self-esteem and negative anticipation and pessimism. Both conditions are familial and belong to a unipolar depressive spectrum with shared familial liability [48]. Each of the chronic variants shows diagnostic specificity as well as nonspecificity in familial aggregation; in particular, the chronic variants, dysthymia and depressive personality disorders, go together whereas the risk for dysthymia in families of probands with depressive episodes in absence of dysthymia is only slightly increased [49]. One extended twin sample proposed a modest genetic influence on dysthymia comparable by magnitude to unipolar depression [21].

Bipolar disorder requires the presence of full-blown depressive and manic episodes (bipolar I disorder). Milder variants of manic episodes are called hypomania and define bipolar II disorder (full depressive episodes and separated hypomanic episodes). Highly recurrent hypomanic and recurrent subthreshold depression define cyclothymia. Are these subtypes genetically distinct entities, or are all of them variants of bipolar I with similar

familial patterns of aggregation? Unfortunately, systematic twin studies exploring these questions have not been published. However, some family studies addressing these issues are available. Those family studies [3,6,50,57] report bipolar II disorder to be familial and observed a coaggregation with bipolar I disorder and unipolar depression. The familial pattern of aggregation among bipolar II probands is more similar to bipolar I than to unipolar depression probands. Simultaneously, bipolar II was most common among relatives of bipolar II probands, which supports bipolar II as being a partially distinct category in a familial genetic perspective. In this vein, some pedigree analyses support familial homogeneity of bipolar II disorder without coaggregation of bipolar I disorder [52]. Thus, it remains unclear if bipolar I and II are alternative expressions of the same liability. A familial relationship between cyclothymia and bipolar disorder was also reported. The familial relationship to unipolar depression seems to be less strong [53–55].

Affective disorders, particularly bipolar disorder, are strongly associated with suicidal ideation and behavior. However, this association cannot explain that suicidal ideation and behavior is familial and genetically influenced. Adoption and twin studies have convincingly demonstrated that the familial genetic basis of suicidal behavior is in large part independent of associated familial mental disorders. Schulsinger et al. [56] and Wender et al. [29] were the first to propose this hypothesis on the basis of a small Danish adoption study covering multiple psychiatric diagnoses. In the meantime, two extended twin studies (one in the U.S. by Fu et al. [57], and one in Australia by Statham et al. [58]) supported this hypothesis and demonstrated that suicidal ideation and suicidal attempts are genetically influenced but most of the variance was explained by shared and nonshared environmental factors. Both studies differ in the magnitude of genetic influence on suicidal attempts vs. ideation. Twin studies also demonstrated that completed suicide is under genetic control [59]. Another family study demonstrated familial genetic links between completed suicide and suicide attempts [60]. Thus, affective disorders with suicidal ideation or behavior might define a genetically distinct and even more complex group than affective disorders as a whole.

E. Familial Genetic Relationship to Other Disorders

Nearly all family studies observed a lack of familial specificity for affective disorders. Most nonspecificity was reported for offspring of probands with affective disorders (mainly bipolar disorder) [61]; cosegregation was particularly observed for various anxiety disorders, attention deficit hyperactivity, and other behavioral problems.

Historically, the relationship between affective and psychotic disorders received most attention. The family and twin studies dedicated to this study question can be summarized by the following conclusions:

1. Schizoaffective disorders are not only at a higher risk for affective disorders but also for a higher risk for psychotic disorders (including schizophrenia) [62].
2. Affective disorders with psychotic symptoms (beyond schizoaffective disorder) were reported to be more common among relatives of probands with schizophrenia than would be expected by chance [63].
3. Most family studies were unable to find a familial relationship between nonpsychotic affective disorders and schizophrenia. In contrast, some controlled family studies propose an elevated risk of nonpsychotic unipolar depression among relatives of probands with schizophrenia but not with bipolar disorder [62,64].
4. Recently, a British twin study ignoring diagnostic hierarchies proposed a rela-

tionship between schizophrenia and mania [65]. An excess of schizophrenia among relatives of probands with affective disorders is not reported in any of the larger controlled family studies, although speculations on anticipation operating in bipolar disorder suggested increased risk of schizophrenia among offspring [66].

Thus, there is some evidence of overlapping familial genetic conditions between affective disorders, on one hand, and psychotic disorders, on the other hand. Linkage studies recently shed new light on this relationship (see below).

The family studies in bipolar disorder proposed a familial genetic subset of patients with comorbid panic disorder (or panic attacks) [67]. The combined diagnosis revealed strong familial clustering but was not associated with increased familial risk for pure panic disorder. This constellation suggests that the comorbid diagnosis defines an etiologically distinct entity and not just the combination of two familial conditions. In those families, panic disorder was similarly intraindividually combined with bipolar I and bipolar II disorders. The validity of this comorbid subtype was further supported by subsequent linkage and association studies (see below). The familial genetic relationship of unipolar depression to anxiety disorders is discussed in more detail in Chapter 9.

The relationship between mood disorders and alcoholism has been explored extensively during the last three decades mainly by family studies. Intraindividual co-occurrence is high not only among the index cases but also among relatives. Overall, the links of alcoholism with bipolar disorder seem to be stronger than with unipolar depression. The conclusions on shared familiality are diverse. Some studies found intrafamilial cosegregation of bipolar disorder or unipolar depression, respectively, and alcoholism [68–70], whereas the majority of investigators were unable to observe this relationship [11,71–76]. One study came to divergent conclusions depending on the mode of data analysis [77]. Furthermore, an extended twin study in the general population found that the observed comorbidity between major depression and alcoholism (with an odds ratio of 2) is nearly exclusively due to common genetic factors [78]. Thus, if there is any communality of familial genetic risk factors between mood disorders and alcoholism, it is of limited degree and, therefore, difficult to detect.

It has been speculated that specific diagnostic subgroups of unipolar depression reveal a stronger familial genetic link to alcoholism; for example, Winokur et al. [79] proposed to subdivide unipolar depression into three groups according to family history—familial loading with unipolar depression only, with alcoholism, and with absence of familial loading. Although the diagnostic distinctions received some subsequent support by the same group [68] they did not hold up in other family studies [80].

Finally, the excess comorbidity between eating disorders and affective disorders stimulated familial genetic inquiries; they often go together in a complex neurobiological relationship. Interest in this relationship recently emerged from the detection of the orphasin gene (with functions in affect, eating, and sleeping). As expected, there is not only cosegregation in families but also a genetic relationship; twin studies have also demonstrated that unipolar depression has similar genetic determinants as anorexia [81]; the relationship to bulimia seems to be less strong [82].

F. Interplay Between Genes and Environment

Epidemiological and clinical research identified several environmental and personal risk factors for unipolar depression [83–86]: (1) female gender; (2) critical life events and

daily hustles in the temporal context of the depressive episode; (3) early emotional environment (e.g., parental warmth); (4) early traumas during childhood; (5) head injury in early adulthood; (6) lack of social support; (7) depressionlike personality factors such as increased neuroticism; and (8) previous psychiatric and somatic diseases (e.g., anxiety disorders, alcoholism, or cardiovascular diseases).

Genetic as well as environmental factors contribute to affective disorders. The mode of their interplay is extensively explored for unipolar depression. Different mechanisms to combine environmental and genetic factors can mediate depressive episodes:

1. Are these risk factors operating independent of the genetic risk factors (independent relationship)?
2. Does their impact depend on the amount of genetic risk (interaction)?
3. Some of these risk factors are genetically influenced: do those genetic influences on risk factors contribute to the hereditary basis of depression (correlation)?

All of these possible mechanisms operate for specific environmental factors in unipolar depression:

1. Independence: Gender as a risk factor neither interacts nor correlates with the genetic risk for depression [87].
2. Interaction: The effect of critical life events on depression is stronger in the presence of increased genetic risk; one family study even found that life events only induce depression in the presence of a history of emotional disorders in parents [41,88].
3. Correlation: Life events reveal some heritability pointing at the genetic mediation of life events that are dependent on the proband's behavior [83]. It has also been shown that this is the case particularly for life events that increase the risk for depression [84]. In addition, the fact that specific environmental risk factors operate independently cannot be excluded.

Which environmental factors have a powerful impact on major depression in the absence of substantial genetic risk? One strategy to approach this question is to compare two discordant monozygotic twins; the rationale is that strong genetic factors would most likely be associated with the presence of depression in both twins. The comparison between affected and unaffected monozygotic twins proposed childhood vulnerability and interpersonal difficulties as most influential environmental determinants [89]; pregnancy and perinatal complications might be of relevance for early-onset bipolar disorders [90].

Thus, the biometric analyses reveal that the interaction of genetic and environmental factors is extremely complex. Further refinement of this puzzle can be expected once specific susceptibility genes have been identified. However, the search for susceptibility genes is severely hampered by the interaction with environmental factors.

II. SEARCH FOR SUSCEPTIBILITY GENES ON A DNA LEVEL

The search for disease genes on a DNA level starts with localization on the genome. For this purpose, two strategies are available: linkage analysis in families with multiple affecteds, and linkage disequilibrium/association studies in case-control or nuclear family samples. Genetic variability occurs so frequently on the genome on a population level that a variant at a specific polymorphic site is in linkage disequilibrium (association) with variants at multiple other loci in close neighborhood. Thus, linkage to a locus or association

with an allele or haplotype does not mean that the linked/associated locus/allele is involved in the etiology of the disease; instead, such a finding identifies a candidate region and stimulates extensive fine mapping and, subsequently, functional studies in order to identify the disease gene.

The most direct approach in the search for disease genes relies on the knowledge of pathophysiology and of involved gene products (candidate-gene approach). Given the linkage disequilibrium along the genome, a genome-wide search for disease genes is possible using high-throughput techniques [91], yet it is technically difficult to perform. In the near future, genome-wide association studies will be technically feasible but will require huge sample sizes. Currently, a genome-wide strategy is only technically feasible with the linkage approach because of the lower spatial resolution of this method (~500 appropriately placed markers required). Unfortunately, genes with minor effect (odds ratio 1:2) have only a low chance of being detected by the linkage strategy. In contrast, the linkage disequilibrium/association strategy is substantially more powerful for small gene effects [92].

Thus, a most promising research strategy is to combine these possibilities and (1) to identify linked candidate regions on the genome by linkage analyses; (2) replicate the linkage finding and, in the positive case, (3) finemap the region using a linkage disequilibrium strategy to further narrow down candidate interval.

A. Genome-Wide Linkage Analysis

Given the stronger genetic influence, bipolar disorder was until now the most frequently investigated affective disorder with regard to linkage analysis. Bipolar disorder was even the first psychiatric disorder where linkage analysis was applied. As early as 1969, bipolar disorder was reported to be linked to a monogenic disease, color blindness, and/or to glucose-6-phosphate dehydrogenase deficiency with genes located on chromosome Xq26-28 [93,94]. In multiple subsequent studies, this finding was only partly replicated [95]. However, a recent linkage scan in a single extended Finnish pedigree proposed linkage to a nearby locus Xq25-26 [96], raising the possibility of genetic heterogeneity. Early enthusiasm motivated by the upcoming molecular genetic opportunities was particularly dampened by the nonreplication of the linkage to chromosome 11p initially reported in an isolate pedigree (Amish population) [97,98].

Meanwhile genome-wide linkage analyses using informative marker systems like microsatellites became feasible. Twelve genome-wide linkage scans have been published; for summary of all studies published till early 2001, see Refs. 99, 100. The chromosomal regions suggested to be linked to bipolar disorder are broad, usually covering 20 to 40 cM. The strength of linkage for all proposed candidate loci is moderate at best, with mostly "suggestive" linkage according to the criteria of Lander and Kruglyak [101]. The maximal lod score observed for bipolar disorder is 3.8 to 10p [100]. Not a single linkage to a specific region received support from all or even the majority of scans. However, at least some of the genome scans found suggestive linkage to some region. Loci emerging from at least two of the published linkage scans are: 1q21-42, 4p16, 10q21-26, 12q23-24, 13q11-32, 18p11, 22q11-12 [102]. Two very recently published genome scans [100,103] provided further support to some of these linkages.

The vast majority of implicated studies are samples of affected pairs of siblings recruited in outbreed populations. Although it is believed that these samples are particularly informative, linkage analysis in isolate families with a high density of affected cases

might accelerate the detection of susceptibility genes: given the homogeneous population background and the possibility that the disease was introduced to the isolate by a single or only a few founders, fewer susceptibility genes, but with stronger effects, are likely to contribute to the disease. Haplotype analysis can efficiently narrow down candidate regions to regions covering less than 10 cM in extended multiplex families. A linkage study in a French-Canadian isolate pedigree demonstrates these putative advantages [104–106].

Limitations of statistical power inherent in the available linkage analyses propose a combination of family samples or a meta-analysis (combination of P values) to select "true" candidate regions. Badner and Gershon [99] performed a meta-analysis across 11 published linkage scans and found strongest evidence for susceptibility loci for bipolar disorder on chromosome 13q11-32 ($p < 6 \times 10^{-6}$) and on chromosome 22q11-12 ($p < 1 \times 10^{-5}$). The other candidate loci did not receive strong support for linkage; on the other hand, a meta-analysis cannot exclude linkage to these regions.

The broad variation of results and high inconsistency between various studies is not an argument against the validity of the linkage approach. Two main reasons explain the diversity of results across studies:

1. The available sample sizes are too small to create consistent results. If multiple genes contribute, the sample size required for the detection of linkage to any of the susceptibility genes is substantially smaller than the sample size required to replicate this specific finding [107]; thus, given the limitations of power and sample size inherent in the published scans, a high rate of nonreplications can be expected.
2. Disorders that are under the influence of multiple genes are likely to be genetically heterogeneous. The genes contributing and their effect sizes might differ across the population of affecteds (e.g., between severe and less severe cases) and by the genetic population background; another possibility is pleiotropy (i.e., multiple different genetic constellations produce the same phenotype). Thus, samples of pairs of affected siblings might differ partly between recruiting sites even within the same country, between countries, and ethnicities. In addition, etiologically different subgroups might be driven by different susceptibility genes as has been recently proposed by Rotondo et al. [108] for bipolar disorder in combination with panic disorder. The variation of proportions of specific subtypes across samples might produce divergent linkage results.

Despite these ambiguities it can be concluded from these results that bipolar disorder is not influenced by a single major gene; instead, multiple susceptibility genes each with only a mild-to-modest effect are likely to operate.

Some of the candidate regions detected for bipolar disorders are overlapping with candidate regions linked to schizophrenia [109]. Some authors interpret this constellation as a hint in favor of a common genetic basis for affective disorder and schizophrenia. Yet, this conclusion is premature. Because there are thousands of genes in these regions, overlap of candidate regions cannot be interpreted as identity of genes being involved for both disorders. On the other hand, the degree of overlap cannot be explained by random fluctuation alone.

Up to now, no genome-wide linkage scan has been performed in unipolar depression; yet, currently, two major linkage efforts are in progress. The only reported linkage studies in unipolar depression focus on candidate genes with no evidence of statistically significant linkage [101] in a sample with "pure depressive disorder" with recurrent episodes [110].

B. From Candidate Regions to Candidate Genes

Given the higher power and the finer spatial resolution of association/linkage disequilibrium studies, this technique has to be applied to polymorphic candidate genes located in regions linked to the disease (positional candidates). In this context, candidate genes that are functionally involved in the pathophysiology of bipolar disorder according to the current knowledge are of interest (positional as well as functional candidates). Some candidates in the linked regions are of particular functional interest and have therefore been explored recently:

1. Myo-inositol monophosphatase (IMPA2), a key enzyme of lithium on chromosome 18p11.2 [111,112].
2. G-protein-coupled receptor kinase-3 on chromosome 22q11, which is differentially expressed in an animal model for psychotic mania [113].
3. Gene coding with multiple mutations for Wolfram syndrome (WFS1), an autosomal recessive disease that is characterized by frequent comorbidity with affective disorders and located on chromosome 4p16 [114–117].
4. Gene coding for Darier's disease, an autosomal dominant dermatological disease with frequent comorbidity with affective disorders on chromosome 12q23-24; this gene expresses the protein calcium-ATPase in the endoplasmic reticulum [118].

Although a few of these studies reported borderline significant associations, a relationship of any of these candidate genes to bipolar disorder has not as yet been established.

C. Genetic Association Studies

Numerous association studies (mainly case-control comparisons) were conducted in affective disorders with a broad range of candidate genes. Recent research preferentially focused on genes coding for receptors, transporters, and enzymes involved in monoaminergic transmission. The results are extremely conflicting with multiple claims for association that could not be replicated. Given the small magnitude of genetic effects to be identified and limitations in sample size, perfect consistence of multiple replication tests cannot be expected even for a "true" positive finding. Meta-analyses are useful under this condition or at least a considerable proportion of successful replication tests of an initially positive finding. In this perspective, several genetic variants of candidate genes reveal some evidence for an association with affective disorders.

The strongest association to bipolar disorder is reported for a variant of the monoamine oxidase A (MAO-A) gene. MAO-A operates as a candidate, given the efficacy of MAO-A inhibitors in antidepressant treatment. There has been suggestive evidence for an association between bipolar disorder and a CA repeat variant in intron 2 [119], although not all replication tests unequivocally confirm this overall (e.g., Refs 120, 121). Unfortunately, association to functional genetic promoter variants was excluded in moderate-sized samples [122].

Most frequently, the relationship to the promoter polymorphism of the serotonin transporter gene has been studied. The polymorphism is functionally relevant, and an association of affective disorders to the short allele has been proposed [123]. A former meta-analysis proposed a small effect of this variant in bipolar disorder [124], with an increased odds ratio of 1.2, but subsequent studies were mainly negative [120,125,126].

The evidence for association of this genetic variant to unipolar disorder is less conclusive. However, heterogeneity might prohibit consistent results (e.g., an association of the small allele with the clinical subtype of seasonal depression was reported [127,128]). The associations of the short allelic variant to the personality factor "neuroticism" appear to be more stable [129–131].

Unfortunately, neither of these two candidate genes with variants in suggestive association with bipolar disorder are located in any of the candidate regions for bipolar disorder found by linkage analysis.

Other polymorphisms, such as the tyrosine hydroxylase (TH) gene, the tryptophan hydroxylase (TPH) gene, the COMT gene, or the serotonin and dopamine receptor genes, provided only inconclusive or negative results in association studies with bipolar disorder or unipolar depression [132].

The view that unipolar depression is under the control of the same genes independent of the age at onset has recently been challenged by an inquiry in late-onset depression: this condition was associated with a mutation in the methylenetetrahydrofolate reductase enzyme gene (MTHFR) in contrast to early-onset depression. The same mutation is predisposing to cerebrovascular diseases and is associated with increased plasma homocysteine and folate deficiencies which were also reported for late-onset depression [133].

D. Cytogenetic Techniques

Besides the main stream of linkage and genetic association studies, a few groups used cytogenetic techniques to search for gene loci that implicate the disease through chromosomal abnormalities (particularly translocations and deletions). A series of regions were found which, unfortunately, did not overlap with regions linked to the disease (for overview see Ref. 132). Of particular interest is a Scottish family with several psychoses, bipolar disorder, and unipolar depression with a balanced translocation between two genes (chromosomes 1q42, 11q14.3). A gene in 11q14.3 disrupted in affected family members (DISC1) with up-to-now unknown function was detected and needs further investigation [134].

E. Search for Genes Using Specific Modes of Transmission

Specific features of the familial aggregation of affective disorders have been proposed.

1. Anticipation

According to some family studies, the vertical transmission of bipolar disorder from one generation to the next goes together with a decrease of the age at onset [135]. This phenomenon can be explained by multiple mechanisms. One possibility in this context is genetically mediated anticipation that can be caused by expansion of trinucleotide repeats at the mutated disease gene locus (dynamic mutations). This kind of mutation codes for the so-called polyglutamine disease (like Huntington's), which shows anticipation on a phenotype level. Using the repeat expansion detection (RED) method, the maximal lengths of trinucleotide alleles were compared for the whole genome between probands with bipolar disorder and controls in multiple studies. Conclusive results were not obtained [136,137]. This negative conclusion does not rule out the hypothesis because the RED method might be too insensitive to detect the majority of dynamic mutations across the genome.

2. Parent-of-Origin Effects

Patients with bipolar disorder have more mothers than fathers with the same disease [135]. Although multiple nongenetic causes might explain this constellation in complex diseases, two genetic mechanisms might account for this pattern:

1. Genetic imprinting: DNA methylization might operate specifically for specific maternal alleles of maternal decent with the consequence that the mutated paternal mutations at a disease locus are preferentially expressed. Indeed, at least one of the proposed linkages to bipolar disorder—18p11—is limited to paternally transmitted pedigrees; however, this specific linkage is not very strong and might be a false positive.
2. Mitochondrial transmission: genetic information is also transported by mitochondria which are exclusively transmitted from the mother. Furthermore, mitochondrial dysfunction is also proposed by other lines of evidence [138]. The whole mitochondrial genome was sequenced in several studies [132,139,140] without a consistently confirmed significant result. Yet, one variant (the 10398A polymorphism) has been found with increased frequency in two studies. However, causal mechanisms might also arise from different haplotype patterns and not only from variations at a single site; this possibility [139] has to be further evaluated.

F. Pharmacogenetics

The broad interindividual variation of response to treatment is partly due to the interindividual variability: multiple functional sequence variation sites in metabolizing enzymes and transport, target, and effector proteins are likely to influence individual side effects and effectiveness [141]. Empirical evidence supports this assumption. Response to long-term lithium treatment in multiple cases within the family was found to be correlated. Also, plasma levels of most antidepressants are influenced by the cytochrome P450 metabolizing system, which reveals broad genetic variation (e.g., resulting in pure and fast metabolizers); plasma levels have some impact on side effects and effectiveness (although a linear relationship or a "therapeutic window" has not been detected).

The field of pharmacogenetics promises to provide DNA markers and haplotypes for prediction of drug response, and to improve the opportunity for individualized treatment [142]. However, progress of knowledge with regard to antidepressants is slow because of the lack of appropriately sized prospective controlled pharmacological treatment programs with a pharmacogenetic component (which are accessible to the academic community). Up to now, few pharmacogenetic studies investigated the impact of the variation in the serotonin receptor or transporter genes and their promoters on response to selective serotonin reuptake inhibitors (SSRIs) and to lithium. The short allele in the promoter of the serotonin transporter gene was associated with poor responses to SSRIs, according to Zanardi et al. [143]; Kim et al. [144] reported a conflicting result with regard to the homozygotes for the short allele. The same allele was associated with response to lithium [145].

Despite the currently disappointing results in the search for genetic predictors of antidepressants, major progress can be expected with the advent of informative clinical trials and new genotyping techniques.

III. CONCLUSIONS

The detection of genes coding for mood disorders turned out to be substantially more difficult than anticipated. This constellation is currently identical for all genetically complex disorders, and the consequences are also the same. The growing opportunities emerging from the progress of human genomics, neurosciences, biostatistics, and epidemiology justify optimistic perspectives. The current research trends in psychiatric genetics support this view.

1. The application of the opportunities for DNA genotyping (e.g., SNPs or chip technology) will increase efficiency, reduce costs, and encourage new strategies as genome-wide case-control studies for the search of susceptibility genes [91]; the huge case-control samples needed for this new approach are currently recruited in many places.
2. The growing knowledge on linkage disequilibrium across the genome and the relevance of population backgrounds will inform the search for disease genes and improve the understanding of the variation of results across samples and populations (for an overview, see Refs. 146, 147). New methods using the diversity of haplotypes across the genome have already been successful in unraveling the genetic basis of complex diseases (e.g., Crohn's disease) [148].
3. The extraction of genetically relevant phenotypes that are more closely related to the genes underlying the disease [149] will accelerate the successful search for disease genes; the clinical diagnosis will not remain the only way to define the phenotype. Clinical subtyping of diagnostic entities is also a new and promising approach to extract biologically meaningful phenotypes (e.g., Ref. 150).
4. The progress of the functional genetics and genomics, of transgenic and proteomic methods allow the detection of new pathways, and improves the understanding of gene function in the brain; new candidate gene families emerge from those innovative research activities (for an overview, see Ref. 151).
5. The emerging collaboration between genetics, neuroscience, epidemiology, and clinical research [1] will replace the small groups of investigators, and will shift the field from a purely hypothesis-free genome-wide search to an informed genome-wide search for disease genes (without focusing on well-established disease genes).

Thus, the lack of knowledge of fully confirmed susceptibility genes for unipolar depression or bipolar disorder will not discourage further research aimed at the genetic architecture of mood disorders. Furthermore, the emerging discipline of pharmacogenetics will contribute to an improvement of antidepressant treatment.

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9

Genetics of Anxiety

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I. INTRODUCTION

Anxiety disorders are common. Like all other common diseases, anxiety disorders are familial and genetically influenced. In contrast to other psychiatric disorders, the familial genetic nature of anxiety disorders were detected recently, with a delay of several decades. It was only about two decades ago—after the advent of the first criteria-based definitions of specific anxiety disorders in DSM-III—that intensive genetic–epidemiological research on anxiety disorders started. It became evident at the same time that anxious behavior in animals reveals strong similarities to that in human behavior, and anxious behavior in animals is under genetic control. Given the face validity of the animal phenotype, genetic animal research became a model for understanding the genetic basis of human anxiety and anxiety disorders.

II. GENETIC EPIDEMIOLOGY BY FAMILY AND TWIN STUDIES

During the last two decades, a series of family and twin studies documented the familial aggregation and genetic determination of generalized anxiety disorders, panic disorders, and phobic disorders. Adoption studies had not been conducted. Recent review studies reported the current evidence in this field [1,2]. Thus, this chapter briefly summarizes the current state of knowledge in Tables 1 and 2.

Although the prevalence rates for anxiety disorders in families of affected subjects and of controls vary considerably across samples, the odds ratios and heritability estimates are less variable. Heterogeneity tests for odds ratios were conducted, and for the heritability rates in twin samples without revealing inconsistencies across samples [2].

Table 1 Family Studies: Meta-Analysis

Disorders	Refs.	Risk (%) among first-degree relatives	Controls	OR compared to controls
Generalized anxiety disorders	4	19.5	3.5	6.6
	5	8.9	1.9	5.0
Panic disorders	6	17.3	4.2	4.8
	5	13.2	0.9	15.6
	7	7.9	2.3	3.6
	8	11.0	1.8	6.7
	9	9.5	3.0	3.4
Agoraphobia	6	11.6	4.2	3.0
	10	10.0	3.0	3.5
Social phobia (generalized)	11	16.0	6.0	2.9
	12	26.4	2.7	12.9
	3—parents	22.4	12.2	4.7
Simple phobias	10	31.0	9.0	4.4

Source: Refs. 2 and 3.

Table 2 Twin Studies: Meta-Analysis

Disorders	Refs.	Sample gender	Proband-wise concordance (%) MZ	DZ	Heritability
Generalized anxiety disorders	13	males/females	60.0	14.3	
	14	males	—	—	0.37
	15	males	22.9	19.5	0.22
	15	females	38.1	40.6	0.22
Panic disorders	16	males/females	30.8	0.0	
	13	males/females	41.7	16.7	
	17	males/females	73.0	0.0	
	18	females	20.7	14.5	0.37
	14	males	—	—	0.43
Agoraphobia	19	females	23.2	15.3	0.39
	20	males	12.2	12.2	0.37
Social phobia	19	females	24.4	15.3	0.30
	20	males	12.6	9.8	0.20
Simple phobias	21	males/females	13.0	8.0	
” , animal	19	females	25.9	11.0	0.32
” ”	20	males	15.9	7.7	0.35
” , situational	19	females	22.2	23.7	—
” ”	20	males	21.2	6.5	0.25
” , medical	22	females	—	—	—
” ”	20	males	15.6	4.1	0.28

Source: Ref. 2.

Two main conclusions can be drawn:

1. Altogether, consistent evidence emerge that all anxiety disorders are strongly familial. The odds ratios indicate an at least threefold excess of morbid risk for any anxiety disorder among first-degree relatives of affected individuals (Table 1). For each of the listed diagnostic categories, the reported odds ratios even exceed the odds ratios for unipolar depression (ranging between 2 and 3) [1].
2. Twin studies clearly demonstrate that all anxiety disorders are under genetic influence (Table 2). The magnitudes of the genetic components range between 0.2 and 0.4 and are comparable by magnitude across the various disorders with a trend for panic disorder showing maximal heritability rates; maximal heritability was reported for panic disorder by Scherrer et al. [14], with 0.43 in males, whereas lowest heritability estimates (15–22%) were calculated for one twin study in generalized anxiety disorder based on only a modest familial aggregation rate [15]. Unipolar depression is influenced by genes to a similar extent. In contrast, schizophrenia and bipolar disorders, as well as dimensional personality traits like neuroticism, are under stronger genetic control (heritability > 0.5).

Overall, the heritability estimates are lower than 50%. Thus, a nongenetic environmental component is apparently more relevant for the manifestation of anxiety disorders than genetic factors. This conclusion might be inappropriate as the environmental component also includes (1) all the measurement errors and (2) the variance due to gene–environment interaction. Consequently, the magnitude of the genetic component is likely to be an underestimate.

The analysis of twin study data allows additional refinements: variance component analysis of twin studies enables the decomposition of the origin of variation because of nongenetic factors in shared and in individual-specific environmental components. Although those calculations are model-dependent, they allow a rough impression on the relevance of putative risk-factor components. Counter to expectancy, the nongenetic variance is nearly exclusively due to individual-specific determinants in anxiety disorders of adults [2]. Common environment, however, might be more relevant in childhood or adolescent anxiety disorders; e.g., a recent twin study [23] found substantial impact for shared environment on separation anxiety disorder, particularly in boys, which decreased with age.

As a limitation, this variance-analytic strategy is not sensitive enough to detect contributions of shared environment of only very modest magnitude ($< 10\%$); e.g., although no impact of shared environment was observed in the global variance analysis in a Virginia sample with more than 2000 twins, the analysis of the specific risk factor "deficient parenting" (coldness, overprotectiveness/rejection, and authoritarianism) revealed a modest, but significant, contribution to the risk of generalized anxiety disorder, phobia, and panic disorder [24]. In this line, a family study in social phobia [3] reported only a borderline significance for parental style. Merikangas et al. [25] were also unable to find a significance impact on parental style (beyond the parental psychopathology) on anxiety disorders in offspring. Thus, also the analysis of specific environmental factors detected only minor effects of environment but could not prove a major contribution of shared nongenetic family influences.

In summary, we can conclude that the family aggregation of anxiety disorders is mainly attributable to genetic factors but not to a common environment. Even without the specification of genes and environments in family and twin studies, these results con-

tribute to the classic discussions on the origin of anxiety disorders; e.g., it was proposed that anxious behavior and particularly social anxiety were learned by modeling (model-learning hypothesis), and that children, in particular, learn this behavior from their parents. According to this hypothesis, children would be exposed to a common, shared risk factor emerging as shared variance from twin studies. Given the lack of evidence for shared nongenetic components in twin studies, the model-learning hypothesis cannot explain a substantial proportion of the variance.

III. MODIFIERS OF THE MAGNITUDE OF RELATIVE RISK

The magnitude of familial loading is broadly varying across families for each anxiety disorder. What are the determinants? Gender, age at onset, and comorbidity with selected disorders have been explored as risk-modifying factors.

Anxiety disorders are generally more common among females than males. Consequently, female relatives of affected probands are more vulnerable to anxiety disorders than male relatives. However, the relative risks of any anxiety disorder are comparable between female and male relatives of affected probands across the various family studies. Similarly, the heritability estimates are not gender-dependent (Tables 1 and 2) [15].

The familial risk of most psychiatric disorders is positively correlated to the age at onset. Similarly, in anxiety disorders, Goldstein et al. [26] observed higher familial loading for early-onset panic disorder. This issue has not been pursued in other anxiety disorders.

Comorbidity of panic disorder with agoraphobia has been proposed to indicate increased severity associated with increased familial risk to panic disorder and agoraphobia [6]. Further research was unable to support this hypothesis [7,27].

Comorbidity of panic disorder with major depression is also considered an indicator of high severity. Despite of earlier contradicting reports, the familial risk for anxiety disorders is apparently unchanged by comorbidity with depression [27].

IV. MODE OF TRANSMISSION

The mode of intrafamilial transmission remains unclear for all anxiety disorders as is the case for all psychiatric and all common diseases. Previous reports on segregation analyses for panic disorders proposing a major gene effect or even a dominant transmission [28] were not replicable in subsequent segregation studies [29]. Thus, all anxiety disorders can be considered genetically complex disorders without a Mendelian mode of transmission.

The most reasonable assumption for all anxiety disorders is that multiple genes are interacting with each other and with environmental factors to produce the disorder (multiple susceptibility/vulnerability genes). Given the results of genome-wide linkage studies in panic disorders (see below), genes with a major effect are unlikely to operate in the majority of families affected by panic disorders (see below). In agreement with this transmission model, Merikangas et al. [25] observed a gene-dose effect: the risk among offspring doubles if both parents suffer from anxiety disorders. More specific knowledge about the transmission of anxiety disorders is only feasible after specific impacting vulnerability genes are identified.

A. The Structure and the Boundaries of the Transmitted Phenotype

What is the genetic architecture of the inherited phenotype? Given that the definitions of anxiety disorders emerge from clinical conventions, it is unlikely that these diagnostic

entities are transmitted in families as distinct phenotypes; a one-to-one relationship between a combination of genetic factors and a specific anxiety disorder cannot be expected. It would come as a surprise if the conventional clinical boundaries would map into distinct genetic entities. Instead, the phenotypes transmitted in families are only correlated to anxiety disorders. Empirical evidence supports this suggestion.

1. Anxiety disorders might be transmitted as part of a continuum.
2. Anxiety disorders might not operate as genetically distinct entities.
3. Anxiety disorders might share susceptibility genes with other disorders.
4. Neurophysiological and neurochemical indicators might be genetically related to anxiety disorders, and present as more direct cues to the susceptibility genes.

1. Transmission of an Anxiety-Related Continuum

Anxiety disorders might be considered as extremes on a continuum of liability to anxious behavior that is driven by a common genetic predisposition (quantitative trait loci—QTL); thus, subthreshold anxiety disorders, anxiety symptoms, and anxious behavior are also influenced by the same genetic variation as anxiety disorders; either a lower environmental or genetic load or the presence of protective genes or environment prevent these subdiagnostic features from becoming more severe and presenting as anxiety disorders.

Empirical research using individual symptoms or dimensional measures of phobia and panic provided only limited evidence for a familial genetic basis for subthreshold conditions. In this context, the magnitude of the relative risk of subsyndromal anxiety in relatives of patients with panic/phobic disorders, and of the heritability estimates remains controversial: particular phobic symptoms have not been found to be highly transmissible by interview-based family studies [30,31]; similarly, only very modest heritability was reported for individual phobic/panic symptoms and factors in a twin study using questionnaires [32]. However, there is also conflicting evidence [33–36]. Several twin studies report heritability estimates for symptoms of specific phobic fears (between 0.45 and 0.55) comparable to phobic disorders particularly among children. Despite these twin studies, it remains uncertain if the number of anxiety symptoms represents a trait that is as heritable as anxiety disorders.

More basic and more traitlike symptoms may produce unpleasant, adverse, or harmful consequences (anxiety sensitivity). Recent research demonstrated that anxiety sensitivity is an antecedent risk factor for panic and anxiety symptoms [37]. A new twin study using a new scale to measure anxiety sensitivity revealed a very substantial heritability rate of nearly 50% [38]. The genetic architecture of this trait, however, remains to be explored.

2. Diagnostic Specificity in Transmission of Specific Anxiety Disorders?

The familial genetic relationship between specific phobias remains ambiguous: on the one hand, a family study in probands with panic disorder and phobia (in absence of comorbidity with nonanxiety disorders) found a diagnosis-specific familial contribution to panic disorder with agoraphobia, social phobia, and simple phobia [10] without excluding partial overlap of the familial components between each of these disorders (especially between social and simple phobias). Another family study found familial coaggregation of social phobia and agoraphobia in probands with panic disorder in the absence of comorbidity with major depression; however, the coaggregation of both conditions is not a consequence

of shared familial genetic factors; instead, social phobia presents more of a consequence of preestablished agoraphobia in relatives [8].

The genetic relationship between panic disorder and phobias has been extensively explored with ambiguous results. A prior family study by Noyes [6] also found panic disorder with agoraphobia and panic disorder without agoraphobia to breed true. Subsequent studies were unable to replicate this relationship [7,27]. Partial identity of genetic vulnerability factors for phobias and panic disorder (as well as bulimia) was also reported in two independent population-based twin studies [14,39]. In contrast, the genes impacting on generalized anxiety disorder and depression did not reveal substantial overlap with genes influencing the manifestation of phobias and panic disorder according to Hickie et al. (40) and Kender et al. [41]. Phobias were not further specified in these two large-scale studies, leaving space for divergent relationships on the level of specific phobias. Another study in offspring of patients found evidence for a familial genetic link between social phobia and depression [3]. A recent twin study by Scherrer et al. [14], however, suggested that at least half of the genetic liability for panic disorder overlapped with generalized anxiety disorder. Thus, it cannot be excluded that specific phobias show differential effects.

Apparently, there is diagnostic specificity as well as unspecificity with regard to the subtype in a family-genetic perspective. Panic disorder and phobic disorder are considerably overlapping whereas generalized anxiety disorder shows the highest degree of distinctness.

3. Genetic Relationship to Other Disorders

Anxiety disorders and depression: The familial genetic relationship between generalized anxiety and depression was the topic of intensive study in several population-based twin studies [42–46]. A very strong genetic relationship between generalized anxiety disorder and major depression emerged from these twin studies: both disorders share the same genetic vulnerability factors; the differential alternative expression as anxiety or as depression is due to distinct environmental factors that still have to be identified. If anxiety and depression are considered quantitatively as two continua, with the disorders defining the extreme of the continuum, the close genetic relationship between both psychopathological conditions was replicated in other population-based twin studies [42–44]. Anxiety disorders and depression in childhood are even more closely correlated than in adulthood [25,47].

There is also consensus in more recent family and twin studies that panic disorders and major depression are not strongly related genetically. Controlled family studies report only modest or insignificant excess of major depression in families of probands with panic disorder without a history of major depression [27,48]. Twin studies [39] report absence of a substantial genetic correlation between panic disorder (and phobias), on the one hand, and depression, on the other hand. In contrast, a family-genetic relationship between bipolar disorder and panic disorder seems to be closer [49].

The observed diagnostic unspecificity of anxiety disorders and depression with regard to familial aggregation has been considered under the model of age-specific expression of common risk factors: anxiety is preferentially expressed in childhood, and depression is preferentially expressed in adulthood [50–52].

Another model, derived from the analysis of a large Australian twin study, proposes that anxiety (mainly in males) and depression (mainly in females) are variable expressions

of a heritable trait of neuroticism (which revealed heritability estimates of more than 50% in multiple twin studies) [53].

The genetic correlations between liabilities to phobias and panic disorder and between generalized anxiety disorder and depression may explain a substantial proportion of the intraindividual comorbidity between these disorders as observed in epidemiological studies [54]. However, genetic factors cannot account for the excess comorbidity between panic disorder and major depression.

Anxiety disorders and addiction: The relationship between anxiety disorders and alcoholism is particularly complex. It is indisputable that an excess rate of alcoholism (abuse or dependence) can be encountered in families of probands with any anxiety disorder. The relationship to anxiety disorders in family studies, however, is heterogeneous: (1) alcoholism in relatives of probands with social phobia is mainly secondary to the relative's primary social anxiety disorder [55]; (2) several studies report that a partly shared genetic-familial diathesis underlies alcoholism and panic disorder [55–57]. However, twin studies only attribute a very modest common genetic component to panic disorder and alcoholism [39].

4. Genetically Correlated Neurobiological Indicators

Diagnostic categories are based on a consensus that does not refer to pathogenic mechanisms or aim at defining the most appropriate phenotype emerging from underlying genes. Genes influence the behavior through multiple complex neurobiological mechanisms (molecular mechanisms, neurotransmitter systems, neurophysiological mechanisms, brain circuits); nongenetic forces do impact those pathways. Thus, the relationship between vulnerability genes and the behavioral phenotype is only indirect, and the effect of a single genetic mutation is likely to influence multiple behavioral qualities simultaneously. Given that genes exert their influence on behavior through neurobiological mechanisms, neurochemical and the neurophysiological indicators represent intermediate phenotypes that are closely related intraindividually to the genetically more complex behavioral phenotype of anxiety. These hypothetical indicators of the genetic diathesis to the disease (1) should occur more often among subjects with increased genetic risk even if these subjects are not affected with the disease, and (2) should be under genetic control. Those vulnerability indicators can be considered as endophenotypes [58] and are most useful for the search of specific underlying genes that also impact on the correlated disorders.

Indicators of particular interest of a diathesis for anxiety are somatic and behavioral reactions to stimuli like lactate infusion, hyperventilation, and CO_2 inhalation [59,60] for panic disorder and phobia, or reactions to the unfamiliar or novelty in children [61] for social phobia, and reactions to threat- and fear-related stimuli (startle) [25] for panic and phobia in children and adults. These response patterns have been shown to precede the development of anxiety disorders (mainly panic disorders and phobias) as indicators of increased risk.

Given that anxiety disorders are familial, the high-risk approach exploring children of affected parents is a particularly promising strategy to evaluate disease-related vulnerability indicators. These indicators have been explored in a series of high-risk studies.

1. Children at high risk for anxiety disorders have increased startle reflex under baseline but even more under various aversive conditions, and increased galvanic skin response particularly subsequent to threat-related stimuli [25].

2. Behavior inhibition to unfamiliar stimuli or situations is associated with shyness among children and occurs consistently more commonly in offspring of parents with social anxiety and other variants of anxiety disorders [51,62]; thus, this reaction type among children was proposed to reflect constitutional "anxiety diathesis" that is familial and predictive of liability to social phobia and panic disorder [63].
3. Sensitivity to CO_2 challenge measured by autonomic reactions is well supported as a vulnerability indicator of anxiety, particularly of panic disorder. Healthy relatives of probands with panic disorder have increased sensitivity [64]. In addition, Bellodi et al. [65] demonstrated genetic influence on this trait by a twin study in healthy volunteers. Response to CO_2 inhalation has been proposed to be associated with the functional promoter polymorphism of the serotonin transporter gene [66]. On this basis, interactive gene x psychological risk factor models have been proposed to explain the pathogenesis of anxiety in vulnerable subjects [66].

Another putative vulnerability indicator is the reaction to lactate infusion that provokes panic attacks in affected subjects. However, in contrast to CO_2, relatives of probands with panic disorder do not consistently respond with panic attacks to lactate [67].

Physiological vulnerability indicators for generalized anxiety disorders have not yet been developed. Automatic (e.g., heart-rate variability) activity and reactions are particularly promising in this respect but have not yet been fully explored as vulnerability indicators.

Besides these physiological functions, behavioral trait measures as anxiety sensitivity or personality traits like neuroticism may also reflect the underlying liability to anxiety disorders more appropriately than the diagnostic categories (see above).

What can be concluded for the transmitted phenotype?

1. Apparently, various categories of anxiety disorders do not breed true, although there is some diagnostic specificity; generalized anxiety is genetically most distinct whereas panic disorders and phobias share genetic risk factors.
2. The diagnostic boundaries are not supported by a differential genetic etiology; the boundary between depression and generalized anxiety is not reflected in distinct genetic etiologies; on the contrary, genetic components for both disorders are strongly correlated. Thus, in a genetic perspective, generalized anxiety is closer to depression than to the other anxiety disorders.
3. Several physiological and behavioral dimensional traits that are closely related to the manifestation of phobias and panic disorder are apparently influenced by the same genes as the anxiety disorders; they might serve as alternative, more appropriate phenotypes from a genetic perspective.

B. Search for Predisposing Genes

Two major strategies for mapping disease genes are feasible: linkage analysis and association studies. In either case, polymorphic loci on the genome are used as markers. The first strategy applies to families with multiple affected subjects, and explores whether genetic markers are transmitted together with the disease. The second strategy applies either to independent cases in comparison to controls or, alternatively, to nuclear families (affected index case with both parents using the not transmitted parental alleles as controls); the

association strategy tests whether a genetic variant (allele) at a specific locus is more common among affecteds compared to controls.

The linkage strategy can be used in a hypothesis-free manner by using a genome-wide approach with a limited number of densely and appropriately placed informative markers; markers linked with the disease indicate a broad candidate region that is likely to host a disease gene. A genome-wide approach by association studies will be technically feasible in forthcoming years; in addition, very extensive case-control samples are needed.

Currently, the association strategy is still restricted to a candidate-gene approach. Candidate genes express gene products (e.g., proteins) that are involved in the pathophysiology or treatment of the disease.

The two strategies have certain advantages and disadvantages: The success of the association strategy depends on the knowledge of the neurobiology of anxiety disorders. Given the limitations of the specific progress in this field, most candidate genes are not yet known. Observed associations with a genetic variant must also be interpreted with caution: two markers in close distance on the same chromosome are in linkage disequilibrium and, therefore, in association; an associated variant, therefore, does not necessarily represent the "true" susceptibility gene variant but may just indicate that a genetic variant in linkage disequilibrium influences the risk. On the other hand, the linkage strategy can only localize genes with a major or at least substantial effect and may be futile in disorders driven by an operation of multiple genes with only small effects that can be detected more easily by the association strategy.

The most critical issue in searching predisposing genes is the appropriate phenotype definition. Up to now, published studies relied nearly exclusively on clinical diagnoses (only exception being anxiety disorders) [68]. However, there are serious uncertainties about the utility of clinical diagnoses in gene-mapping studies (see above). Latent behavioral and/or biological traits (like anxiety sensitivity or reaction to CO_2 inhalation) might be more appropriate.

1. Genome-Wide Linkage

In an influential paper, Risch [69,70] demonstrated that linkage studies for mapping of disease genes using pairs of affected siblings are more powerful the higher the relative risk of the disease among first-degree relatives. Given that the odds ratio is higher than 3.0, all anxiety disorders are promising candidates for genome-wide linkage analysis; in this respect they are better suited for linkage studies than unipolar depression with lower relative risk.

Three genome-wide linkage scans were conducted in samples of families with panic disorder: in 23 families by Knowles et al. [71], which were extended to 34 families by Weissman et al. [72]; in 23 families by Crowe et al. [73]; and in 20 pedigrees by Gelernter et al. (74). Linkage signals with only modest effects were found in each of these studies, proposing that only genes with a small or modest effect influence panic disorder. Consequently, multiple susceptibility genes with small to modest effects impact on the manifestation of panic disorder.

The signals found in one family sample were, unfortunately, not replicable in the two other family samples, although very similar selection criteria for probands and families and similar tools for phenotype characterization were applied. Given the difficulties of replication of small or modest linkage signals under the condition of relatively small family samples, this inconsistency does not come as a surprise [75]. However, the comparison of two of the three studies brought some consensus: a locus on chromosome 7p showed

suggestive linkage in two samples: maximal lod score in Knowles' et al. sample 1.71 [71], in Crowe et al.'s sample 2.23 [73]; similarly, another locus on chromosome 1 revealed suggestive linkage in Crowe et al.'s sample [73] with a maximal lod score of 1.1, and of 2.0 in Gelernter et al.'s sample [74]. Despite not reaching the level of statistical significance, and despite the lack of incomplete consensus between the three genome scans, these results are remarkable given the low likelihood of obtaining overlapping signals in different genome scans with very limited sample sizes. These promising candidate regions await additional replication as well as fine-mapping, particularly in candidate genes located in these areas; unfortunately, current knowledge does not propose interesting positional candidate genes. Linkage studies for other anxiety disorders are currently not available.

Two innovative approaches to linkage deserve special attention, although the empirical results are not yet replicated. In the sample of families with multiple members with panic disorder recruited for linkage [72] coincidence of panic disorder with a series of somatic diseases (affecting kidney, bladder, thyroid, and mitral valve) in individual family members was noticed. After restriction of the sample to families with at least one member reporting panic together with one of these somatic diseases, the lod score at a single locus on chromosome 13 went up to a highly significant magnitude.

Motivated by the observation of the excess comorbidity between panic disorder and joint laxity in a specific population, Gratacos et al. [76] collected families with a high prevalence of panic disorder or phobias and joint laxity. Before starting the genome scan, an initial cytogenetic examination in family members with both disorders was performed; an interstitial duplication on chromosome 15q (DUP25) was found in the majority of affected subjects. At the DUP25 locus, a highly significant linkage signal was detected in the multiplex families. The proposed vulnerability gene variant DUP 25 is of particular interest because of its non-Mendelian transmission [76].

2. Candidate Genes

The selection of candidate genes primarily focuses on neurochemical hypotheses to the disease. Anxiety and its disorders are thought to be related to serotonin. Other candidate genes refer to targets of anxiety-provoking agents.

Serotonin transporter genes. The serotonin transporter is instrumental in the regulation of serotonergic activity. The polymorphism in the promoter region of the serotonin transporter gene is a most promising candidate for negative emotions, anxiety, as well as anxiety disorders. After the first report of an association of the allele with reduced in vitro transporter expression with neuroticism and other personality-bound, anxiety-related traits [77], a bulk of studies tried to replicate the results with limited success: in favor of the original finding [78–80]; partly in favor [81]; not in favor [82–84]. Using clinical diagnoses (panic disorder) as phenotypes, neither linkage nor association have been found in family-based studies [85].

Serotonin receptor genes. Another candidate is the serotonin receptor 5HT2c as agonists to this receptor may provoke anxiety (e.g., mCPP) whereas antagonists (e.g., ritanserine) are anxiolytic agents. Deckert et al. [86] identified polymorphisms in regulatory units of this gene and found a significant association of one variant with panic disorder in one but not in another population.

Animal studies (knockout models), in particular, propose the 5HT1a receptor as a candidate for genetic studies [87]. However, common variants of this gene have not been found up to now which could explain a relevant proportion of the genetic variance [88].

Serotonin-metabolizing enzymes: monoamine oxidase and COMT genes. Monoamine oxidase inhibitors are powerful drugs for panic disorder and social phobia. Although the relationship between MAO and anxiety disorders remains obscure [89], the genes coding for the two variants of MAO are promising candidates; both genes carry functional genetic variants in their regulatory units. Deckert et al. [90] first reported an association between a promoter variant for MAO-A on the X-chromosome and panic disorder in two independent populations. A subsequent replication test in a family-based association sample was unable to support the initial report [91].

The gene encoding another monoamine-metabolizing enzyme, catechol-O-methyltransferase (COMT), carries a functionally relevant variant that is hypothesized to impact on several psychiatric disorders and their clinical presentations. Until now, there has been no evidence for an association with anxiety disorders [92]. Lack of evidence for association with the COMT variants was also reported for the anxiety-related traits of behavior inhibition and neuroticism [93].

Target of anxiolytic drugs: GABA and its receptors. The GABAergic systems are implicated in the pathophysiology and treatment of anxiety disorders: GABA-A agonists like benzodiazepines are anxiolytic and there is evidence for a decreased benzodiazepine receptor function in anxious patients; in temporal correlation with the onset of anxiolytic action, mRNA of specific GABA-receptor subunits is expressed by benzodiazepines in critical areas in the brain. The genes of GABA-receptor subunits carry multiple polymorphisms. Unfortunately, candidate gene studies of GABA-receptor subunits did not form replicable associations with panic disorder [94]. However, recently an association study with behavior inhibition, an anxiety-related phenotype, found modest evidence that an isoform of the glutamic acid decarboxylase (GAD65) was less common in affected subjects. While this finding requires replication, it is noteworthy that the GAD65 knock-out mouse revealed increased anxiety-like behavior [68].

Anxiety-provoking substances and their targets: CKK and adenosine receptors. Cholecystokinin-tetrapeptide triggers panic attacks in patients with panic disorder more often than in controls; this effect can be blocked by antipanic substances (e.g., imipramine). The CKK gene carries a polymorphism in the promoter region. It has been tested for association with panic disorder with positive results [95], which were not consistently replicable [96]. Caffeine can also provoke anxiety in panic disorder probands through its binding to adenosine receptors. Several silent mutations were identified in the two subtypes of adenosine receptors A_1 and A_2 AR. Some evidence for an association with A_2 AR gene variants was found by Deckert et al. [97] for panic disorder. A replication test could not confirm the initial result [98].

C. Genetic Animal Models

Anxiety as a reaction to threatening situations is not unique to humans. Avoidance and anxiety-like behavior is a physiological reaction in all rodents and serves a protective function; anxiety behavior is an evolutionary conserved phenotype (in contrast to depression). Thus, we can use animal models in order to understand mechanisms underlying human anxiety. Multiple behavioral models in animals were developed in the past with the following results: as in humans, there is a broad variation of anxiety-related behavior among members of the same species and the same strain; selective breeding experiments have clearly demonstrated that genetic factors control the degree of anxiety-like behavior also among mice and rats. Because of the apparent homology between human and animal

behavior, these studies provided valid animal models for anxiety disorders by defining extreme behavioral variants as pathological anxiety. In this context, anxiety-like behavior can be observed and quantified by avoidance behavior and behavior inhibition in aversive conditions, and by conditioned fear and potential startle [99].

More recently, the inactivation of specific genes became feasible among mice using transgenic technology (knock-out lines) in order to study the impact of a specific gene on the behavior and underlying neurobiological circuits. By these means, new promising candidate genes can be identified beyond our current knowledge of the pathophysiology of the disease (what is particularly advantageous, given the limited knowledge on the pathophysiology of anxiety disorders). A large number of genes contributing to neuronal messengers, receptors, and intracellular regulations have been targeted in knock-out lines and tested for anxiety-like behavior (see overview, Ref. 100). Apart from confirming the involvement of serotonin, gamma-aminobutyric acid, and corticotropin-releasing hormone as major mediators of anxiety and stress-related behaviors, two novel groups of anxiety-relevant molecules have been revealed. The first group consists of neurotrophic-type molecules, such as interferon-gamma, neural cell adhesion molecule, and midkine, which play important roles in neuronal development and cell-to-cell communication. The second group comprises regulators of intracellular signaling and gene expression, which emphasizes the importance of gene regulation in anxiety-related behaviors. Defects in these molecules are likely to contribute to the abnormal development and/or function of neuronal networks, which leads to the manifestation of anxiety disorders.

V. CONCLUSION

All anxiety disorders are under genetic control probably with multiple genes impacting on all disorders. The contributing genes still have to be identified. The search for susceptibility genes is hampered by the uncertainty of what is transmitted in families. The familial genetic transmission reveals diagnostic specificity as well as unspecificity between the various subcategories of anxiety disorders as well as with depression and alcoholism. Thus, diagnostic subcategories, even if they are diagnosed with maximal precision and reliability, will not present the most appropriate phenotypes for the detection of the underlying genes. The availability of the expanding technical potentials to map the variability of the human genome, and to explore the relationship to genetically influenced phenotypes, will not automatically result in a successful identification of genes accounting for anxiety disorders. In order to reduce the complexity of the phenotype-genotype relationship and to use forthcoming technical potentials, two critical steps have to be achieved: (1) the development of a genetic nosology [1] using behavioral and neurobiological vulnerability indicators and endophenotypes; [2] the recruitment of large samples of nuclear families (with affected index cases) and/or of cases and controls in order to use the new prospects of genome-wide association studies.

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10

Stress-Responsive Neurohormones in Depression and Anxiety

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I. INTRODUCTION

Stress is classically defined as a threatening of homeostasis to which the organism, in order to survive, responds with a large number of adaptive responses. According to work by Cannon, this mainly implicates the sympathetic nervous system and hormones of the adrenal medulla. Selye was the first to suggest that neuroendocrine factors play a decisive role and he considered the pituitary adrenal system to be the major organizer of the nonspecific responses to stress. Any type of emotional or physical stressor sets into motion a cascade of processes that fine-tune the adaptive response according to specific demands. Sympathetic pathways are activated to allow for enhanced alertness and focused attention, whereas vegetative functions such as feeding, sleep, and sexual drive are decreased. Peripherally, the humoral and neural systems support the most pressing requirements by elevating heart rate, blood pressure, respiratory rate, and gluconeogenesis. An integral part of adaptation to a stressor is the protection of the organism against an overreaction and the curtailment of the response following termination of the stressor. If the organism is incapable of terminating the reponse to stress at the end of the exposure, or if it is exposed to chronic stress, adaptive mechanisms can lead to pathological changes. Defects in this counterregulatory mechanism may be either genetically encoded, acquired during premorbid life, or they may be a scar imprinted by previous disease episodies or traumatic events. Whatever their origin, there is plausible evidence that changes in stress-adaptive mechanisms are involved in the development, treatment, and prevention of depression and anxiety disorders.

II. THE STRESS RESPONSE

In response to acute physical or psychological stress, parvocellular neurons of the paraventricular hypothalamus (PVN) produce increased amounts of corticotropin-releasing hormone (CRH), which is released into portal vessels activating the secretion of corticotropin (ACTH) from anterior pituitary cells. In turn, ACTH enters the circulation and elicits corticosteroids from the adrenal cortex. This rapid activation of the hypothalamic pituitary adrenocortical (HPA) system can be life sustaining because of the metabolic effect of elevating blood glucose levels. Other stress-related responses needed for life-sustaining adaptations encompass a number of behavioral reflexes elicited by activation of the HPA system, presumably by an increase in CRH release. Activation of the stress response may be affected by ascending aminergic input from the locus coeruleus and the raphé nuclei. However, limited direct input to the PVN and dense innervations of central limbic structures further suggest indirect pathways in the modulation of the stress response. In addition, GABA-, glutamate-, and possibly acetylcholine-containing neurons play a role in excitation of the PVN.

A. Corticotropin-Releasing Hormone (CRH), CRH Receptors, and Natriuretic Peptides

CRH is a 41-residue peptide originally isolated by Vale and colleagues from ovine hypothalamus [1]. Sequences for human and rat CRH were subsequently determined and found to be identical to each other, and they differed from ovine CRH in 7 of the 41 amino acid residues [2]. Two important CRH-containing neuronal tracts originate in the PVN of the hypothalamus: CRH neurons with an endocrine role coproduce arginine vasopressin (AVP) and terminate at the median eminence. CRH neurons involved in autonomic functions produce CRH alone and project to the brain stem and the spinal cord. CRH is also found in the cerebrocortex, limbic system, locus coeruleus, and olfactory bulb, although at lesser concentrations. Besides its role as the principal secretagogue of ACTH, CRH has neurotransmitter-like actions outside the hypothalamus as well. Of note, CRH neurons innervate noradrenergic centers in the pons (locus coeruleus) and the central nucleus of the amygdala, areas of recognized importance in anxiety and stress response.

The effects of CRH are mediated by two specific G-protein-coupled seven-transmembrane domain receptors called CRH-R1 and CRH-R2. Furthermore, two splice variants of the CRH-R2 receptor have been characterized in the rat brain [3]. A nearly exclusive expression of CRH-R1 has been observed in frontal cortical areas, the cholinergic basal forebrain, the brainstem cholinergic nuclei, and superior colliculus, the basolateral nucleus of the amygdala, the cerebellum, the trigeminal nuclei, and the anterior pituitary. CRH-R2α, on the other hand, is more strongly expressed in the paraventricular nucleus, the lateral septum, the cortical and medial nuclei of the amygdala, and the serotonergic raphe nuclei. Mixed receptor populations have been reported for the olfactory bulb, the hippocampus, the entohirnal cortex, the bed nuclei of the stria terminalis and the periaqueductal gray [4]. Interestingly, low-to-moderate CRH-R1 signals have further been reported for the central nucleus of the amygdala and the substantial nigra [5]. Moreover, recent evidence points toward expression of CRH-R1 in the locus coeruleus, at least in the nonhuman primate brain [6]. However, differences among species have to be considered when predictions about clinical implications of drugs acting at CRH receptor subtypes are based on manipulations of rodent CRH receptors [7].

Most studies exploring behavioral effects of CRH in animals have used intracerebroventricular or site-specific effects of CRH, and all agree that CRH mediates numerous anxiogenic and fear-related aspects of stress. These include the CRH-induced potentiation of acoustic startle, suppression of social interaction, and an increase in stress-induced freezing behavior [8]. This is further supported by transgenic mice overexpressing CRH. These mice have deficits in emotionality and were used as a genetic model of anxiogenic behavior [9]. As shown in Figure 1, most of the signs and symptoms induced by CRH administration correspond to symptoms of today's diagnostic algorithms for depression and anxiety disorders.

Decreased CRH neurotransmission has been studied by administering antisense oligodeoxynucleotides corresponding to the start-coding region of CRH mRNA. The application of this kind of gene therapy to stressed rats produced a decrease in CRH biosynthesis and led to the reduction of anxiety-related behavior [10]. Comparison of the behavioral effects of antisense probes that were either directed against CRH-R1 or against R2-receptor mRNA suggested that CRH-R1 is more likely to convey anxiety and, possibly, depression-related signaling [11,12]. Complementary evidence was provided by the generation of CRH-R1-receptor-deficient mouse mutants, which proved to be less anxious than normal

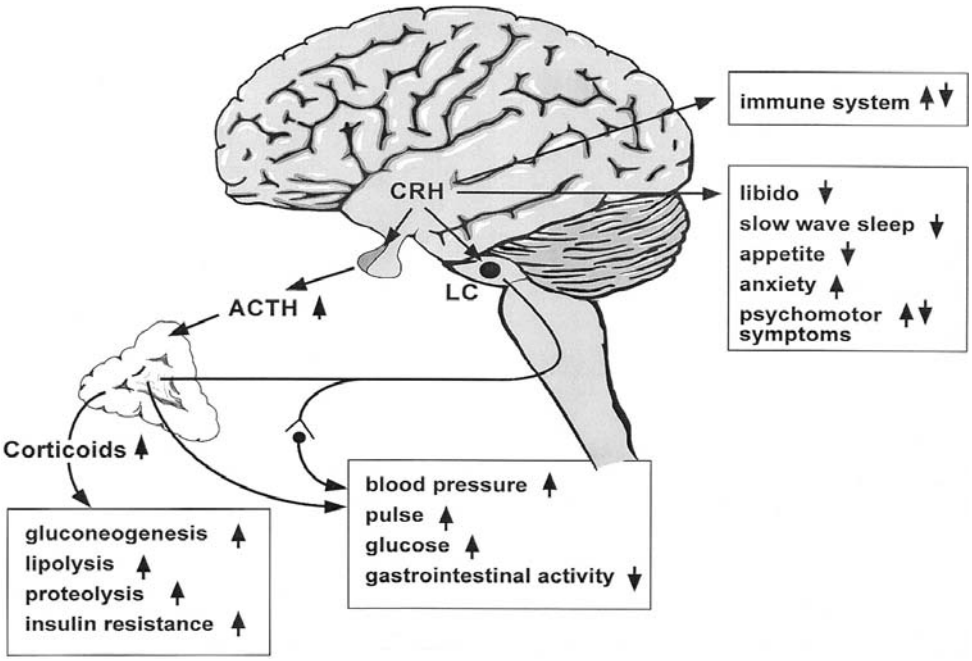


Figure 1 Animal studies in which CRH was injected intracerebroventricularly or CRH synthesis was disrupted (knockout mice or antisense oligodeoxynucleotide treatment of rats) or CRH receptor function was manipulated through antagonists, gene therapy or gene targeting are in accord with indirect clinical evidence that many signs and symptoms present in major depression can be attributed to enhanced central activity of CRH (according to Ref. 7).

mice [13,14]. Conflicting results with respect to anxiety-like behavior were described for CRH-R2-receptor knockout mice [15,16].

Whereas several peptides besides AVP are known to act synergistically with CRH, the only peptide candidate in humans that inhibits the HPA system at all regulatory levels of the system seems to be atrial natriuretic peptide (ANP). ANP has been shown to inhibit the stimulated release of CRH and ACTH in vitro and in vivo. This can be observed in humans as well, where ANP inhibits the CRH-induced ACTH [17], prolactin [18], and cortisol secretion [19]. ANP is not only synthesized by atrial myocytes [20] and released into the circulation, but also is found in neurons of different brain regions [21] where specific receptors have been found. ANP receptors and immunoreactivity have been found in periventricular and paraventricular hypothalamic nuclei, the locus coeruleus, and the central nucleus of the amygdala. HPA modulation by ANP is shown in Figure 2.

Intracerebroventricular administration of ANP elicited anxiolytic activity in the open-field, social interaction, and elevated plus-maze tests [22,23]. The effects of central and peripheral administration of atriopeptin-II, a 23-amino-acid residue peptide of ANP (Ser^{103}-Arg^{125}), was furthermore investigated in the elevated plus-maze test in rats previously exposed to a social defeat stress. Results show that the intracerebroventricular, intra-amygdala, and intraperitoneal administration of atriopeptin-II produced anxiolytic effects without affecting spontaneous locomotor activity [24]. In line with opposite endocrine effects of C-type natriuretic peptide (CNP) [25], intracerebroventricular administration of CNP had a clear anxiogenic effect in the elevated plus-maze [26]. The blockage of this anxiogenic effect of CNP by a CRH receptor antagonist [27] gives further evidence for

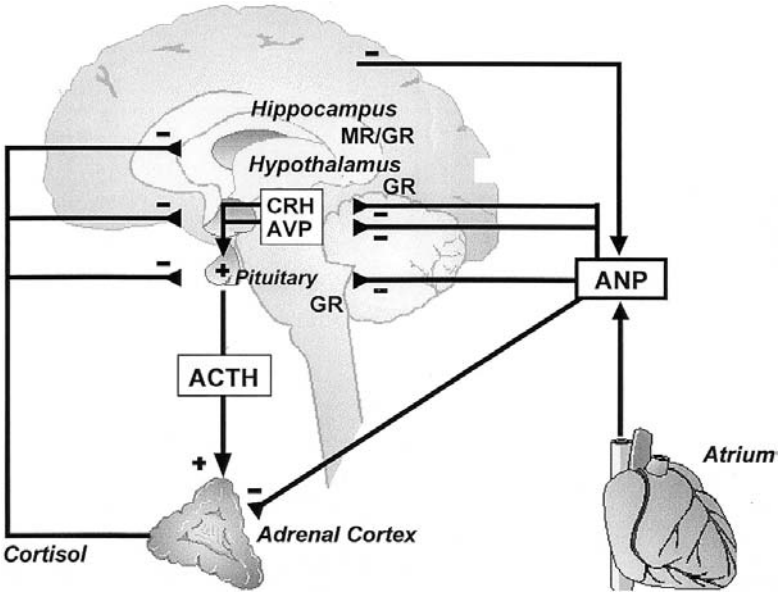


Figure 2 Preclinical and clinical studies show that ANP inhibits the HPA system at the hypothalamus, the pituitary, and the adrenal cortex. ANP is released from the heart but is found in the CNS as well. *GR, glucocorticoid receptor; MR, mineralocorticoid receptor.*

a bidirectional interaction of CRH and natriuretic peptides, with respect not only to the HPA-system, but also to anxiety-related behavior.

B. Glucocorticoids, Glucocorticoid Receptors, and Neuroactive Steroids

Corticosteroids act on neurons by two principal mechanisms: (1) genomic actions, where the steroid enters the cell and binds to cognate receptors that transform into transcription factors, modulating the expression of steroid hormone-regulated genes; and (2) nongenomic actions, where steroids bind to sites at synaptic membranes, affecting ion conductance (Figure 3).

The central role of corticosteroids in the maintenance of basic functions and in adaptation and survival under stressful conditions has led to the evolution of two distinct receptor systems, the mineralocorticoid receptors (MR) and the glucocorticoid receptors (GR) [28]. These two receptors, the MR with about 10-fold higher affinity to cortisol than the GRs, provide sufficient control over tonic (MR) and stress-response (GR) mechanisms in the hippocampus allowing for physiological responsiveness over a wide concentration range of cortisol. Under baseline conditions, the MRs are 90% occupied by cortisol,

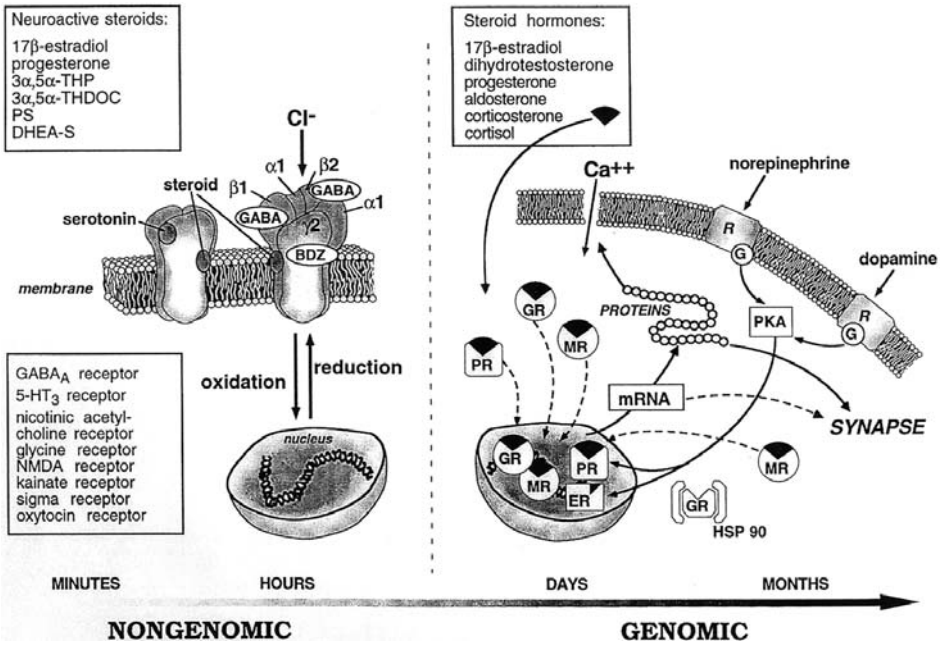


Figure 3 Nongenomic and genomic effects of neuroactive steroids. The list in the upper left-hand corner of the figure shows steroids that fulfill the criteria for neuroactive steroids. The lower list gives neurotransmitter receptors that are targets for steroid modulation. The right-hand side of the figure describes the classical model of steroid-hormone activation via the steroid-receptor cascade at the genomic level, which takes place over minutes to hours. The list on the right-hand side of the diagram gives typical steroid hormones. *BDZ, benzodiazepines; ER, estrogen receptor, G, G-protein; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PS, pregnenolone sulfate; R, receptor.* (From Ref. 33.)

whereas the GRs are only 50% occupied. In the early morning, when HPA activation occurs, or following stress, the GRs become more fully occupied in order to curtail HPA-activating mechanisms. Continuing corticosteroid hypersecretion leads to overexposure of GRs and MRs which in turn are downregulated. The most important control mechanism of the HPA system is an autoregulatory feedback by corticosteroids that can inhibit ACTH secretion, both directly and indirectly by rapid (within minutes) and delayed (more than 2 h) effects [29]. At the DNA level, where positive and a few negative (including ACTH suppression) gene regulations occur, GR and MR bind as heterodimers. Whenever colocalyzed as in the case in hippocampal cells, GR and MR can heterodimerize, which expands the repertoire of cellular responses to fluctuating corticoid levels [30].

In the last decade, considerable evidence has emerged indicating that certain steroids may alter neuronal excitability via their action at the cell surface via interaction with certain neurotransmitter receptors. For steroids with these particular properites, the term "neuroactive steroids" has been used (31–33). Other authors use the term neurosteroids, which can be misconstrued, because these steroids are not specifically synthesized in neurons. Whereas it seems attractive to differentiate genomic from nongenomic actions upon neurons, it could be demonstrated that transitions may also exist between these two modes of action [34]. Steroids that are believed to have limited effects at membrane sites, such as allopregnanolone (3α,5α-THP) and allotetrahydrodeoxycorticosterone (3α,5α-THDOC), can be oxidized intracellularly and then exert genomic actions through progesterone receptors. Thus, the steroid molecule provides a rather flexible structure that can be modified, depending on the tissue, to satisfy specific demands.

The first behavioral observations related to these steroids date back to Selye, who, over 50 years ago, reported that progesterone and deoxycorticosterone (DOC) have a strong sedative action through their A-ring-reduced metabolites. These two steroids, 3α,5α-THP and 3α,5α-THDOC, bind at γ-aminobutyric acid$_A$ (GABA$_A$) receptors to enhance GABA-induced chloride currents. In rats, 3α,5α-THDOC and 3α,5α,THP are elevated in cortical and hypothalamic tissue after stress [32], and they have been shown to be anxiolytic and hypnotic, respectively, as predicted by electrophysiology, where a benzodiazepine-like action was demonstrated [34]. Several other neuroactive steroids have opposite effects. For example, the sulfated form of pregnenolone has been observed to antagonize GABA$_A$-receptor-mediated chloride currents by reducing the channel open frequency [31] and therefore being proconvulsant. Interestingly, 3α,5α-THP dampens the activity of the HPA system and counteracts CRH-induced anxiety. In addition, neonatal treatment of rats with 3α,5α-THDOC abolishes the behavioral and neuroendocrine consequences of adverse early life events [35].

III. DEPRESSION AND ANXIETY DISORDERS

Today, there is a trend to create an increasing number of diagnostic categories, which is reflected by the fact that the official diagnostic manual of the American Psychiatric Association for diagnoses released in the 1950s (DSM-I) contained 106 diagnostic categories, while the current version of this manual (DSM-IV) has been expanded to over 400 categories. In this light, any attempt to validate a psychiatric diagnosis with laboratory findings must be frustrating. Instead of syndromes or diagnoses being contrasted with HPA measures, the latter should be considered as one of the features of the clinical phenotype. Ideally, these abnormalities should be incorporated as signs and symptoms into a multiaxial diagnostic scheme [36].

A. Major Depression

Approximately 50 to 60% of patients with major depression show distinct baseline changes in ACTH and cortisol secretion. Studying the pulsatile activity and circadian rhythmicity, it can be demonstrated that in depression the number of ACTH pulses is increased, while for cortisol it was not the number but the amount released per burst that was increased [37]. The coincidence of increased ACTH burst frequency and enhanced cortisol secretory amplitude is probably a secondary consequence of the hypersensitivity of the adrenal glands, which is further supported by increased cortisol surges after ACTH administration [38], the normal cortisol response despite blunted ACTH secretion following CRH stimulation [39] and an enlarged adrenal size during depression [40].

The dexamethasone suppression test (DST) has received considerable attention in psychiatric research because of its ease of use and its suggested potential for identifying patients with endogenous or melancholic depression. A large number of studies have shown that depressives frequently escape from the suppressive effect of 1 to 2 mg dexamethasone (DEX) [41–43], but to date the DST is no longer regarded as a diagnostic test [44]. The most promising application of the DST remains its use as a state marker, which can be applied longitudinally to follow-up treatment response [44].

After CRH became available for clinical studies, several groups consistently reported that ACTH response was blunted, regardless of whether ovine or human CRH was used [39,45] (Fig. 4). In some studies, an inverse relationship was found between baseline cortisol concentration and post-CRH ACTH concentrations, and it was concluded that elevated baseline cortisol concentrations account for ACTH blunting via negative feedback. However, other factors, such as CRH receptor desensitization of corticotrophs, altered processing and storage of ACTH precursors and alternative processing of POMC may also contribute to this phenomenon.

A surprising finding emerged when patients with depression were given DEX and were then challenged with CRH in the afternoon of the following day. Inadequately suppressed cortisol concentrations and DEX together should be additive in blunting ACTH release via negative feedback. Against expectation, depressed patients showed a paradoxical pattern insofar as DEX pretreatment resulted in increased ACTH and cortisol responses to CRH despite combined endogenous (cortisol) and exogenous (DEX) glucocorticoid concentrations [46,47] (Fig. 4). This abnormality disappears after successful antidepressant treatment [48]. In those patients where the neuroendocrine abnormalities persisted, the risk of relapse was much higher [49,50]. The combined DEX/CRH test proved particularly useful as a predictor of increased risk for relapse [48–53]. Results of a study in which different doses of DEX were administered prior to CRH showed that ACTH and cortisol suppression occurs at higher DEX dosages in the depressives than in matched controls [54]. This shift of the dose-response curve to higher DEX dosages corroborates the view that negative feedback mechanisms through glucocorticoid receptors to which DEX binds are impaired in major depression [55].

In major depression as well as in chronic stress there is a shift to a gradually intensifying vasopressinergic regulation of the HPA system. Dexamethasone, which does not bind to corticosteroid-binding globulins, exerts its effect on the HPA system primarily at the pituitary corticotrophs and does not suppress the expression of hypothalamic CRH and vasopressin as effectively as endogenous corticosteroids. A transient GR desensitization gradually develops, and vasopressin expression, which reacts differentially to changes in glucocorticoid concentrations than does CRH expression, becomes less efficiently sup-

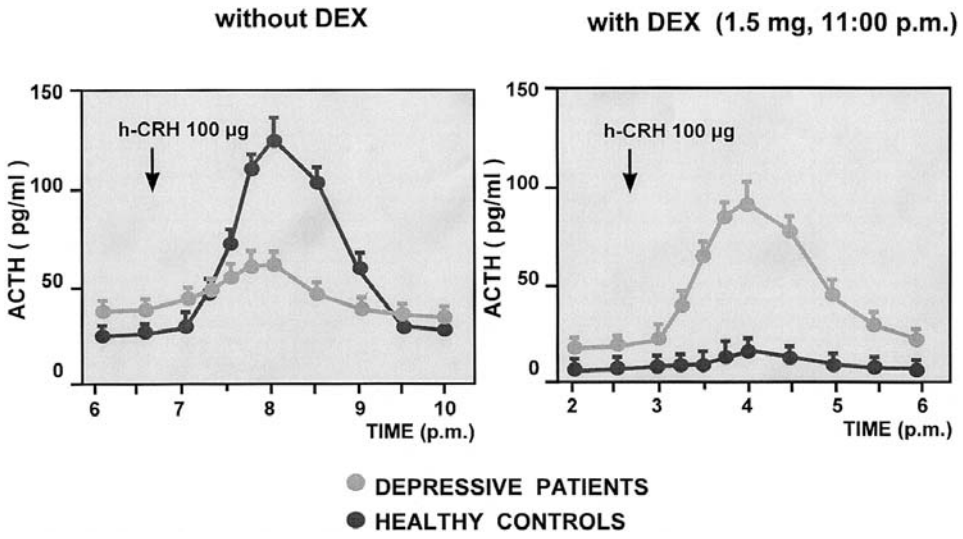


Figure 4 Patients with major depression usually have a blunted ACTH reponse to CRH, which is believed to be secondary to desensitized CRH1 receptors (left). After pretreatment with a low dose of dexamethasone, healthy controls do not show a substantial ACTH release, whereas the ACTH response in patients with major depression is comparable to the response of healthy controls not pretreated with dexamethasone (right).

pressed than CRH by circulating corticosteroids. In control subjects, only CRH and AVP administered together are able to override DEX suppression of the HPA system [56]. In patients with major depression, corticosteroid receptor function is genetically altered, as the Munich Vulnerability Study [57,58] suggests, or acquired as a result of impaired coping with stress, and this is why patients with major depression show a much less restrained release of central ACTH stimulants than healthy controls or depressive patients in remission.

The consequences of impaired regulation of cortisol concentrations are manifold, ranging from untoward effects in peripheral tissues (e.g., osteoporosis) to changes in the central nervous system. The latter are believed to comprise effects on morphology as well as on cognitive function [59]. Studies by Sheline et al. [60,61] and Bremner et al. [62] suggest that recurrent major depression is associated with hippocampal volume loss and that the degree of this change is determined by the duration of the illness. It has been proposed that the neuroendocrine changes in depression may account for the changes in hippocampal volume. Importantly, these reductions in hippocampal volume, which are also seen in patients with schizophrenia [63], a disease not particularly associated with hypercortisolemia, do not necessarily reflect cell death [62]. Moreover, in post-traumatic stress disorder, decreased hippocampal volume is associated with normal or even reduced plasma and urine cortisol concentrations [64]. Factors other than glucocorticoids also account for stress-induced reversible or permanent morphological changes in the hippocampus. For example, a study in nonhuman primates, where high dosages of glucocorticoids were given for 12 months to aged macaques no evidence was found for decreased hippocampal volume, subfield volumes, subfield neuronal density, and subfield total neuronal number [65]. This finding is in accordance with a report of postmortem brains of patients

with major depression and of patients treated with synthetic corticosteroids, where morphological changes or signs of cell death were absent [66].

B. Panic Disorder

Only subtle changes in baseline HPA activity occur in panic disorder patients. Abelson and Curtis [67] showed that panic disorder patients tend to show elevated cortisol concentrations at night, a greater amplitude of ultradian secretory episodes, but a similar number of secretion pulses compared to controls. Interestingly, these HPA system alterations in panic disorder patients are modulated by illness severity and treatment-seeking behavior: patients who were actively in treatment had higher cortisol concentrations than patients who were enrolled in the study through advertisement. Most studies, however, show that compared to patients with major depression, panic disorder patients have decreased [68] and, compared to control subjects, unchanged baseline cortisol concentrations [69]. Studies in panic disorder patients with agoraphobia have been more inconsistent: some studies demonstrate mild hypercortisolism while other studies do not [70,71].

Compared to patients with major depression, lower rates of nonsuppression following a standard DST has been found in panic disorder patients. Furthermore, compared to control subjects, panic disorder patients in general do not have a higher rate of nonsuppression in the DST [71,72]. Like patients with major depression, panic disorder patients have a blunted ACTH response to CRH [73,74], although it has been suggested that this attenuation has different reasons [75]. After DEX pretreatment, panic disorder patients have higher CRH-induced ACTH and cortisol concentrations than control subjects and lower levels than a reference group of depressed patients [76]. CSF concentrations of CRH are not altered in patients with panic disorder [77].

Although it seems unlikely that CRH is not secreted during panic attacks, there are no empirical data demonstrating this release directly. Only few investigations have managed to study the HPA system during naturally occurring panic attacks: while spontaneous panic attacks in the laboratory are not accompanied by an increase in cortisol concentrations [78], spontaneous attacks in the natural environment are reported to be accompanied by increased cortisol concentrations [79]. In the context of exposure therapy, situationally triggered panic attacks were not accompanied by an increase in cortisol concentrations [80]. Panic disorder patients who experience a panic attack in response to lactate demonstrate significantly higher plasma cortisol concentrations immediately preceding lactate infusion compared with nonpanicking patients and normal control subjects [81]. This activation of the HPA system correlates with self-reported fear, and may be related to anticipatory anxiety. While lactate-induced panic attacks are not accompanied by an activated HPA system, cholecystokinin tetrapeptide (CCK-4)-induced panic attacks are accompanied by an exaggerated ACTH secretion [82], providing evidence for an enhanced pituitary ACTH secretion during panic attacks despite unchanged cortisol concentrations and points to a possible role of CRH in these attacks.

Negative feedback loops seem to be involved in the HPA-system alterations of patients with panic disorder as well. Compared to patients with major depression, panic disorder patients have a significantly increased number of lymphocyte glucocorticoid receptors [83]. Furthermore, a significantly accelerated ANP release has been described in patients with lactate-induced panic attacks [84] and it has been suggested that this increase contributes to the paradoxical blunting of ACTH and cortisol secretion during lactate-induced and most likely spontaneous panic attacks as well.

C. Post-Traumatic Stress Disorder

Compared with normal controls and in contrast to other psychiatric disorders, patients with post-traumatic stress disorder (PTSD) have lower mean 24-h urinary cortisol secretion [85], lower baseline plasma cortisol concentrations [86], increased glucocorticoid binding in lymphocytes [84], and increased sensitivity to the HPA-suppressive effects of DEX [87], which, taken together, indicate enhanced negative feedback capacity in these patients. Further studies will have to clarify whether these abnormalities are a consequence of the trauma and the disorder or whether they represent premorbid alterations, increasing the risk for PTSD after trauma exposure. Preliminary data demonstrated that a history of a previous assault leads to attenuated cortisol concentrations in the immediate aftermath of a subsequent rape in women [88].

Like patients with major depression, PTSD patients have increased CSF CRH concentrations [89] and a blunted ACTH response to the CRH stimulation test [90]. Further evidence for a hypersecretion of CRH in PTSD comes from a study showing an increased ACTH release in response to metyrapone [91], which impedes adrenal steroidogenesis by blocking the conversion of 11-deoxycortisol to coritsol. This finding further underscores the role of increased sensitivity of the glucocorticoid receptors in HPA-system abnormalities in PTSD.

IV. STRESS-RESPONSIVE NEUROHORMONES IN THE TREATMENT OF DEPRESSION AND ANXIETY

$GABA_A$-receptor modulation by benzodiazepines has long been practiced in the short-term treatment of depression and anxiety disorders. However, side effects, tolerance development, and dependence limit their use to highly selected patients in the early phase of drug treatment as an adjunct to antidepressants [92]. Inhibition of CRH release by benzodiazepines has been described in the hypothalamus [93] and the median eminence [94]. In panic disorder patients, benzodiazepine treatment resolves pretreatment HPA-system abnormalities [95]. Although neuroimaging studies are indicative of changes in benzodiazepine–$GABA_A$-receptor function in panic disorder [96–98], the hypothesized shift in the benzodiazepine receptor "set-point" [99] could not be substantiated [100,101]. At the moment, long-term pharmacological treatment of depression or anxiety disorders is best performed with antidepressants. Their effects on stress-responsive neurohormones and possible new treatment approaches will be reviewed in more detail.

A. Antidepressant Treatment

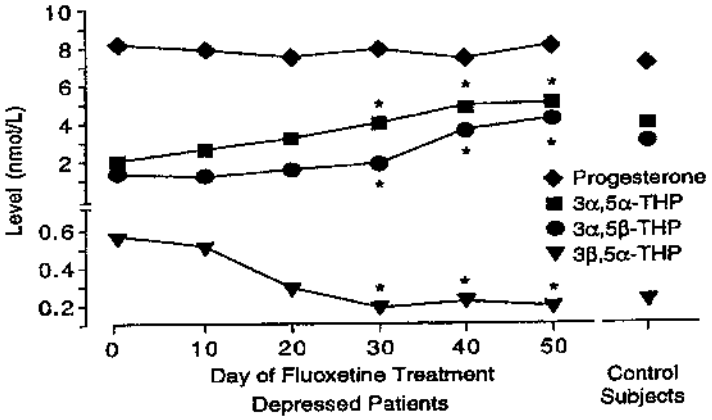
Antidepressants are clinically effective not only in depression, but also in panic disorder and PTSD. In contrast to their clinical effectiveness, elucidation of the mechanisms of action of these drugs has been less successful. The hypothesis that has dominated the field is based on the assumption that a biogenic amine-deficiency underlies mood disorders and that this can be remedied by inhibition of presynaptic reuptake transporters of serotonin and/or norepinephrine, thus increasing the transmitter concentrations at presynaptic sites. This hypothesis, however, does not explain why it takes at least weeks before antidepressants become effective, whereas reuptake inhibition occurs immediately. Two new hypotheses, which are complementary rather than mutually exclusive, have been forwarded to explain how antidepressants work. One hypothesis, developed by Duman et al. [102], focuses on the effects of activation of the cAMP cascade through cell membrane receptors,

followed by enhanced induction of CREB and hippocampal brain-derived neurotrophic factors (BDNF). The other hypothesis submits that antidepressants act through improving GR function [57,103]. In the CNS, these modulations also affect brain regions not or only indirectly connected to the peripheral HPA system, which regularly stabilizes under the influence of antidepressants.

In vivo experiments showed that rats, when treated chronically with antidepressants, displayed decreased baseline and stress-induced levels of plasma ACTH and corticosterone. Upon analyzing the capacity of MR and GR in the hippocampus of these rats, it was found that the first change was seen in MR binding, which increased after 1 week of treatment [104,105]. In the light of inhibitory effects of MRs on HPA activity, which is reflected by studies employing MR antagonists in rats and humans or MR antisense in rats [106], the observed upregulation of MR capacity seems to be the first step necessary for the inhibition of hypothalamic CRH neurons. This effect is followed by increased GR transcription capacity [104,105,107]. The MR upregulating effects of antidepressants and the subsequent reduction of HPA overactivity in depressed patients underscore the importance of appropriate MR function. This is further supported by a clinical trial in which antidepressant response to amitriptyline in major depression was impared by co-administration of spironolactone, an MR antagonist (Hundt et al., unpublished results, cited in Ref. 7).

The effects of antidepressants on GR function have also been studied using transgenic mice expressing GR antisense: after long-term treatment with moclobemide, a reversible MAO-A inhibitor, these mutated mice not only show normalized HPA activity but also changes in several tests of anxiety and memory [108]. These findings support the notion that antidepressants act by improving the negative feedback capacity of the HPA system at various levels and that the setpoint of the HPA-system activity is modified in a way that "buffers" the hormonal response to stressors. This may represent one mechanism by which patients who have recovered from depression or an anxiety disorder may be protected by long-term antidepressant treatment against further stress-induced relapses.

A new line of research in regard to the mechanisms of antidepressants was stimulated by the observation that in animal studies the selective serotonin reuptake inhibitor (SSRI) fluoxetine, which is widely used for the treatment of depression and anxiety, may enhance the concentrations of 3α,5α-THP in the rat brain [109]. At the molecular level, it has been demonstrated that SSRIs shift the activity of the 3α-hydroxysteroid oxidoreductase, which catalyzes the conversion of 5α-DHP into 3α,5α-THP, toward the reductive direction, thereby enhancing the formation of 3α,5α-THP [110]. Additionally, 3α,5α-THP has been suggested to possess antidepressant-like effects in mice using the Porsolt forced swim test [111]. These preclinical findings suggest that 3α-reduced neuroactive steroids such as 3α,5α-THP may play a role in the treatment with antidepressant drugs. Indeed, the concentrations of GABA-agnostic neuroactive steroids 3α,5α-THP and 3α,5β-THP were reduced in plasma of depressed patients, while there was an increase in 3β,5α-THP, an antagonistic isomer of 3α,5α-THP [112,113], as shown in Figure 5. In contrast to preclinical data, also tri- and tetracyclic antidepressants interfered with the composition of neuroactive steroids in a similar way as did SSRIs [112]. In addition, antidepressants do not generally shift the activity of the 3α-hydroxysteroid oxidoreductase toward the reductive direction. The concentrations of 3α,5α-THDOC were elevated during depression, probably as a consequence of hypercortisolemia, and reduced by fluoxetine [114], but not by tri- or tetracyclic antidepressants [115]. Thus, the effects of antidepressants on neuroactive steroids also appear to be substance substrate-specific. So far no data are available whether



[a]Levels at day 0 and during fluoxetine treatment were compared by tests with contrasts in repeated measures ANOVA.
*$p<0.05$.

Figure 5 Mean plasma concentrations of progesterone, 3α,5α-THP, 3α,5β-THP and 3β,5α-THP in patients with major depression during fluoxetine treatment and in control subjects. (From Ref. 112.)

similar effects on the concentrations of neuroactive steroids can be achieved by nonpharmacological interventions such as electroconvulsive treatment or psychotherapy. Furthermore, no clinical data on the potential antidepressant activity of 3α-reduced neuroactive steroids are available.

While no data on the role of a 3α-reduced neuroactive steroids in the treatment of PTSD are published until now, in panic disorder patients, opposite changes to those seen in major depression emerged. At baseline, patients with panic disorder had significantly increased concentrations of the positive allosteric modulators 3α,5α-THP and 3α,5β-THP together with significantly decreased concentrations of 3β,5α-THP, a functional antagonist for $GABA_A$-agonistic steroids, which might result in an increased GABA-ergic tone. SSRI treatment did not influence these changes of neuroactive steroid concentrations [116]. Most strikingly, during experimentally induced panic attacks, drastic changes of neuroactive steroid concentrations occur, paralleling psychopathological changes and resulting in a dramatically reduced GABA-ergic tone [117] supporting the idea that the increased baseline concentrations of $GABA_A$-agonistic neuroactive steroids may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks. Major depression and panic disorder show distinct changes in the concentrations of neuroactive steroids and the effects of antidepressant treatment. When attempting to pharmacologically modify the equilibrium of neuroactive steroids as a treatment for psychiatric disorders [118], consideration should also be given to baseline concentrations and possible counterregulatory mechanisms.

C. Possible Treatment Approaches

If one assumes that normalization of an altered HPA setpoint is an essential mechanism for antidepressant drug action, the question arises as to how this goal is achieved. An alternative treatment approach would, therefore, be either to suppress the behavioral symptoms of enhanced CRH by CRH receptor antagonists or ANP receptor agonists or to block

the many untoward effects of excessive corticosteroid secretion by administration of GR antagonists. Furthermore, modulation of the concentration of neuroactive steroids has been suggested as a new psychopharmacological tool as well [33,119].

The most straightforward strategy to restrain the depressionogenic and anxiogenic effect of excessive CRH production and relase is the administration of CRH-R1 antagonists. One of these compounds (R121919), a pyrazolopyrimidine has been tested [7]. The first open-label trial with this substance in depressed patients significantly reduced depression and anxiety scores as was seen in both clinican and patient ratings, suggesting that this type of compound may have considerable therapeutic potential [120]. The question of whether CRH-R1 antagonists resolve the entire depressive syndrome or only several stress-related symptoms, such as pathological anxiety, loss of appetite and sexual drive, sleep disturbance, psychomotor and cardiovascular changes, etc., as found in animal experiments, remains to be validated in controlled clinical trials. Although especially promising until now, there are no data on the therapeutic potential of CRH-R1 antagonists in the treatment of panic disorder or PTSD.

In preclinical studies with two selectively bred rat lines, in high-anxiety-related behavior (HAB) R121919 reduced anxiety-related behavior in a dose-dependent manner, whereas it had virtually no behavioral effect in low-anxiety-related behavior (LAB) rats [121]. In contrast, the stress-induced activity of the HPA system, as determined by simultaneously measured plasma ACTH levels, was similarly blunted by R121919 in both HAB and LAB animals. Because CRH gene expression was observed to be increased in the locus coeruleus of HAB rats and activation of the brain area is associated with stress and anxiety in rats, the anxiolytic effect of R121919 is consistent with an action of the CRH-R1 antagonist at this brainstem locus [122].

An alternative approach focuses upon reduction of cortisol either by cortisol synthesis inhibitors or by blocking their action by antagonizing their effects at receptors. To date, only few studies with small sample size exist, which limit their clinical acceptance. Of particular interest is the use of metyrapone, which results in an inhibition of hydroxylation at position C11 of the steroid molecule, thus preventing the synthesis of cortisol. Metyrapone treatment has produced antidepressant-like behavioral changes in two frequently used rodent models for screening antidepressive compounds, the forced swim-test and the olfactory-bulbectomized rat [123]. Numerous exploratory studies and one small placebo-controlled trial suggest that metyrapone has antidepressant potential [124]. This clinical effect seems to contradict the notion that decreased cortisol-mediated feedback may elicit increased CRH in all those brain areas where CRH gene expression is controlled by GR. However, the additional bioavailability of non-C11-hydroxylated adrenal steroids increases the pool of neuroactive steroids [125], which can exert a number of neuroendocrine and behavioral symptoms such as a reduction of anxiety-like behavior and suppression of CRH expression [35,126]. Ketoconazole, in addition to blocking adrenal steroid synthesis, inhibits cytochrome P450-dependent enzymes and has direct inhibitory effects on other pituitary cells than corticotrophs [127], thus, for example, affecting gonadal steroid secretion as well. Consistent with predictions from open-label studies, a recent placebo-contolled trial suggested that ketoconazole is superior to placebo among hypercortisolemic depressed patients [128]. Again, it is difficult to dissociate the effects of ketoconazole from the effects of other adrenal steroids that are produced at much higher rates because of the enzyme blockade.

Blockade of GR as a treatment strategy has been poorly studied due to the lack of specific antagonists. Preliminary results suggested that mifepristone (RU486) may be a

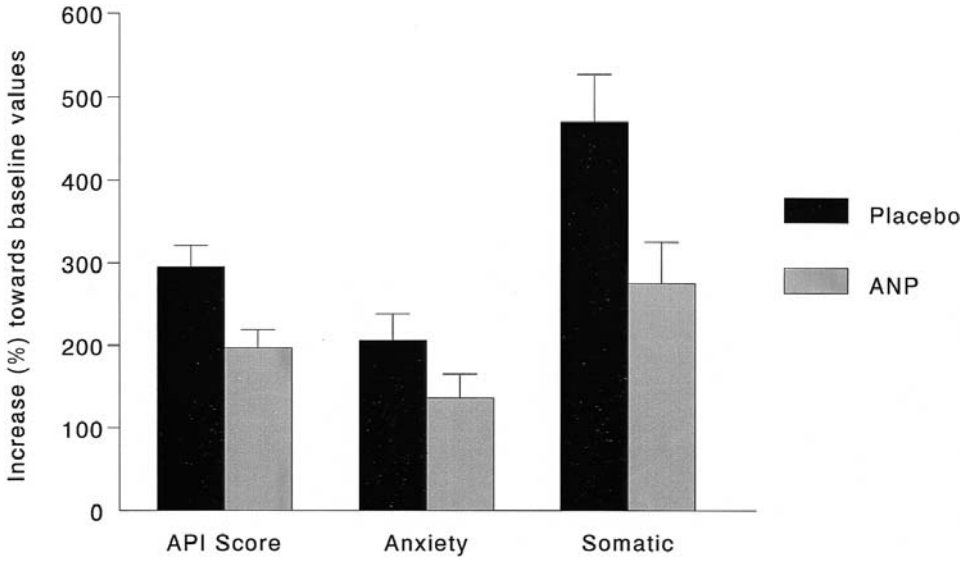


Figure 6 Normed CCK-4-induced increase in the API score, and the anxiety and somatic sub-scores in patients with panic disorder. Compared to placebo, ANP inhibits the CCK-4 induced increase in the API score and the anxiety and somatic subscores, giving evidence for an anxiolytic activity of ANP in patients with panic disorder. (From Ref. 131.)

useful treatment approach in major depression [129]. At the moment it is not fully clear how disturbed corticosteroid-receptor signaling can be influenced therapeutically. Conservatively, administration of a CRH-R1 antagonist is expected to shorten the onset of coadministered antidepressants. Such drugs may also work as antidepressants when administered as monotherapy [120]. Similarly, GR antagonists might not be effective enough as monotherapy, but they may be a worthwhile adjunct to antidepressants. Perhaps the most intriguing approach is the administration of GR antagonists to patient with psychotic depression. It has been submitted by Schatzberg and coworkers [130] that hypercortisolemia enhances dopaminergic transmission to an extent that results clinically in psychotic symptoms. Thus suppressing cortisol effects at GR may limit this dopamine excess and thus resolve psychotic symptoms. It is of note that psychotic depression is almost always associated with HPA overactivity.

Natriuretic peptides are specifically involved in the modulation of the HPA system at all regulatory levels [19]. Neuroanatomical distribution of the natriuretic peptide system supports a role of these peptides in anxiety modulation as well. And indeed, in rodents ANP was found to be anxiolytic [24], whereas CNP was anxiogenic [26,27]. In patients with panic disorder, basal ANP concentrations are lower when compared to healthy control subjects, but ANP concentrations are faster and more pronounced during experimentally induced panic attacks [17]. In line, there is recent evidence for an anxiolytic activity of ANP in humans: ANP decreases CCK-4-induced panic anxiety in patients with panic disorder [131] (Fig. 6) and healthy control subjects and attenuates the HPA-system activity by decreasing ACTH and cortisol stimulation [132]. Nonpeptidergic ANP receptor ligands may ultimately be used in the pharmacological treatment of anxiety disorders, like panic disorder.

VIII. SUMMARY

Clinical and preclinical studies have gathered substantial evidence that stress-response alterations play a major role in the development of major depression, panic disorder, and post-traumatic stress disorder. The stress response, the hypothalamic pituitary adrenocortical (HPA) system and its modulation by CRH, corticosteroids, and their receptors, as well as the role of natriuretic peptides and neuroactive steroids, are described. We review the role of the HPA system in major depression, panic disorder, and post-traumatic stress disorder, as well as its possible relevance for treatment. Impaired glucocorticoid-receptor function in major depression is associated with an excessive release of neurohormones, like CRH, to which a number of signs and symptoms characteristic of depression can be ascribed. In panic disorder, a role of central CRH in panic attacks has been suggested. Atrial natriuretic peptide (ANP) is involved in sodium lactate-induced panic attacks. Furthermore, preclinical and clinical data on its anxiolytic activity suggest that nonpeptidergic ANP-receptor ligands may ultimately be used in the treatment of anxiety disorders. Recent data further suggest a role of 3α-reduced neuroactive steroids in major depression, panic attacks, and panic disorder. Post-traumatic stress disorder is characterized by a peripheral hyporesponsive HPA system and elevated CRH concentrations in CSF. This dissociation is probably related to an increased risk for this disorder. Antidepressants are effective both in depression and anxiety disorders and have major effects on the HPA system, especially on glucocorticoid and mineralocorticoid receptors. Normalization of HPA-system abnormalities is a strong predictor of the clinical course, at least in major depression and panic disorder. CRH-R1 or glucocorticoid-receptor antagonists and ANP-receptor agonists are currently being studied and may provide future treatment options more closely related to the pathophysiology of the disorders.

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11

Neuropeptide Alterations in Depression and Anxiety Disorders

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I. INTRODUCTION

In the search for the underlying pathophysiology of the major psychiatric disorders, neuropeptides, in general, and hypothalamic-releasing factors, in particular, have been scrutinized closely. Our growing understanding of the brain over the past few decades has suggested complex interactions between classic monoamine neurotransmitters, such as dopamine (DA), norepinephrine (NE), and serotonin (5HT), and the growing numbers of neuropeptides found throughout the brain. Neuropeptides—molecules that contain two or more amino acids linked by peptide bonds—function as critical chemical messengers and are heterogeneously distributed through the peripheral and central nervous system (CNS). Many of these peptides exert diverse actions, functioning as hypothalamic hypophysiotropic-releasing factors, neuromodulators, and/or neurotransmitters. As understanding of the behavioral effects of these neuropeptides continues to grow, it has become increasingly apparent that dysregulation of the proper functioning of neuropeptide systems may be relevant to particular psychiatric disorders. In this chapter, we briefly review basic neuropeptide neurobiology most relevant to psychiatric disorders and summarize preclinical and clinical studies implicating neuropeptide alterations in the pathophysiology of mood and anxiety disorders.

II. RATIONALE FOR EXPLORING NEUROPEPTIDE MODULATION IN AFFECTIVE AND ANXIETY DISORDERS

Undoubtedly one early rationale for the intensive study of neuropeptide systems in patients with primary psychiatric disorders was the higher than expected psychiatric morbidity in patients with primary endocrine disorders such as Addison's disease or Cushing's syndrome. Disturbances in the feedback regulation of the hypothalamic–pituitary–end-organ axes are of considerable interest. Because hypothalamic hypophysiotropic neuropeptides ultimately control the activity of these neuroendocrine axes, considerable effort has been expended into the search for evidence of dysfunction in these systems. However, outside of the neuroendocrine axes, the ongoing discovery and characterization of new neuropeptides and multiple subtypes of neuropeptide receptors and their heterogeneous distribution have often suggested these peptides may modulate behaviors directly relevant to psychiatric disease. Because neuropeptides account for neurotransmission at a large percentage of CNS synapses and indirectly or directly modulate the activity of a diverse number of hormones and neurotransmitters that have been implicated in the pathogenesis of affective and anxiety disorders, their scrutiny may allow for elucidation of the primary pathophysiological deficits in these devastating illnesses.

One of the primary motivations for the continuing investigation of the major endocrine axes is the so-called "neuroendocrine window strategy." This strategy is based on a large literature that indicates that the secretion of the target endocrine organs (e.g., the adrenal cortex or thyroid) is largely controlled by their respective pituitary trophic hormones, which in turn are controlled primarily by the secretion of their respective hypothalamic release and/or release-inhibiting hormones. There is now considerable evidence that the secretion of these hypothalamic hypophysiotropic hormones is controlled, at least in part, by the classic neurotransmitters including 5HT, acetylcholine (ACh), and NE, all previously posited to play a preeminent role in the pathophysiology of affective, anxiety, and/or psychotic disorders.

However, the hypothesis that one can infer information about higher CNS neuronal activity as, for example, the activity of serotonergic neurons in a particular disease state solely by measuring the function of a specific endocrine axis, is, however far from proven and fraught with difficulty. Many factors other than a single neurotransmitter system affect the activity of the various endocrine axes. Nevertheless, taken together with other measures of neurotransmitter function, this approach can indeed provide useful information.

The contrasting behavioral and neurobiological effects of antidepressants [tricyclic antidepressants or monoamine oxidase inhibitors (MADs)], anxiolytics (SSRIs, SNRIs, and benzodiazepines), and antipsychotics as well as those drugs which induce or worsen depression (such as reserpine), anxiety (flumazenil, cholecystokinin), and psychosis (psychostimulants, phencyclidine) have provided yet another impetus for scrutiny of neuroendocrine and neuropeptide pathophysiology in the major psychiatric illnesses because virtually all of these agents alter the activity of one or more endocrine axis. This second core assumption, the "psychopharmacological bridge technique" posits that if a drug produces therapeutic effects and has specific biochemical actions, an etiological relationship between the therapeutic effects, biochemical changes, and the primary cause of the syndrome may exist [1]. For example, tricyclic antidepressants block reuptake of NE and 5HT, MAO inhibitors inhibit the metabolism of catecholamines and 5HT and, moreover, downregulation (decrease in the number) of β-adrenergic receptors that is associated with most antidepressant treatments occurs in association with the clinically successful treatment of de-

pression. The pharmacological bridge technique and "neuroendocrine window strategy" together suggest that alterations of a variety of endocrine axes exist within patients with major psychiatric disorders, and that many clinically efficacious agents act upon one or more of the neuropeptide circuits in the CNS. The pharmacological bridge technique also suggests altered neurotransmitter transporter or receptor-mediated signal transduction in depression and other psychiatric disorders. Whether alterations in peripheral endocrine organ hormone secretion contribute primarily to the pathogenesis of psychiatric disorders, and/or whether altered secretion of pituitary and hypothalamic hormones primarily contribute to the signs and symptoms of a specific mental illness remains a subject of considerable controversy. In this chapter, we briefly outline the major findings concerning the putative roles of neuropeptides and hypothalamic-release factors in mood and anxiety disorders.

III. CURRENT STRATEGIES FOR EVALUATING A SPECIFIC ROLE FOR NEUROPEPTIDES IN PSYCHIATRIC DISORDERS: PRECLINICAL AND CLINICAL STUDIES

A. Preclinical Studies

Preclinical studies have been essential in providing useful and novel peptide circuit targets for drug discovery and in understanding some of the possible roles of neuropeptides and their interactions with other brain systems. Most of these studies involve directly injecting the peptide of interest into the brains of laboratory animals and studying its effect on behavior and on other known peptide and hormonal systems. One example of the usefulness of this strategy is in identifying potential behavioral effects of cholecystokinin (CCK), which was originally discovered in the gastrointestinal tract. CCK injections in laboratory animals can induce many behavior responses characteristic of panic attacks, which has led to scrutiny of a potential role for CCK and its receptor systems in panic disorder.

B. Clinical Studies

As noted above, during the 1970s and 1980s peripheral neuroendocrine markers were often used to indirectly assess CNS function because the brain was relatively inaccessible for study, with the exception of cerebrospinal fluid (CSF) and postmortem studies. With the emergence of the monoamine theories of mood disorders and schizophrenia, many investigators attempted to draw conclusions about the activity of specific neurotransmitter circuits in patients with various psychiatric disorders by measuring the basal and stimulated secretion of pituitary and end-organ hormones in plasma. Clinical studies, however, are often confounded by the normal circadian rhythms and the pulsatile release of many of the hypothalamic–pituitary–end-organ axes components that are often not taken into account when these experiments are designed. Further, differences in assay sensitivity, gender, inclusion criteria for patients, and severity of symptoms all may potentially generate confounding or at least quite variable results. Nevertheless, considerable progress about the neurobiology of psychiatric disorders has been discovered through such an approach.

Before proceeding into a review of the relevant clinical literature, a brief overview of the most common methodologies employed merits discussion. Most clinical studies in humans are based on one of three methodologies: (1) postmortem studies in which the concentration of neuropeptides and neuropeptide receptors are measured; (2) studies mea-

suring the concentrations of peptides in biological fluids such as plasma and CSF; and (3) studies measuring the biological and behavioral changes following administration of the neuropeptides (i.e., challenge tests).

Postmortem studies are advantageous in that they allow peptide levels to be sampled directly in brain tissue and from distinct brain regions. Methodologically there are major obstacles to overcome in obtaining and studying postmortem tissue. Because peptides and their receptors and mRNA are degraded postmortem, differences between the elapsed time between death of the subject and harvesting of the tissue, and hence in postmortem decay, as well as other neurochemical confounds associated with preserving and handling the tissue render conclusions concerning the relations between peptide circuit alterations and psychiatric disorders tentative and unable to stand on their own.

Many investigators have measured peptides in plasma and CSF. The concentration of neuropeptides in CSF is thought by some, but not all, investigators to accurately measure extracellular fluid concentration in brain, and therefore has an advantage over postmortem studies that are thought to measure both the releasable and nonreleasable pools of neuropeptides. Although CSF concentrations may be an index of the mean activity of a neuropeptide system, they may not accurately represent regional variations in extracellular availability of these peptides that may be of great pathophysiological importance. Plasma concentrations of neuropeptides are also commonly determined, although they are confounded by the presence of binding proteins as, for example, with corticotropin-releasing factor (CRF), and the impossibility of differentiating between CNS and peripheral sources of the neuropeptide being measured.

Another important strategy has been the administration of neuropeptides or synthetic neuropeptide agonists or antagonists directly to patients with affective and anxiety disorders, and measuring subsequent neuroendocrine and/or behavioral changes. In these so-called stimulation or provocation tests, hypothalamic and/or pituitary-derived factors or their synthetic analogs are exogeneously administered, and the hormonal response to this "challenge" is assessed. For example, in the standard CRF stimulation test, a 1 μg/kg dose of human or ovine CRF is administered intravenously, and the adrenocorticotropin (ACTH) and cortisol response is measured over a period of 2 or 3 h. This test is a very sensitive measure of hypothalamic–pituitary–adrenal (HPA) axis activity, and changes in the magnitude and/or duration of the response relative to normal control values are characteristic of one or another type of dysregulation of the HPA axis. One must be cautious, however, about making assumptions about what, if any, CNS deficits are responsible for the pattern of HPA axis activity observed.

IV. CORTICOTROPIN-RELEASING FACTOR IN MOOD AND ANXIETY DISORDERS

A. Basic Biology

The HPA axis has been extensively scrutinized in patients with psychiatric diseases. An abundance of evidence dating back over 100 years has demonstrated that dysfunction of the HPA axis may lead to specific psychiatric disturbances. At the pinnacle of this system is the neuropeptide CRF, also known as corticotropin-releasing hormone (CRH), a 41-amino-acid peptide that, as a hypothalamic-releasing factor, controls the activity of the HPA axis.

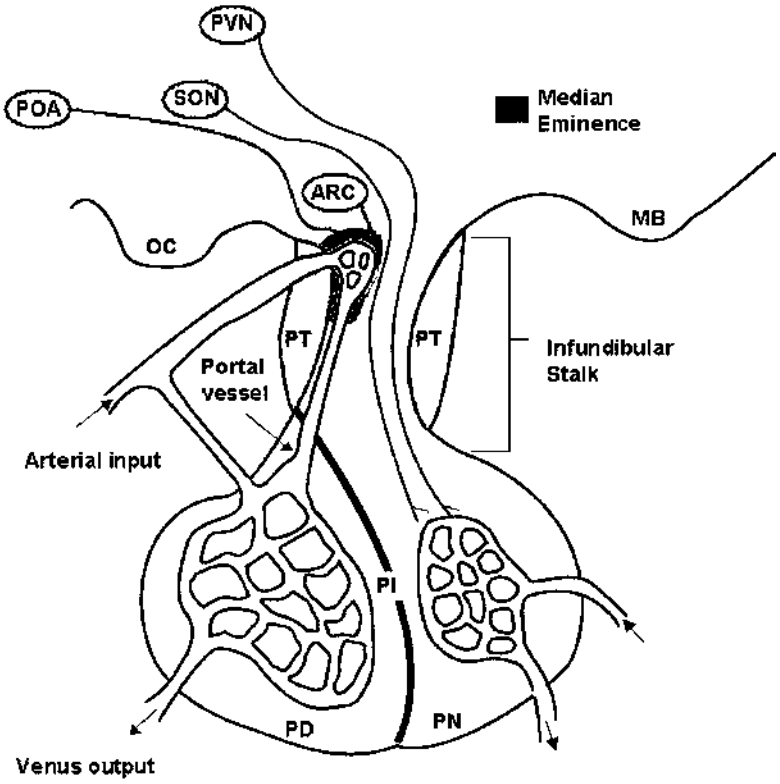

Figure 1 Diagram of the neurovascular anatomy of the hypothalamic-pituitary axis. PVN, paraventricular nucleus. SON, supraoptic nucleus. POA, preoptic area. ARC, arcuate nucleus. PT, pars tuberalis. PI, pars intermedia. PD, pars distalis. PN, pars nervosa, MB, mamillary body, OC, optic chiasm.

Within the hypothalamus, CRF is primarily synthesized in the parvocellular neurons located in the paraventricular nucleus (PVN) [2]. CRF neurons in the PVN (Fig. 1) receive input from a variety of brain nuclei, including the amygdala, bed nucleus of the stria terminalis, and other brain stem nuclei [2]. Hypothalamic CRF-containing neurons, in turn, project to the median eminence [3]. In response to stress, this neural circuit is activated releasing CRF from the median eminence into the hypothalamo-hypophyseal portal system where it binds to CRF receptors on corticotrophs in the anterior pituitary, which promotes the synthesis of propiomelanocortin (POMC) and the release of its post-translation products, adrenocorticotropic hormone (ACTH), β-endorphin, and others. ACTH released from the anterior pituitary into the systemic circulation then stimulates the production and release of cortisol by acting on ACTH receptors in the adrenal cortex. (For a general schematic of HPA axis regulation, see Fig. 2.).

Although Saffron and Schally identified a crude extract that promoted the release of ACTH from the pituitary in 1955 [4], CRF was not isolated and chemically characterized until 1981. Working with extracts derived from 500,000 sheep hypothalami, Vale and colleagues at the Salk institute isolated, synthesized, and elucidated the structure of

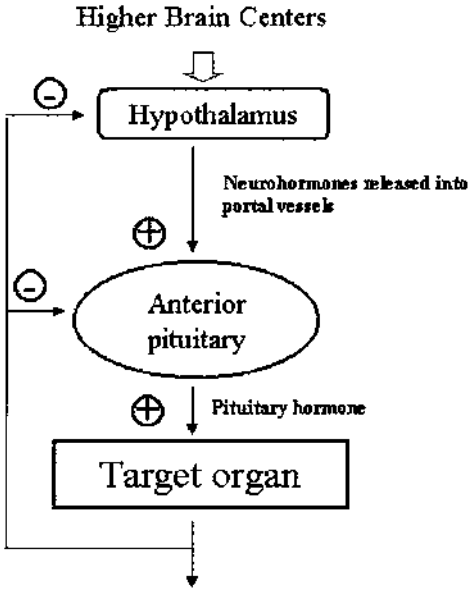

Figure 2 Overview of the common organizational motif of the neuroendocrine axis. The neurosecretion of hypothalamic factors into hypophyseal portal vessels is regulated by a set point of activity from higher brain centers. Neurohormones released from the hypothalamus into hypophyseal portal vessels in turn stimulate cells in the pituitary. These adenohypophyseal hormones then regulate the hormone output from the end-organ. The end organ then exerts negative feedback effects at the pituitary and hypothalamus to prevent further neurohormone and pituitary hormone release via "long-loop" negative feedback. Short-loop negative feedback may also occur where pituitary hormones feed back directly on hypothalamic neurons to prevent further neurohormone release.

CRF [5]. This discovery led to the availability of synthetic CRF, which finally permitted a more comprehensive assessment of HPA axis activity to be developed. It is now clear that CRF coordinates the endocrine, immune, autonomic, and behavioral responses of mammals to stress.

Two CRF receptor subtypes, CRF_1 and CRF_2, with distinct anatomical localization and receptor pharmacology, have been identified [6–10] in rats and humans. Both receptors are G-protein-coupled receptors and are positively coupled to adenylyl cyclase via G_s. The CRF_1 receptor is predominantly expressed in the pituitary, cerebellum, and neocortex in the rat [11]. A growing body of evidence from animal studies has shown that CRF_1 receptors may specifically mediate some of the anxiogenic-like behaviors observed after administration of CRF [12,13]. The CRF_2 receptor family is composed of two primary splice variants, CRF_{2A} and CRF_{2B}. The CRF_{2A} receptor is more prevalent in subcortical regions, such as the ventromedial hypothalamus, lateral septum, and dorsal raphe nucleus, whereas CRF_{2B} is more abundantly expressed in the periphery. In addition to CRF, several other endogeneous peptide ligands for CRF receptors have recently been discovered including urocortin [14], urocortin II, and urocortin III, and perhaps others [15,16]. With the discovery of several new putative endogenous ligands, much of the pharmacology and functional interactions between these ligands and receptors remains to be discovered [17,18].

B. HPA Axis Abnormalities in Depression

Evidence linking HPA axis abnormalities and psychiatric symptoms, as noted above, dates back more than 100 years. The greater than expected prevalence of depression and other psychiatric symptoms in both Cushing's and Addison's disease served as an impetus for researchers to scrutinize HPA axis function in patients with psychiatric disorders. Based on the work of research groups led by Board, Bunney, and Hamburg, as well as by Carroll, Sachar, Stokes, and Besser, literally thousands of studies were conducted in this area, most during the 1970s.

Some of the earliest studies demonstrated abnormalities in glucocorticoid function in depressed patients, including elevated plasma and CSF cortisol concentrations in depressed patients [19,20], increased 24-h urinary free cortisol concentrations, and increased levels of cortisol metabolites in urine [21]. One of the major advances, of course, came with the discovery of the chemical identity of the neuropeptide CRF, the primary physiological secretagogue of ACTH, and ultimately of cortisol (Table 1).

In order to accurately assess HPA axis function, several functional challenge tests have been developed, both provocative and suppression tests. The most commonly used test to measure HPA axis function is the dexamethasone suppression test (DST). In this standardized test, 1 mg of dexamethasone is administered orally at 11 P.M., and blood samples are obtained at 8 A.M. the following morning for cortisol measurement. Dexamethasone is a synthetic steroid similar to cortisol; it suppresses ACTH secretion and subsequently cortisol release in healthy volunteers. Nonsuppression of plasma glucocorticoid levels following the administration of dexamethasone is common in depressed patients. The rate and magnitude of cortisol nonsuppression after dexamethasone administration generally correlates with the severity of depression [22]; in fact, nearly all patients with major depression with psychotic features exhibit DST nonsuppression [23,24]. Since Carroll's initial report [25,26] and subsequent claims for diagnostic utility [27], the dexamethasone suppression test has generated considerable controversy [28] as to its diagnostic utility. Diagnostic issues notwithstanding, the overwhelming conclusion from a myriad of studies demonstrates that a sizeable percentage of depressed patients exhibit HPA axis hyperactivity.

Shortly after the isolation and characterization of CRF, a standardized CRF stimulation test was developed to further assess HPA axis activity. In this paradigm, CRF (ovine or human) is administered intravenously (usually in a fixed dose of 100 μg or as a 1 μg/kg dose), and the ensuing ACTH (or β-endorphin) and cortisol response is measured at 30-min intervals over a 2 to 3-h period [29]. Numerous studies have now demonstrated

Table 1 HPA Axis Changes in Depression

↑ Corticotropin-releasing factor (CRF) concentrations in cerebrospinal fluid[a]
↓ Adrenocorticotropic hormone response (ACTH) to CRF stimulation
↓ Density of CRF receptors in frontal cortex of suicide victims
Enlarged pituitary gland in depressed patients[a]
Adrenal gland enlargement in suicide victims and depressed patients
↑ Plasma cortisol during depression[a]
↑ Urinary free cortisol concentrations[a]
Nonsuppression of plasma cortisol and ACTH after dexamethasone administration[a]

[a] State-dependent.

a blunted ACTH and β-endorphin response to exogenously administered ovine CRF (oCRF) or human CRF (hCRF) in depressed patients when compared to nondepressed subjects, though the cortisol response in depressed patients and nondepressed control subjects does not consistently differ [30–34]. The blunted ACTH response to CRF occurs in depressed DST nonsuppressors, but not in DST suppressors [35]. The attenuated ACTH response to CRF is presumably because of either chronic hypersecretion of CRF from nerve terminals in the median eminence, which results in downregulation of CRF receptors in the anterior pituitary, and/or to chronic hypercortisolemia. This receptor downregulation results in a reduced responsivity of the anterior pituitary to CRF, as has been demonstrated in laboratory animals [36–40]. Following recovery from depression, the documented disturbances in the HPA axis generally remit.

A combined dexamethasone/CRF test has also been developed by Holsboer and colleagues. In this test, 1.5 mg of dexamethasone is administered orally at night (23:00 h), and subjects receive an IV bolus of 100 μg of human CRF at 15:00 h the following day. Patients with HPA axis dysfunction, which is frequently encountered in 40 to 60% of depressed patients, display a paradoxically increased release of ACTH and cortisol following CRF challenge relative to controls. These abnormalities disappear following remission of depression, and normalization of HPA axis function seems to precede full clinical remission [41,42]. The combined DEX/CRF test appears to have much higher sensitivity for detecting subtle alterations in HPA axis function; approximately 80% of patients with major depression exhibit an abnormal response to the DEX/CRF test. In contrast, only approximately 44% of patients with major depression demonstrate an abnormal response when the dexamethasone suppression test is administered alone [41,42]. Furthermore, otherwise healthy individuals with first-degree relatives with an affective illness, which greatly increases their own risk for psychiatric disorders, demonstrated cortisol and ACTH responses to the DEX/CRF test that were higher than a control group, but less than patients currently suffering from major depression. One interpretation of these findings is that a genetically transmittable defect in corticosteroid receptor function may render these individuals more susceptible to developing affective disorders by increasing baseline and evoked HPA axis activity [43].

C. Extrahypothalamic CRF and Depression

The studies thus far discussed focused primarily on dysregulation of the HPA axis, but perhaps of even greater relevance to psychiatric disorders is the fact that CRF controls not only the neuroendocrine, but also the autonomic, immune, and behavioral responses to stress in mammals. Moreover, results from both clinical studies, and a rich body of literature conducted primarily in rodents and lower primates, have indicated the importance of extrahypothalamic CRF circuits [13,44]. In rodents, primates, and humans, CRF and its receptors have been heterogeneously localized in a variety of regions including the amygdala, thalamus, hippocampus, and prefrontal cortex, and others [45–48]. These brain regions are known to be important in regulating many aspects of the mammalian stress response, and in regulating affect. The presence of CRF receptors in both the dorsal raphe (DR) and locus coeruleus (LC), the major serotonergic and noradrenergic cell-body-containing regions in the brain, respectively, also deserve comment. Because most available antidepressants, including the tricyclics and selective serotonin reuptake inhibitors (SSRI), are believed to act primarily via modulation of noradrenergic and/or serotonergic

systems, the neuroanatomical proximity of CRF and monoaminergic systems provides plausibility for interaction between CRF systems and antidepressants.

Involvement of extrahypothalamic CRF systems in the pathophysiology of depression is suggested by numerous studies showing elevated CRF concentrations in the cerebrospinal fluid (CSF) of drug-free depressed patients [49–53], although a discrepant report has appeared [54]. Elevated cisternal CSF CRF concentrations have also been detected in depressed suicide victims [49]. A reduction in concentrations of CRF in CSF has been observed in healthy volunteers treated with the tricyclic antidepressant desipramine [55] and in depressed patients following treatment with fluoxetine [56] or amitryptiline [57], providing further evidence of a possible interconnection between antidepressants, noradrenergic neurons, and CRF systems. Similar effects have been reported after electroconvulsive therapy (ECT) in depressed patients [58]. Elevated CSF CRF concentrations appear to represent a state, rather than a trait, marker of depression (i.e., a marker of the state of depression rather than a marker of vulnerability to depression) [58]. Furthermore, high and/or increasing CSF CRF concentrations, despite symptomatic improvement of major depression during antidepressant treatment, may be the harbinger of early relapse [59], as our group previously reported for DST nonsuppression [60].

While the exact mechanism contributing to CRF hyperactivity remains obscure, studies from our group and others have documented long-term persistent increases in HPA axis activity and extrahypothalamic CRF neuronal activity after exposure to early untoward life events—for example, neglect, and child abuse, respectively, in both laboratory animals (rat and nonhuman primates) and patients [43,61–63]. Early-life stress apparently permanently sensitizes the HPA axis and leads to a greater risk of developing depression later in life. To measure HPA axis responsivity to stress in humans, the Trier Social Stress Test (TSST) was developed. This laboratory paradigm involves a simulated 10-min public speech and a mental arithmetic task. The TSST has been validated as a potent activator of the HPA axis in humans [64]. Recently, our group has reported increased plasma ACTH and cortisol concentrations, presumably because of hypersecretion of CRF, after exposure to the TSST in women (both depressed and nondepressed) who were exposed to severe physical and emotional trauma as children [65]. The depressed women both with and without early-life stress exhibited a blunted ACTH response to CRF, whereas the women with early trauma alone exhibited an exaggerated ACTH response. These data provide further evidence for functional hyperactivity of CRF systems that may be influenced by early adverse life events.

D. CRF and Anxiety Disorders

Involvement of CRF in anxiety disorders has been well documented in both animal and human studies. As reviewed by Arborelius and colleagues [66], patients with post-traumatic stress disorder (PTSD) (i.e., Vietnam combat veterans) exhibit significantly elevated CSF CRF concentrations [67,68], as well as alterations in the ACTH response to CRF challenge [69]. A recent elegant study using an in-dwelling catheter in the lumbar space, which allows repeated sampling of CSF for several hours after the initial, and presumably stressful, lumbar puncture, demonstrated elevated CSF CRF levels in PTSD combat veterans [68]. In contrast, low serum cortisol and urinary free cortisol levels have been repeatedly, yet unexpectedly, detected in PTSD, especially after dexamethasone administration [70,71]. One possible mechanism that has been proposed by Yehuda and colleagues to

explain these findings is heightened negative glucocorticoid feedback within the HPA axis in chronic PTSD patients [72].

Although CSF CRF concentrations are not increased in panic disorder patients [73,74], a diminished ACTH response to CRF administration has been observed [75]. Increased [76] or normal concentrations [74,77] of CSF CRF have been documented in patients with obsessive-compulsive disorder (OCD), although significant decreases in CSF CRF concentrations occur with a therapeutic response to clomipramine [78]. Patients with generalized anxiety disorder (GAD), however, exhibit similar CSF CRF concentrations in comparison to normal controls [74,79]. Not surprisingly increased concentrations of CSF CRF occur in alcohol withdrawal, a condition of sympathetic arousal and increased anxiety [80,81]. In contrast, CSF CRF concentrations are reduced [82] or are normal [83] in abstinent chronic alcoholics with normal plasma cortisol concentrations. Although HPA axis hyperactivity exists in patients with certain anxiety disorders, such perturbations do not exist in the patterns suggestive of CRF hypersecretion as documented in patients with major depression [66]. Moreover, in the majority of these studies, careful assessment of comorbidity of mood and anxiety syndromes and symptoms has not been conducted.

E. CRF in Depression and Anxiety: Conclusions

Space constraints do no permit an extensive review of the preclinical literature; however, several additional points are worth highlighting. Numerous studies have documented that when CRF is directly injected into the CNS of laboratory animals, it produces effects reminiscent of the cardinal symptoms of depression, including decreased libido, reduced appetite and weight loss, sleep disturbances, and neophobia. Based on this premise, newly developed CRF_1 receptor antagonists represent a novel putative class of antidepressants. Such compounds show activity in nearly every preclinical screen for antidepressants and anxiolytics currently employed. Recently, a small open-label study exmaining the effectiveness of R121919, a CRF_1 receptor antagonist, in major depression was completed [84] in which standard severity measures of both anxiety and depression were reduced in the depressed patients after R121919 treatment. Although this drug is no longer in clinical development, it is clear that CRF_1 antagonists potentially represent a new class of psychotherapeutic agents to treat anxiety and affective disorders. Finally, the early trauma data raises the question of whether childhood physical and sexual abuse, common among depressed patients, is responsible for some or all of the changes reported in the HPA axis and CRF circuits in patients with major depression.

V. THYROTROPIN-RELEASING FACTOR, THE THYROID AXIS, AND DEPRESSION

A. Biology

Dysfunction of the HPT axis has been associated with numerous psychiatric disturbances, ranging from mild depression to overt psychosis. The HPT axis controls the release of thyroid hormones, T_3 and T_4, and is regulated by two peptide hormones, thyrotropin-releasing hormone (TRH), a tripeptide, and thyroid-stimulating hormone (TSH) or thyrotropin, a large protein. TRH was the first of the hypothalamic-releasing hormones to be isolated and characterized. TRH is synthesized predominantly in the paraventricular nucleus in the hypothalamus and stored in nerve terminals in the median eminence where it is released into the vessels of the hypothalamic-hypophyseal portal system. TRH is then

transported to the sinusoids in the anterior pituitary, where it binds to TRH receptors on thyrotropes and promotes the release of TSH into the systemic circulation.

TSH is a 28-kDA glycoprotein composed of two noncovalently linked protein chains, TSH-α, which is identical to the α subunit contained in other pituitary hormones, including follicle-stimulating hormone, luteinizing hormone, human chorionic gonadotropin, and TSH-β. Upon stimulation by TSH, the thyroid gland releases the iodinated amino acids L-triiodothyronine (T_3) and thyroxine (T_4). Beside their well-known metabolic effects, T_3 and T_4 provide feedback to the hypothalamus and adenohypophysis to regulate the synthesis and release of TRH and TSH, respectively [85]. Following its initial isolation, the heterogeneous distribution of TRH in extrahypothalamic sites strongly suggested a role for this peptide as a neurotransmitter or neuromodulator, as well as a releasing hormone. It is now established that TRH itself can produce direct effects on the CNS independent of its actions on pituitary thyrotrophs.

B. Psychiatric Manifestations of Thyroid Dysfunction

Primary thyroid axis disorders are often associated with numerous psychiatric manifestations ranging from mild depression and anxiety to overt psychosis. Regardless of the etiology, hypothyroidism leads to a number of clinical manifestations, including slowed mentation, forgetfulness, decreasing hearing, cold intolerance, and ataxia. Decreased energy, weight gain, depression, cognitive impairment, or overt psychosis ("myxedema madness") may also result. Because of the overlapping symptoms with depression, thyroid hormone deficiency must be ruled out when evaluating patients with depression.

Early studies from the late 1960s and early 1970s scrutinized psychiatric comorbidity in patients with hypothyroidism, and a substantial number of these patients demonstrated signs of depression, anxiety, and/or cognitive disturbances [86,87]. Furthermore, these symptoms were often improved or resolved following resolution of the thyroid condition alone. Later studies have demonstrated varying degrees of cognitive disturbance in up to 48% of psychiatrically ill hypothyroid cases [88], and approximately 50% of unselected hypothyroid patients have symptoms characteristic of depression [88]. Anxiety symptoms are also common, occurring in up to 30% of unselected patients. Mania and hypomanic states have rarely been reported in hypothyroid patients. Finally, although psychosis is the most common reported symptom in the case literature on hypothyroidism (52.9%), it only accounts for approximately 5% of the psychiatric morbidity in an unselected sample [88], presumably because more severe symptoms are more likely to be documented in the clinical literature.

C. TRH and TSH in Depression

Considerable evidence has revealed an increased incidence of HPT axis dysfunction in patients with major depression (Table 2). More than 25 years ago research groups led

Table 2 Hypothalamic-Pituitary-Thyroid (HPT) Axis Alterations in Depression

↑ CSF TRH concentrations in depressed patients
↓ Nocturnal plasma TSH
Blunted or exaggerated TSH in response to TRH stimulation
Presence of antithyroglobulin and/or antimicrosomal thyroid antibodies

by Prange [89] and Kastin demonstrated that approximately 25% of patients with major depression exhibit a blunted TSH response to TRH [89,90]. This is clearly not secondary to hyperthyroidism but presumably because of hypersecretion of TRH from the median eminence, which leads to TRH receptor downregulation in the anterior pituitary and reduced sensitivity of the pituitary to exogeneously administered TRH. This hypothesis seems plausible in light of evidence showing elevated TRH concentrations in CSF of drug-free depressed patients [91] and an inverse relationship between the blunted TSH response to TRH and CSF TRH concentrations [92]. Depressed patients have also been shown to have an increased prevalence rate of symptomless autoimmune thyroiditis (SAT), defined by the abnormal presence of antithyroglobulin and/or antimicrosomal thyroid antibodies consistent with grade 4 hypothyroidism [93].

Although there is a preponderance of evidence suggesting elevated TRH release in some depressed patients, as of yet it is unclear what precise role this factor plays in depression. Interestingly, a lumbar intrathecal infusion of 500 μg of TRH into medication-free inpatients with depression produced a clinically robust, but short-lived, improvement in mood and suicidality, which led the authors to propose that elevated TRH levels might be a compensatory response in depression [94]. Although this work is preliminary, it raises the interesting possibility that a systematically administered TRH receptor agonist may represent a novel class of antidepressant agents.

D. Bipolar Disorder and HPT Abnormalities

HPT axis abnormalities have also been reported in bipolar disorders. Both elevated basal plasma concentrations of TSH and an exaggerated TSH response to TRH have been demonstrated [95,96]. There is also evidence that bipolar patients with the rapid cycling subtype have a higher prevalence rate of hypothyroidism (grades I, II, and III) than bipolar patients who do not [97,98]. A blunted or absent evening surge of plasma TSH, a blunted TSH response to TRH [99,100], and the presence of antithyroid microsomal and/or antithyroglobulin antibodies [101,102] have also been demonstrated in bipolar patients.

E. Thyrotropin-Releasing Hormone Alterations in Anxiety Disorders

Evidence demonstrating alterations of TRH circuits in anxiety disorders is relatively limited. A blunted TSH response to TRH has been observed in panic disorder patients [103,104]. However, in one of these studies, this effect was only demonstrated in patients with concurrent major depression [104]. Another study indicated that the TSH reponse to TRH is normal in panic disorder patients [105]. CSF TRH concentrations in patients with panic disorder, generalized anxiety disorder, or obsessive-compulsive disorder are unchanged compared to control subjects [106].

VI. ARGININE-VASOPRESSIN

A. Biology

Arginine-vasopressin (AVP), also known as antidiuretic hormone (ADH), is a nonapeptide synthesized in the lateral magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus, and is released directly into the bloodstream from axon terminals in the posterior pituitary [107]. These AVP-containing neurons terminate in the neuro-

hypophysis and secrete AVP into the systemic circulation, although they send collaterals to the hypothalamo-hypophyseal portal system as well. Another group of AVP-containing neurons project from the medial parvocellular subdivision of the paraventricular nucleus (PVN) to the median eminence. Within the median eminence, the parvocellular-derived AVP is released from axon terminals, secreted into the hypothalamo-hypophyseal portal circulation, and carried to the anterior lobe of the pituitary gland [3]. Moreover extrahypothalamic AVP-containing neurons lie within limbic structures such as the septum and amygdala, as well as in the brainstem and spinal cord [108]. AVP-containing neurons also receive afferent innervations from many different neuronal cell groups and send axonal projections from the cerebral cortex throughout the CNS. It is thought that AVP and the other well-known posterior pituitary hormone, the nonapeptide oxytocin (OT), play a role in modulating neural activity in hypothalamic, limbic, and autonomic circuits.

AVP has prominent roles in controlling fluid balance via its effects on the kidney, in regulating blood pressure by its vasoconstrictive effects on blood vessels, and can directly promote the sensation of thirst. AVP also can act synergistically with CRF to promote the release of pituitary POMC-derived peptides (i.e., ACTH and β-endorphin) in humans [109] and animals [110] following stressful stimuli [111]. Chronic stress or adrenalectomy increases the activity of the parvocellular AVP system [112,113]. CRF and AVP are colocalized in the parvocellular cells of the human hypothalamus and may be secreted together into the human hypothalamic-hypophyseal portal circulation [114]. The ratio of AVP and CRF released into the hypothalamic-hypophyseal portal circulation varies in different species [110] and according to the nature of the stress [115,116].

B. AVP in Anxiety and Depression

Similar to the clinical investigations regarding CRF, a variety of patient groups have been studied. Alterations of CSF AVP concentrations have been reported in patients with major depression, bipolar disorder, schizophrenia, anorexia, obesity, alcoholism, Alzeheimer's disease, and Parkinson's disease [117,118]. CSF AVP concentrations in patients with major depression are reportedly reduced in comparison to control subjects, although the source of CSF AVP is likely extrahypothalamic and not an index of PVN AVP secretion [119–121]. Basal plasma concentrations of AVP in depressed patients are also reportedly decreased in comparison to age-matched controls [122], although others have found no difference [123]. Interestingly AVP secretion in response to an infusion of hypertonic saline is diminished in depressed patients as compared to controls [123].

A mildly blunted ACTH response to exogenous AVP administration has been reported in depressed patients [33], but the finding was not replicated in two other studies [124,125]. Remarkably, an increase in the number of PVN AVP neurons colocalized with CRF cells has been reported in depressed patients compared to controls in a postmortem tissue study [126,127]. This is of interest in view of the ability of AVP to potentiate the actions of CRF at the corticotrope. In contrast to the findings suggestive of diminished hypothalamic-vasopressinergic activity in depressed patients are the findings suggestive of hypersecretion of AVP in bipolar patients during their manic phase. Elevations in CSF AVP concentrations have been documented in manic patients [118], as well as significant increases of plasma AVP in comparison to patients with unipolar depression and controls [128]. Clearly, hypothalamic and extrahypothalamic AVP circuits are regulated independently. Whether the perturbations of AVP secretion in patients with neuropsychiatric disorders are state- or trait-dependent requires further elucidation.

VII. ENDOGENOUS OPIOID PEPTIDES

A. Biology

The rationale for studying opioids in depression and anxiety disorders is based on the observation that opiates possess antidepressant and anxiolytic effects. Opium has been known to have mood-elevating properties, and Krapelin proposed opium as a treatment for depressed patients as early as 1905. It is now known that the endogenous opioid peptide system is composed of three groups of peptides, including methionin-enkephalin and leucine-enkephalin [129], β-endorphin [130], and the dynorphins [131]. These peptides have been shown to play a variety of physiological roles, including regulation of pain, mood, respiration, cardiovascular function, gastrointestinal activity, satiety, sexual behavior (see review, Ref. 132).

At the genomic level, there are three genes responsible for the precursors of opioid peptides: pro-opiomelanocortin (POMC), proenkephalin, and prodynorphin. Consequently, there are at least three classes of opioid peptides with different biosynthetic and neuronal pathways: the endorphins, enkephalins, and dynorphins. In the adenohypophysis [133], POMC is processed to yield only ACTH and β-lipotropin. β-lipotropin is then processed to yield at least three compounds, including β-γ, and α-endorphin. The second endogenous opioid system is composed of met- and leuenkephalin, whose precursor is proenkephalin. Derivatives of prodynorphin are the third group of endogenous opioid peptides, including dynorphin A, dynorphin B, and neoendorphin, which are located almost exclusively in the posterior pituitary.

B. Opioids in Depression

Early open trials with β-endorphin indicated an improvement in depressed patients following intravenous β-endorphin administration [134], which were later substantiated by controlled clinical trials [135,136]. However, these results were not confirmed, and there is conflicting evidence as to whether intravenously administered β-endorphin permeates the blood-brain barrier (see Ref. 137 for a comprehensive review of these studies). Also, the highly addictive nature of opioids limits their usefulness as a potential treatment for depression. Several studies have also shown that the potent mu opiate receptor antagonist naloxone does not directly affect mood [138–140], further weakening a strong role of the endogenous opioid system in affective disorders. Two later studies, using very high doses, showed an increase in anxiety in normal controls [141] or an augmentation of symptom severity in depressed patients. Nevertheless, these studies did not provide evidence for a role for opioid peptide systems in depression.

The studies investigating CSF β-endorphin immunoreactivity in depression and anxiety disorders by and large also have not substantiated any major role for endogenous opioid peptides in depression or anxiety disorders. Most investigators [142–146], but not all [147], have reported normal concentrations of CSF β-endorphin in patients with major depression. One study also showed elevated CSF β-endorphin levels in patients diagnosed with panic disorder [148], although this effect was not demonstrated in a later study [149]. Because of these negative findings and a lack of corroborating evidence, extensive scrutiny of CSF enkephalin and dynorphin concentrations in patients with affective disorder has not been conducted.

Numerous studies have also measured plasma concentrations of β-endorphin in patients with affective disorders. Because both ACTH and β-endorphin are coreleased from

the pituitary following stress and share a common precursor, POMC, these studies are particularly interesting in light of the consistent alterations in HPA axis function seen in depression [150]. Studies investing plasma levels of β-endorphin have yielded somewhat discrepant results. Some investigators have shown increased concentrations [147,151,152], whereas others have found normal concentrations [34,153–156].

Not surprisingly, similar to the ACTH response to i.v. CRF challenge (*vida supra*), the β-endorphin response to exogenously administered oCRF is blunted in depressed patients compared to normal subjects [34]. Morever, nonsuppression of plasma β-endorphin occurs in depressed patients in a manner similar to cortisol nonsuppression after dexamethasone administration. β-endorphin nonsuppression to dexamethasone has been observed even in those patients whose baseline β-endorphin levels were similar to those of normal controls [156–158]. In these patients, postdexamethasone levels of cortisol and β-endorphin were strongly correlated [156,158]. In contrast, depressed patients have been reported to exhibit increased secretion of β-endorphin in response to cholinergic stimulation [147], thyrotropin-releasing hormone (TRH), and luteinizing-hormone-releasing-hormone (LHRH) in comparison to controls [159].

VIII. NEUROPEPTIDE Y

Originally cloned from a pheochromocytoma by Minth and colleagues in 1984, neuropeptide Y (NPY) is a 36 amino acid–containing peptide whose gene is expressed in cells derived from the neural crest [160]. Neurons displaying NPY immunoreactivity are abundant within several of the limbic areas of the CNS [161,162]. NPY is also present within neurons of the hypothalamus, brainstem, and spinal cord. Present in most sympathetic nerve fibers, NPY can be detected in vascular beds throughout the body and occurs in parasympathetic nerves as well [163]. Receptors for NPY are also widely distributed. Not only do NPY-containing neurons innervate CRF-containing cells of the PVN [164], but NPY administration increases hypothalamic CRF levels [165] and promotes its release as well [166]. The relationship of NPY to CRF is further substantiated by the partial blockade of the NPY-stimulated ACTH response by a CRF receptor antagonist. Moreover, NPY potentiates the effects of exogenously administered CRF in animals [167]. NPY itself may have anxiolytic properties. Recently developed NPY overexpressing mice have demonstrated an attenuated sensitivity to the behavioral consequences of stress [168].

Although an initial investigation [169] did not find significantly diminished CSF NPY concentrations in depressed patients, Widerlov and colleagues [170] subsequently reported that patients with major depression do exhibit decreased CSF NPY concentrations in comparison to sex- and age-matched controls. A recent study has also demonstrated decreased levels of NPY in the plasma of patients who attempted suicide, and patients who had repeatedly attempted suicide had the lowest plasma NPY levels [171]. Negative correlations have been also observed between dimensional anxiety ratings and CSF NPY levels in depressed patients [172]. Marked reductions in brain-tissue concentrations of NPY have also been reported in suicide victims, with the most dramatic decreases in those patients diagnosed with major depression [173]. Efforts toward development of NPY receptor-specific agonists and antagonists continue, but the field has been greatly hampered because of the lack of availability of synthetic nonpeptide NPY receptor agonists [174]. NPY-ergic medications may have significant benefit in the treatment of affective disorders and/or eating disorders.

IX. SUBSTANCE P

Mammalian members of the peptide tachykinin family are known as neurokinins [175], including neurokinin A, neurokinin B, and substance P. The most well known and abundant of the neurokinins, the undecapeptide substance P, was discovered in 1931 by von Euler and Gaddum, but not isolated in pure form until 1970 by Chang and Leeman. Substance P (SP) binds to the neurokinin-1 (NK-1) receptor, neurokinin A (NKA) to the neurokinin-2 (NK-2) receptor, and neurokinin B (NKB) to the neurokinin-3 (NK-3) receptor. Within the CNS, SP is localized within the limbic, hypothalamic, and brainstem areas (amygdala, hypothalamus, periaqueductal gray, locus coeruleus, and parabrachial nucleus) [176] and is colocalized within norepinephrine- and serotonin-containing cell bodies as well [177–180]. Furthermore, substance P and other tachykinins serve as pain neurotransmitters in primary afferent neurons [181] and exert a variety of other peripheral actions, including bronchoconstriction, vasodilatation, salivation, and smooth muscle contraction in the gut [182,183].

Preclinical studies have provided much of the impetus to continue investigation of the potential efficacy of substance P receptor antagonism in psychiatric disorders, particularly when these agents have not been effective as analgesics [184]. Substance P (or substance P agonist) administration to animals elicits behavioral and cardiovascular effects resembling the stress response and the so-called "defense reaction" [185]. Moreover, preclinical studies documented reduction of behavioral and cardiovascular stress response by administration of substance P receptor antagonists [186,187]. A breakthrough study had indicated that the substance P receptor antagonist MK-869 is more effective than placedo, and is as effective as paroxetine in patients with major depression with moderate-to-severe symptom severity [187].

Future clinical investigations will determine whether brain and CSF substance P concentrations are altered in patients with major depression [188] (one study showed elevated levels [189]) and whether there are significant changes in CSF substance P concentrations after treatment [190]. Moreover, we await convincing studies that reveal whether substance P antagonists will play a substantive therapeutic role in patients with anxiety disorders or schizophrenia [191] or asthma, irritable bowel syndrome, and migraine.

X. GROWTH HORMONE AND SOMATOSTATIN

A. Biology

A great deal of evidence has accumulated demonstrating abnormalities in both growth hormone secretion and somatostatin concentrations in CSF in depression and, to a lesser extent, anxiety disorders. Growth hormone (GH) is synthesized and secreted from somatotrophs located in the anterior pituitary. Its release is unique in that it is controlled by two peptide hypothalamic hypophysiotropic hormones, growth-hormone-releasing factor (GHRF), and somatostatin, and secondarily by classic neurotransmitters such as Ach, DA, NE and 5HT that innervate the releasing factor–containing neurons. Somatostatin, also known as growth hormone-release-inhibiting hormone (GHIH) or somatotrophin-release inhibiting factor (SRIF), was first isolated from ovine hypothalamus in 1974 [192]. It is a tetradecapeptide containing a disulfide bridge linking the two cysteine residues. Somatostatin is released predominantly from the periventricular and paraventricular nucleus of the hypothalamus and inhibits GH release. Somatostatin also inhibits the release of both CRF and ACTH [193–195]. Somatostatin has a wide extrahypothalamic distribution in

brain regions, including the median eminence [196,197], limbic system, cerebral cortex, hippocampus, hypothalamus [198], and amygdala.

GHRF was characterized and sequenced in 1981 after considerable difficulty. The long-postulated GHRF was discovered several years after the elucidation of the structure of somatostatin from extracts of an ectopic tumor associated with acromegaly. GHRF is a 44-amino-acid peptide, and has the most limited CNS distribution of all the hypothalamic-releasing hormones that have been identified. GHRF-containing neurons are concentrated in the infundibular and arcuate nuclei of the hypothalamus and stimulate the synthesis and release of GH. Dopamine, NE, and 5HT innervate GHRF-containing neurons thereby modulating GH release. Both GHRF and SRIF are released from the median eminence into the hypothalamo-hypophyseal portal system where they act on somatotrophs in the anterior pituitary to regulate GH release. Negative feedback is provided by GH, which stimulates somatostatin release preventing further GH release. The GH axis is unique in that it does not have a single target endocrine gland but instead growth hormone acts directly on multiple tissue targets including bone, muscle, and liver.

Growth hormone is released in a pulsatile fashion, with its highest release occurring around the time of sleep onset and extending into the first non-REM period of sleep [199]. A variety of stressors, including starvation, exertion, or emotional stress, also promote growth hormone release in humans [200]. Growth hormone is necessary for the longitudinal bone growth that occurs during the late childhood; appropriately, GH levels are high in children, reach their peak during adolescence, and decline throughout adulthood. In addition to its effects on the long bones, growth hormone has predominantly anabolic effects and leads to increased muscle mass and decreased body fat. GH stimulates the release of somatomedin from the liver as well as insulin-like growth factors.

Growth hormone is released after treatment with L-dopa, a DA precursor [201], apomorphine, a centrally active DA agonist [202,203], clonidine [204], a central α_2 adrenergic receptor agonist, NE [205], and the serotonin precursors L-tryptophan [206] and 5-hydroxytryptophan [207]. Serotinin receptor antagonists, methysergide and cyproheptadine, interfere with the GH response to hypoglycemia [205]. In contrast, phentolamine, a nonspecific α-adrenergic receptor antagonist, inhibits GH secretion [208] (Table 3).

B. Growth Hormone and Depression

Several findings indicate dysregulation of GH secretion in depression. A blunted nocturnal GH surge in depression has been reported [209], whereas daylight GH secretion seems to be exaggerated in unipolar depressed patients [210]. A number of studies have also demonstrated a blunted GH response to noradrenergic agents (clonidine, desipramine) [211–217] and dopaminergic agonists (apomorphine) [218] in depressed patients. Siever et al. [217] demonstrated that the blunted GH response to clonidine was not related to age or sex, and this study provided evidence that the diminished GH response to clonidine may be secondary to decreased α_2-adrenergic receptor sensitivity in depression [217].

A GHRF stimulation test has also been developed and studied in depressed patients. Two groups observed a blunted GH response to GHRF in depressed patients [219–221]. However, Krishnan and colleagues [222,223] found minimal differences in serum GH response to GHRH between depressed and control patients. A comprehensive review of GHRF stimulation tests in depression, anorexia nervosa, bulimia, panic disorder, schizophrenia, and Alzheimer's disease suggested that the results of this test are not always consistent and in some cases contradictory [224]. Contributing factors include the variabil-

Table 3 Releasing and Inhibiting Factors for Growth Hormone

Growth hormone releasing factors
- Growth-hormone releasing hormone (GRF of GHRH)
- Dopamine
- L-DOPA
- Apomorphine (dopamine receptor agonist)
- Norepinephrine
- Clonidine (α_2-adrenergic receptor agonist)
- Serotonin
- L-tryptophan
- 5-hydroxytryptophan (5HTTP)

Factors that inhibit growth hormone release
- Somatostatin (SRIF)
- Phentolamine (nonspecific alpha-adrenergic receptor antagonist)
- Methysergide (serotonin $5HT_{1,2}$-receptor antagonist)
- Cyproheptadine (serotonin $5HT_{1,2a,2c}$-receptor antagonist)

ity of GHRF-stimulated GH among controls, lack of standard outcome measures, and age- and gender-related effects. Further studies using GHRF will help develop a standard stimulation test to further clarify the GH response to GHRF in depression and other psychiatric disorders. With the characterization of the genes encoding GHRF and its receptor, alterations in the CNS of depressed patients that underlie the diminished GH response to NE and DA agonists can now be studied in post-mortem tissue (Table 4).

Using a GHRF stimulation test, our group has demonstrated a slight exaggeration of GH response to GHRF in depressed patients compared to controls, although this group difference was mainly attributable to 3 of the 19 depressed patients who exhibited markedly high GH responses to GHRF [222]. Others, however, have reported a blunted GH response to GHRF in depressed patients. Thus it is unclear whether the blunted GH response to clonidine seen in depression is because of a pituitary defect in GH secretion, further implicating a subsensitivity of α-adrenergic receptors in depression, or to a primary GHRF deficit which leads to a secondary blunted GH response. Recently a diminished GH response to clonidine was demonstrated in children and adolescents at high risk for major depressive disorder. When considered with evidence demonstrating GH dysregulation in childhood depression [225], it suggests the blunted GH response seen in high-risk adolescents may represent a trait marker for depression in children and adolescents [226]. Arguably, the blunted GH response to clonidine seen in depression may be the most reproducible and specific finding in the biology of affective disorders.

C. Somatostatin and Depression

Several studies have demonstrated decreased SRIF concentrations in the CSF of patients suffering with depression [143,227–231], schizophrenia [227], and dementia, including

Table 4 Growth Hormone (GH) Axis Changes in Depression

↑ Circulating daily GH levels (uni- and bipolar depression)
↓ Nocturnal GH in depression
↓ Response of GH to noradrenergic or dopaminergic agents

Alzheimer's disease [232,233]. Reduced CSF SRIF concentrations have been reported in patients exhibiting dexamethasone nonsuppression (whether schizophrenic or depressed), and are negatively correlated with the maximum post-dexamethasone cortisol plasma concentration in patients with major depression [234]. Although some investigators have reported normalization of CSF SRIF concentrations after recovery from depression [228,235,236], others have noted no significant changes in CSF SRIF concentrations of depressed patients after clinical improvement with antidepressants [59] or ECT treatment [58]. Interestingly, administration of certain psychotropic medications is known either to decrease CSF SRIF concentrations (e.g., carbamazepine [235], diphenylhydantoin, and fluphenazine [237]), increase CSF SRIF concentrations (e.g., haloperidol [238]), or have no effect (e.g., desipramine or lithium [239]). Somatostatin also inhibits the release of both CRF and ACTH [193–195] indicating a direct interaction between the growth hormone and HPA axes. No differences in CSF SRIF levels have been observed in patients with generalized anxiety disorder compared to normal controls [79]. No published studies measuring GHRH concentration and GHRH mRNA expression have been conducted in post-mortem tissue of depressed patients and matched controls which, in light of the evidence presented here, is of interest. Similarly, CSF studies of GHRH are also lacking.

Obvious alterations of GH and SRIF concentrations and function exist in major depression, although whether these changes represent fundamental contributors to this syndrome or are merely epiphenomena remains to be determined. Diminished concentrations of the inhibitory neuropeptide SRIF might plausibly allow CRF hypersecretion and increased HPA axis activity. Further elucidation of SRIF receptor function and the effects/utility of SRIF receptor agonists and antagonists will provide important information regarding the pathophysiology of major depression and neurodegenerative disorders such as AD.

XI. CHOLECYSTOKININ

A. Biology

Cholecystokinin (CCK) was first identified in the gastrointestinal tract as a 33-amino-acid peptide [240] and was later discovered in the mammalian CNS in 1975. Utilizing gastrin antisera that avidly cross-reacts with CCK, Vanderhaeghen et al. [241] found abundant gastrin-like material in the brain of many vertebrate species, including humans. Amino acid sequence analysis determined this substance to be the carboxyl-terminal amidated peptide CCK 8 [242]. In the gut, CCK exists predominantly in its larger forms of CCK, 22, 33, 39, and 58 with smaller quantities of CCK 8. In the brain, its major amidated form is CCK 8. Although first identified in the gastrointestinal track, CCK is found in higher concentrations in the brain than in the gut. In the brain only neuropeptide Y exists in higher concentrations than CCK. CCK and high densities of its receptors exist in areas of the mammalian brain associated with emotion, motivation, and sensory processing, such as the cortex, striatum, hypothalamus, hippocampus, and amygdala [243–248]. CCK is often colocalized with DA in the ventrotegmental neurons that comprise the mesolimbic and mesocortical DA circuits. Of the two major subtypes of CCK receptors that exist, the CCK_A subtype is primarily found in the lower gastrointestinal tract, pancreas, and gallbladder, whereas the CCK_B receptor predominates in the brain.

CCK has been reported to reduce the release of DA [249–251]; conversely, the release of CCK is modulated by DA [252,253]. Moreover, preclinical studies indicated that DA neuronal activity may be either facilitated or inhibited by CCK [254–257]. Because of

the interactions between DA and CCK, initial investigations had focused on a putative role for CCK in the pathophysiology of schizophrenia with little evidence forthcoming to support the hypothesis. Investigation of possible perturbations of CCK function in patients with mood disorders has similarly demonstrated rather disappointing findings. There is a single report of diminished concentrations of CSF CCK in patients with bipolar [258], but not unipolar, depression [119,143,259,260].

B. CCK and Panic Disorders

An impetus for the study of a role of CCK in the pathophysiology of panic disorder (PD) and other anxiety disorders was stimulated by the finding that i.v. injection of cholecystokinin tetrapeptide (CCK-4) induced panic symptoms in healthy individuals [261]. In a subsequent double-blind study, patients with PD experienced panic attacks after i.v. CCK but not following saline challenge [262]. Furthermore, in comparison to normal controls, patients with PD exhibit an increased sensitivity to CCK-4, a preferential CCK_B receptor agonist [263–265], although both PD patients and controls experience panic attacks with increasing doses of CCK-4 [263,264]. These findings have been extended to investigations in which panic attacks were provoked in patients with PD, and to a lesser extent in patients with generalized anxiety disorder [266], and normal controls with pentagastrin, another CCK_B receptor agonist [267,268]. Patients with PD have been reported to exhibit diminished CSF CCK concentrations in comparison to controls [269].

The development of CCK_B receptor antagonists may lead to a potentially novel treatment for PD and other anxiety disorders. Certain CCK_A or CCK_B receptor antagonists have demonstrable anxiolytic [270–273], antidepressant [274], or memory-enhancing [275] effects in animals. Moreover, in patients with panic disorder, administration of L-365,260, a benzodiazepine-derived CCK_B receptor antagonist, blocks CCK-4-induced panic [276]. In normal controls, L-365,260 did not exhibit an anxiolytic effect but did not induce adverse changes in mood, appetite, or memory [275]. A larger placebo-controlled, double-blind clinical trial with L-365,320 failed to find any clinical improvements in patients with panic disorders [277]. Another compound, CI-988, has been studied in patients with generalized anxiety disorder but was no more effective than placebo as an anxiolytic [278]. Nevertheless, efforts continue toward the development of an alternative, effective anxiolytic that does not have the adverse sedative, dependence liability, and cognitive effects of benzodiazepines.

XII. CLINICAL IMPLICATIONS AND CONCLUSIONS

The last three decades of neuropsychophysiological exploration has yielded a plethora of new findings regarding alterations of CNS circuits containing neuropeptides and hypothalamic releasing factors in certain psychiatric disorders. These studies have led to major advances in biological psychiatry by helping us further understand the brain circuits involved in the pathophysiology of mood and anxiety disorders. Although the balance of evidence indicates that multiple neuropeptide systems within the CNS are altered in major depression and anxiety disorders, determination of the activity or dysfunction of these systems within the brain remains relatively difficult. Not only may there be differences between hypothalamic and extrahypothalamic circuit involvement in a particular disorder, but it remains unclear as to which compartment (or both) CSF sampling accesses. Furthermore, there is also discordance between CNS and more "peripheral" sources of several

neuropeptides, such as CCK. Peripheral plasma concentrations of a neuropeptide or hypothalamic releasing factor are determined not only by the rate of release, but also by local metabolic degradation, the presence of specific binding proteins (as, for example, in the case of CRF), and redistribution into other extravascular spaces [279]. For example, plasma CRF concentrations can be measured, but may not truly represent CNS secretion because of the factors noted above and the contribution by the adrenal medulla and spleen, which also synthesize the peptide.

The importance of neuropeptides in the pathophysiology of psychiatric illness is most evident in the large literature indicative of CRF hypersecretion in patients with major depression. This theory is supported by evidence from a variety of disciplines and has led to the development of a novel therapeutic approach for the treatment of anxiety and depression, namely, CRF receptor antagonists. This is one of the few instances where preclinical evidence has led to a rational target for drug discovery in the hopes of treating psychiatric disease. Furthermore, this work has provided a mechanism to explain the increase in depression seen in patients exposed to trauma early in life, first postulated by Freud in the early part of the twentieth century. If CRF truly is the "black bile" of depression, CRF receptor antagonists may represent a novel class of antidepressants with a unique mechanism of action distinct from other commonly used antidepressants.

Virtually all of the neuropeptide and neuroendocrine axis alterations in patients with major depression thus far studied are state-dependent (Table 5). However, nearly all the studies noted in this chapter are "cross-sectional" in design (i.e., the psychiatric disorder and alterations of neuropeptide or hypothalamic releasing factor were determined at approximately the same time). Clinical investigators of the twenty-first century will extend understanding of whether certain neurobiological alterations provide fundamental pathophysiological contributions to the behavioral manifestation of a particular psychiatric disorder, or are merely epiphenomena (i.e., diminished CSF concentrations of SRIF in patients with Alzheimer's disease). Furthermore, present efforts guided by the "neuroendocrine window strategy" and the "pharmacological bridge technique" may provide information as to whether the secretion of neuropeptides and hypothalamic-releasing factors are associated with alterations in the activity of putative neurotransmitters, such as 5HT, DA, and ACh, in a particular disease state. The availability of selective ligands that can be utilized with positron-emission tomography (PET) will mark the next major leap in our understanding of the peptidergic involvement in psychiatric disorders. The ability to deter-

Table 5 Alterations of Neuropeptides and Hypothalamic Releasing Factors in Various Psychiatric Disorders

Major Depression
Hyperactivity of the HPA axis
Dysregulation of GH secretion
Diminished SRIF activity
Diminished NPY secretion
Bipolar Disorder-Manic Phase
Hypersecretion of AVP
Anxiety Disorders
Increased sensitivity to CCK-4, a preferential CCK_B-receptor agonist

mine peptide-receptor alterations in the brain and pituitary of patients with psychiatric disorders will contribute immensely to our understanding of the neurobiological underpinnings of such disorders.

A clearer understanding of the neuroendocrinology of depression and anxiety may well lead to the development of novel pharmacological agents for the treatment of these major mental disorders. We await confirmation of the initial report documenting the effectiveness of the substance P (NK-1) receptor antagonist, MK-869, in patients with major depression. Early studies of novel CRF receptor antagonists suggest efficacy in the treatment of depression. A selective CCK_B antagonist with anxiolytic activity offers a new psychotropic modality in the treatment of panic disorder. Progress during the last three decades has been nothing short of remarkable and the concatenation of present findings undoubtedly adumbrates further progress in these disorders. In conclusion, the elucidation of biochemical abnormalities in psychiatric disorders has led to an increased comprehension of the neurochemical basis of psychiatric disease, with the ultimate goal being the development of novel pharmacological and behavioral techniques to treat these devastating disorders.

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12

Immunology in Anxiety and Depression

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I. INTRODUCTION

The objectives of psychoneuroimmunology are to elucidate the reciprocal influences of the nervous system and the immune system and their effects on behavior and health. This field covers in vitro studies of tissue and lymphocytes; investigations of the influence of stress, stress coping, and personality traits on the function of the immune system, as well as the role of psychological factors during pathogenesis, and the course of tumor disorders and their psychotherapeutic treatment. In addition, human behavior studies and animal experiments, such as conditioning studies [1] or investigations into the dependency of coupling behavior from the human leukocyte antigen (HLA) system [2], also belong to the category of psychoneuroimmunology.

Cytokines mediate information between cells of the peripheral immune system and the central nervous system (CNS). In part, they are actively transported through the blood–brain barrier, but they are also released from activated astrocytes and microglial cells. Interleukin (IL)-1, IL-2, IL-6, and tumor necrosis factor-alpha (TNF-α) are the most relevant cytokines known to act on the CNS. Cytokines belong to a network, and they can activate cells to produce other cytokines. Recent findings indicate the important role of cytokines in psychiatric disorders, which may be because of their influence on neurotransmission.

From a clinical point of view, depression shows several parallels to autoimmune

disorders. These include early onset in many cases, genetic vulnerability, waxing and waning course, and female preponderance.

The influence of an immune process on the pathogenesis of psychosis has been under discussion since it was proposed that autoimmune disorders of the CNS, such as lupus erythematosus, might lead to severe depressive states. The more it became evident that the noradrenaline [3,4] and serotonin hypotheses [5] of depression were not sufficient to explain depressive disorders, the more other pathological agents came into the focus of interest.

Moreover, the increasing role of psychoneuroimmunology is also due to the rapid development of immunological methods. Thanks to the recent immunological and molecular biological investigative techniques, our knowledge of the function, mechanisms of regulation, and interaction of the immune system is growing, as shown in Figure 1. More accurate estimation of the highly differentiated and variable immune system are made possible by recent methodological advances.

The induction of severe depressive syndromes by immune processes is shown by psychiatric disturbances that occur during different autoimmune disorders, such as lupus erythematosus [6], scleroderma [7], Sjögren syndrome [8], and antiphospholipid syndrome [9]. Additional evidence is given by the characteristic depressionlike side effects of interferon-α (IFN-α) treatment in patients suffering from hepatitis C or malignant melanoma [10,11].

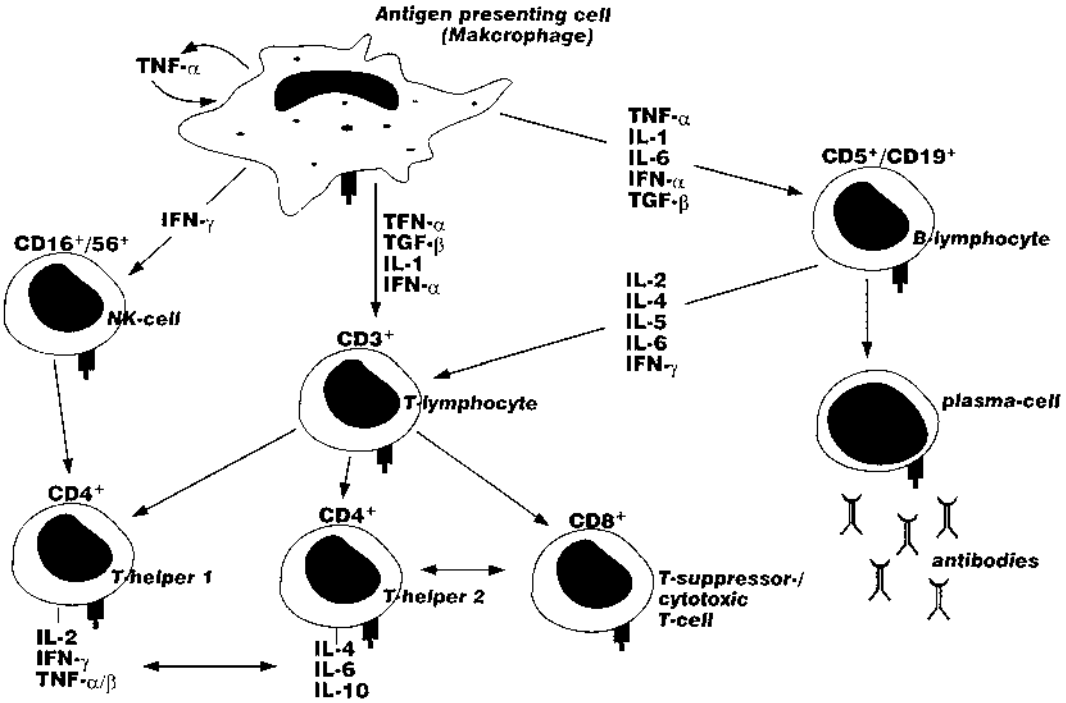

Figure 1 Overview of important cell types and their products (cytokines) of the highly differentiated immune system.

Modern immunological methods at first led to the investigation of lymphocyte subpopulations by monoclonal antibodies. Different cell surface markers point to different functions of the lymphocytes. During the 1980s, several researchers studied the cellular immune system in different psychiatric disorders [12–18]. The findings in the cellular immune system, in particular in affective disorders, indicated that there was an immune activation in these disorders, that in turn, raised the question as to the function of cytokines, a key factor in the activation of the immune system.

II. THE CYTOKINE NETWORK IN THE CNS

Recent studies have revealed the close connection between the CNS, endocrine system, and immune system [19]. Cytokines in the CNS are involved in the following different regulatory mechanisms:

1. Initiation of an immune process in the CNS during an inflammatory disease.
2. Regulation of the blood–brain barrier.
3. Developmental and repair mechanisms after injury.
4. Regulation of the endocrine system in the hypothalamus–pituitary–adrenal (HPA) axis.
5. Different stimulatory and inhibitory influences on the dopaminergic, serotonergic, noradrenergic, and cholinergic neurotransmission.

Cytokines activate CNS cells in different ways. First, several cytokines such as IL-1 [20], IL-2 [21], and TNF-α [22], can be transported from the blood into the CNS by active transport mechanisms, as shown in in vitro studies. Second, glia cells secrete cytokines after activation by an antigenic challenge. Finally, it was recently reported that cytokine secretion in the CNS can be stimulated by neurotransmitters [22a]. Noradrenaline stimulates the release of IL-6 from astrocytes in vitro in a dose-dependent manner, an effect that can be antagonized by blocking the adrenergic receptors. Since IL-6 is closely linked to the function of other cytokines (e.g., IL-1, IL-2, and TNF-α), this finding indicates that neurotransmitters can activate the cascade of cytokines [23]. This represents possibly a relevant psychoneuroimmunological regulative mechanism affecting (auto-)immune disorders, susceptibility to infections, and psychiatric disorders. Noradrenaline, released during stress [24], may act as a cytokine-activating stimulus, which in turn activates immune phenomena mediated by the cytokine cascade.

It is unclear, however, whether cytokines have different functions depending on whether they are released from the periphery or from the CNS. Nevertheless, it is likely that there is at least a functional synergism. The localization of interleukin receptors, in particular IL-1 receptors in glia cells around arterioles and in the plexus choroideus, suggests that there is close communication between CNS IL-1 receptors and IL-1 invading the CNS from the periphery [25].

III. ASTROGLIA, MICROGLIA, AND NEURONS AS CARRIERS OF THE CNS IMMUNE FUNCTION

Astrocytes form the largest cell population in the CNS, exceeding neurons by more than tenfold [26]. Astrocytes are located immediately beside neurons, often enfolding them. This localization underlines the close relationship between astrocytes and neuronal func-

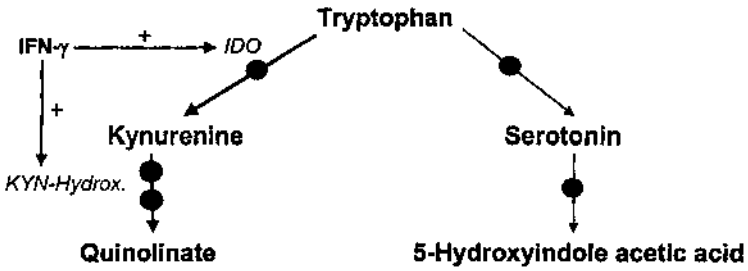

Figure 2 The relationship between the TH-1 cytokine IFN-γ and serotonin metabolism. IDO = indoleamine 2,3-dioxygenase; KYN-Hydrox. = kynurenine hydroxylase.

tion. About 10% of the glia cells are microglia cells [26]. Both microglia cells and astrocytes can produce and release cytokines after activation, as shown schematically in Figure 2. However, they not only do this in different ways, but also they produce different patterns of cytokines. This indicates a functional difference in how they activate the immune process in the CNS.

Viruses and viral particles stimulate microglia to release cytokines. This mechanism initiates an immune reaction after viral infection and expresses cellular surface structures for recognition [27].

The existence of cytokine receptors on neurons suggests that cytokines have a direct influence on neuronal function. IL-1–containing neurons have been found in different areas of the CNS, including the hypothalamus and hippocampus in animal investigations [28–30]. A larger amount of messenger-RNA for IL-2 receptors (IL-2R)—the gene transcript for the protein synthesis of IL-2R—was found in neurons than in microglia or astrocytes. Thus, IL-2 seems to directly influence neuronal functions [31], too.

Astrocytes and microglia are also able to express receptor structures of the immune system, such as HLA molecules [32] or T-cell receptors after activation [33,34], and they can act as antigen-presenting cells. Cytokines also play an important role during the physiological development of the CNS [35], which may be strongly altered by an over- or underproduction of cytokines.

IV. EFFECTS OF CYTOKINES IN THE BLOOD AND IN THE CNS

IL-1, IL-2, IL-6, and TNF-α are examples for cytokine functions in the CNS, although many more cytokines are known to be released in the CNS.

Psychotropic effects were first described for IL-1. IL-1 is released by macrophages, stimulates T-cells to proliferate, and is present everywhere in the blood. During an inflammation, IL-1 levels increase locally. IL-1 is actively transported into the CNS [21], but it can also move passively into the CNS via the circumventricular organs [36]. It is produced by astrocytes or microglia cells [35].

The localization of IL-1 in neurons of the hippocampus [25] underlines its influence on both neuronal processes and psychological phenomena. One psychotropic effect of IL-1 is a psychomotor retardation; IL-1 also induces fever, sleep disturbance, loss of weight, and loss of appetite, suggesting that it has an anorectic effect. IL-1 also plays a pronounced role in "sickness behavior" [37].

Via hypothalamic neurons, IL-1 upregulates the hormones of the HPA axis. For

example, the release of corticotropin-releasing hormone (CRH) and growth hormone releasing hormone (GHRH) is stimulated by IL-1 [38,39]. Since IL-1 release in the hypothalamus is upregulated by stress [40], one factor in the glucocorticoid stress reaction may be IL-1, as Besedovsky et al. [38] mentioned. The central IL-1 upregulation, which leads to stimulation of CRH, the HPA axis, and the sympathetic nervous system [41,42], may cause immunosuppression induced by CNS IL-1 effects. The upregulation of IL-1 in hypothalamic neurons was thought a conclusive link to immunosuppression during stress, historically a focus of experimental investigations in psychoneuroimmunology [43,44]. In vivo studies also show that the exacerbation and the course of viral disorders are influenced by stress (e.g., herpes simplex) [45,46]. In parallel, antibody titers of neurotropic viruses [47] and the onset and course of several autoimmune disorders [48] are modulated by stress.

Like IL-1, IL-6 is known to interact strongly with the HPA axis, too. As our knowledge of the function of cytokines in the CNS grows, the concept of specificity of the cytokine action on the HPA axis needs to be revised in favor of an unspecific central downregulating action of cytokines to the HPA axis [49]. IL-2 is produced in blood by activated T lymphocytes; in the CNS, it is produced mainly by activated microglia cells. Considering psychiatric disorders, IL-2 effects in the CNS are of particular interest for three reasons: the highest concentration of IL-2 receptors is found in the hippocampus [50], psychotic phenomena occur after application of IL-2 [51], and IL-2 has an immense effect on the dopaminergic neurotransmission [52–54].

IL-6 is a pleiotropic cytokine, which is released from different cell types in the blood (macrophages, monocytes, T and B cells). The largest amount of IL-6 in the blood is macrophage-derived. One function of IL-6 is to activate B cells to synthesize antibodies [55]. In the CNS, IL-6 is produced by activated astrocytes and microglia. The IL-6 production, however, differs depending on the cell type. For example, TNF-α and IL-1, as well as norepinephrine, induced IL-6 production in astrocytes, but not in microglia [56], whereas IL-6 production in microglia is stimulated by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), as in vitro studies show. Astrocytes express more adrenergic receptors than neurons [57]. The strong effect of noradrenaline on astrocytes seems to be because of this fact. Several findings suggest that IL-6 may mediate the exacerbation of autoimmune disorders in the CNS [57] (e.g., IL-6 supports the differentiation of B cells, local IgG synthesis in the CNS, and blood–brain barrier disturbance [58,59]). In the hypothalamus, IL-6 can induce the release of GHRH and thyroid-stimulating hormone (TSH), as does IL-1; moreover, IL-6 stimulates in vitro the secretion of prolactin, growth hormone (GH), and luteinizing hormone (LH) from pituitary cells [49].

TNF-α is a cytokine that acts synergistically with several other cytokines and has strong toxic effects. In the CNS, primarily astrocytes release TNF-α, but also microglia can release TNF-α after stimulation. The release of TNF-α from activated CNS cells can be due to several stimuli, such as neurotropic viruses [27], but also bacterial toxins. Thus, glial-derived cytokines up- and downregulate each other, providing an endogenous feedback mechanism [35].

V. THE CONCEPT OF INNATE AND ADAPTIVE IMMUNITY IN HUMANS

The immune system has been developed during evolution over millions of years. In order to guarantee successful defense against numerous varying invading, life-threatening micro-

Table 1 Components of the Unspecific Innate and the More Specific Cellular Adaptive Immune Systems in Humans

Components	Innate	Adaptive
Cellular	Monocytes Macrophages Granulocytes NK cells γ/δ cells	T and B cells
Humoral	Complement, APP, mannose binding lectin (MBL)	Antibodies

organisms like bacteria, viruses, or parasites, a highly differentiated system consisting of different lines of defense was established. A widespread heterogeneity was the consequence: two functionally different immune systems both representing different types of barriers and each consisting of cellular and humoral immune components. The innate immune system is the phylogenetically older, primitive one. Its cellular arm is represented by monocytes/macrophages, granulocytes, and natural killer (NK) cells. The humoral arm consists of acute phase proteins and the complement system. This unspecific immune system represents the first line of defense.

The specific part of the immune system of higher organisms, including humans, is the adaptive immune system, consisting of the cellular arm of the T and B cells and the humoral arm of specific antibodies. This system includes higher functions, such as memory, and can be conditioned. In case of a reexposition to a specific antigen, this system can specify the enemy and initiate a specific immune answer (Table 1).

The innate and adaptive immune systems are functionally balanced. Within the adaptive immune system is another balance regarding the activation of the cellular and the humoral immune system. The cellular arm of the adaptive immune system is mainly activated by the T-helper-1 (TH-1) system—helper cells, which produce the activating immunotransmitters IL-2, IL-12, IFN-γ, and TNF-α. The humoral arm of the adaptive immune system is mainly activated via the TH-2 system—helper cells, which produce mainly IL-4, IL-10, IL-13, and IL-6.

VI. MAJOR DEPRESSION: THE DISCRIMINANT POWER OF TH-1 AND TH-2 FOR SUICIDALITY

An immunological model of major depression (MD) is sickness behavior, the nonspecific reaction of the organism to infection and inflammation. Sickness behavior is characterized by weakness, malaise, listlessness, inability to concentrate, lethargy, decreased interest in surroundings, and reduced food intake—all of which are depressionlike symptoms. The sickness-related psychopathological symptomatology during infection and inflammation is mediated by cytokines such as IL-1, IL-6, TNF-α, and IFN-γ. Their active pathway from the peripheral immune system to the brain is via afferent neurons and direct targeting at the amygdala and other regions of the brain after diffusion at the circumventricular organs and choroid plexus [60]. Undoubtedly, there is a strong relationship between the cytokine system and the neurotransmitter system, but a more differentiated analysis may be required to understand the distinct mechanisms in the heterogeneous disease entity of

major depression. Blood levels of several cytokines have already been investigated in major depression, and their interpretation based on the TH-1/TH-2 concept may help to generate hypotheses for discrimination between subgroups of MD.

VII. TH-1 CYTOKINES IN MAJOR DEPRESSION: THE TH-1 SEROTONIN LINK AS A BIOLOGICAL BASIS FOR SUICIDALITY

Data on IL-2 in major depression are mainly reduced to some measurements of its soluble receptor in peripheral blood. Those blood levels of sIL-2R were repeatedly described to be increased in MD patients [61–63]. One single study dealt with CSF measurement of sIL-2R levels and reported a highly significant reduction in MD patients compared to healthy control subjects [64].

Production of both IL-2 and IFN-γ is the typical marker of TH-1 cells. IFN-γ is produced in higher amounts by lymphocytes of patients with MD than in healthy controls [65]. Higher plasma levels of IFN-γ in depressed patients, accompanied by lower plasma tryptophan availability, were described in 1994 [66]. Mendlovic and colleagues discriminated between suicidal and nonsuicidal MD patients in a small study. They found distinct associations between suicidality and TH-1-type immune response on the one hand and a predominance of TH-2 parameters in nonsuicidal patients on the other hand [67]. This combination of psychopathological symptoms and their immunological correlates may represent a very successful strategy in immunological research of major depression. We want to introduce a line of evidence for a relationship between the TH-1 cytokine IFN-γ, tryptophan metabolism, and suicidality.

The essential amino acid tryptophan is the precursor of two distinct metabolic pathways, leading to the products serotonin or kynurenine (see Fig. 2). The enzyme indolamine 2,3-dioxygenase (IDO) metabolizes tryptophan to kynurenine, which is then converted to quinolinic acid by the enzyme kynurenine hydroxylase. Both IDO and kynurenine hydroxylase are induced by IFN-γ. The activity of IDO is an important regulatory component in the control of lymphocyte proliferation [68]. It induces a halt in the lymphocyte cell cycle due to the catabolism of tryptophan [69]. The TH-2 cytokines IL-4 and IL-10 inhibit the IFN-γ-induced tryptophan catabolism by IDO [70]. The enzyme IDO is located in several cell types, including monocytes and microglial cells [71]. An IFN-γ-induced, IDO-mediated decrease of central nervous tryptophan availability may lead to a serotonergic deficiency.

One of the most consistent findings in biochemical research dealing with mental disorders is that some patients with low 5-hydroxyindoleacetic acid (5-HIAA)—the metabolite of serotonin—in CSF are prone to commit suicide [72–74]. This gives additional evidence for a possible link between the TH-1-like cytokine IFN-γ and the IDO-related reduction of serotonin availability in the CNS in suicidal patients.

On the basis of epidemiological data it has been hypothesized that high IL-2 levels are associated with suicidality [75]. Accordingly, clinical studies have observed an activation of the TH-1 system that might be related to suicidality. Increased levels of serum sIL-2R have been described in medication-free suicide attempters disregarding the psychiatric diagnosis [76]. Treatment with high-dose IL-2 has been associated with suicide in a case report [77]. Recently, a small study showed that T cells of suicidal patients with major depression have TH-1 characteristics, while T cells of nonsuicidal depressed patients showed TH-2 characteristics [67].

We believe that the possible involvement of IFN-γ-induced IDO activity in the pathophysiology of suicidality in major depression should be considered in future studies.

VIII. CYTOKINES AND THE CELLULAR IMMUNE SYSTEM IN MAJOR DEPRESSION: FOCUS ON IL-6 AND ACUTE PHASE PROTEINS

Former studies show an increase of T-helper cells ($CD4^+$ cells) and an increased $CD4^+$/$CD8^+$ ratio in depressive disorders [78,79]. This finding points to an immune activation and was the starting position for a series of further studies.

Further investigations of the cellular components of the immune system focused on monocytes and macrophages. Increased numbers of peripheral mononuclear cells have been described by different groups of researchers [80–82].

Neopterin is a sensitive marker of cell-mediated immunity. The main source of neopterin are monocytes/macrophages. According to the findings of increased monocytes/macrophages, an increased secretion of neopterin has been described by several groups of researchers [66,83–85].

As a product of monocytes and macrophages, IL-6 is one of the most frequently investigated immune parameters in patients suffering from major depression. Most publications report a marked increase of in vitro IL-6 production [86] or serum IL-6 levels in depressed patients [61,87–91]. Most of these studies also report elevated plasma levels of acute phase proteins (APPs)—indicators of the unspecific (innate) immune system. Contradictory results are very few, indicating reduced [92], or not altered serum IL-6 levels [62,92a]. An age-related increase of IL-6 serum levels was reported in patients with major depression [93]. From a methodological point of view, the potential influence of possibly interfering variables such as smoking, gender, recent infections, and medication prior to IL-6 release and concentration must be considered [94].

IL-6 production is stimulated by prostaglandin E2 (PGE2) [95]. The central nervous system expression of the PGE2-producing enzyme cyclooxygenase-2 (COX2) is, on the other hand, induced by IL-6 [96]. Therefore, an increased secretion of PGE2 could be expected in depressive disorders. Indeed, several studies described increased PGE2 in the cerebrospinal fluid, serum, and saliva of depressed patients [97–99]. Moreover, in vitro studies show an increased PGE2 secretion from lymphocytes of depressed patients compared to healthy controls [91]. Further evidence for a functional relationship between PGE2 and depression is given by the fact that COX inhibitors are able to reduce the lipopolysaccharide-induced sickness behavior in an animal model of depression [100].

Data of IL-6 and the IL-6 system in the CSF are still rare. The only available data on CSF IL-6 have been published by our group. Herein, we found markedly decreased levels of IL-6 and its soluble receptor subunit IL-6Rα in elderly patients with MD compared to matched healthy controls [101].

As stated above, IL-6 is a highly important inducer of antibody production (TH-2 immune response) and, indeed, some data show increased antibody titers in MD. As in schizophrenia, a great heterogeneity of antigen specificity of the antibodies such as antinuclear antiphospholipid, antithyroidal, or antiviral antibodies was found [86,102,103].

There is no doubt that IL-6 is involved in modulation of the HPA axis [55]. Activation of the HPA axis is one of the best-documented changes in major depression [104]. Furthermore, the relationship between psychological or physical stress and an enhanced IL-6 secretion in the peripheral immune system seems to be well established [105–108]. An impaired ability of stress coping is often observed in depressed patients. Thus, the

high number of data showing elevated peripheral IL-6 levels in MD patients may be related to psychological stress. On the other hand, there is evidence for a relationship between high peripheral IL-6 levels and elevated central nervous system serotonin availability. Intravenous or intraperitoneal administration of IL-6 induced, not only an activation of the HPA axis, but also an increase in brain tryptophan and serotonin metabolism, whereas norepinephrine metabolism was unaffected in an animal model [109]. Accordingly, IL-6 seems to mediate the activation of the HPA axis and of the central nervous serotonin system after administration of the endotoxin lipopolysaccharide (LPS) [110]. Thus, elevated plasma levels of IL-6 do not fit with the hypothesis of a serotonin deficiency in MD.

On the other hand, there is a report about a correlation of the increased IL-6 production in vitro with decreased tryptophan levels in depressed patients that emphasizes the influence of IL-6 on the serotonin metabolism [86]. Serotonin synthesis in the CNS is at least partly dependent on the availability of free plasma tryptophan [110a].

It should be recognized that an inherent heterogeneity exists in the etiology of depression and different neurotransmitter systems may be disturbed. Norepinephrine is the second major biogenic amine that has been proposed to be causally involved in the pathophysiology of major depression [3,111] and in the mechanism of antidepressant drug action [112]. Whether MD patients with elevated peripheral IL-6 are suffering primarily from a CNS norepinephrine deficiency—in contrast to the suicidal patients with marked serotonin deficiency and possible TH-1-dominated immune response—needs to be investigated.

Studies on other TH-2 cytokines are lacking and the current data on IL-6 alone are insufficient to establish any TH-2-related hypothesis on major depression. However, the question remains, is a TH-2 response the common immunological hallmark of both nonsuicidal depression and negative schizophrenia—two different disease concepts but showing certain similarities in the psychopathology.

IX. DEPRESSION: A HETEROGENEOUS SYNDROME WITH DIFFERENT IMMUNE PATHOLOGIES

Major depression is a common disorder showing a lifetime prevalence between 5% and 25%, twice as frequent in women compared to men. From the standpoint of many researchers, the diagnosis of MD reflects different etiological and pathophysiological subgroups as older diagnostic manuals differentiated entities such as endogenous depression, neurotic depression, or psychoreactive depression. Moreover, different etiologies have been suggested for monopolar and bipolar depression. Therefore, it could be expected that different immunological patterns are found in different types of depression.

Only recently, immunological investigations have been performed on different types of MD. A group of patients suffering from MD were investigated, a part of them fulfilling the criteria of melancholic-type depression. Melancholic patients had normal leukocyte, lymphocyte, and NK-cell counts, while the nonmelancholic depressed patients showed increased leukocytes, lymphocytes, and NK cells [82].

On the other hand, melancholic patients showed decreased production of IL-2, IFN-γ, and IL-10, while nonmelancholic patients had cytokine production comparable to healthy controls [82]. During antidepressive treatment, the monocyte count stayed higher in nonmelancholic patients after 2 and 4 weeks of treatment. Accordingly, alpha-2 macroglobulin, another marker of inflammation, was increased in nonmelancholic patients before therapy and after 2 and 4 weeks of treatment. There were no differences between healthy

Table 2 Markers of TH-1/TH-2 Responses in Major Depression

Site of cytokine expression	TH-1	TH-2
In vitro production	IFN-γ ↑	IL-6 ↑↑
Peripheral	sIL-2R ↑↑ IFN-γ ↑ IFN-γ ↑ ⇒ TRP ↓	IL-6 ↑↑
CSF	sIL-2R ↑	IL-6 ↓ sIL-6R ↓
Hypothesis	A TH-1 serotonin link in suicidal MD?	A TH-2 dominance or an overactivation of monocyte/macrophage system in nonsuicidal MD?

↑↑ or ↓↓ means repeated consistent data for elevation or decrease respectively; ↑ or ↓ means less consistent data.

controls and both melancholic and nonmelancholic depression regarding C-reactive protein, haptoglobin, and in vitro production of IL-1β [113].

These results show that different types of depression—melancholic versus nonmelancholic—are associated with different immune states. Moreover, suicidality seems to show a further distinct immune pattern.

These results point to a methodological pitfall regarding studies of immunity and depression: the heterogeneity of the results of immunological studies might at least be due in part to different types of depression, including suicidality. Perhaps the finding of increased sIL-2R and IL-1 concentrations in depression might be due to a patient group more often exhibiting the melancholic type of depression.

However, regarding these findings it is doubtful that the pleiotropic cytokine IL-6 and the other components of the IL-6 system (such as the IL-6 receptor and the signal transducing molecule gp130) are specifically altered in major depression. Moreover, increased IL-6 levels have also been observed in patients suffering from schizophrenia. Increased levels in schizophrenia are associated with the duration of the disease and the paranoid psychopathology [114,114a].

Taken together, there might be a relationship between immunological, neurochemical, and clinical variables in MD patients: suicidality in MD may be related to a central nervous serotonin deficiency, possibly induced by an IFN-γ (TH-1)-mediated IDO activation, whereas elevated levels of IL-6 (TH-2) might indicate a distinct group of MD patients without deficiency of the serotonergic system. The possible predictive value of immunological parameters on antidepressant therapy regimen has to be unraveled in future studies (Table 2). However, future immunological studies of MD should emphasize the role of clinical and neurochemical variables.

X. CYTOKINES AND EFFECTS OF ANTIDEPRESSANTS

Only very few investigations have been carried out regarding the effects of antidepressant medication on immune parameters [115]. However, attention has focused on the interac-

tion of the serotonergic system and the immune system. It has been suggested that serotonin has an inhibitory action on antibody production, since an inverse relationship between brain serotonergic concentration and antibody synthesis has been demonstrated in previous studies [116]. The suggested role of the cytokines in depression leads one to expect that antidepressants would have an inhibitory effect to certain activating cytokines. Studies using modern immunological methods observed an inhibitory function of the serotonin system on the IFN-γ-induced MHC expression [117] and on mitogen-induced T-cell proliferation [118].

During the last years, a modulatory, mostly inhibiting, effect of serotonin reuptake–inhibiting drugs to activating immune parameters has been demonstrated in animals [119,120]. Moreover, inhibiting effects of selective serotonin reuptake inhibitors on the acute phase proteins were observed in animal investigations as well [121], and there are several hints that the severe side effects of zimeldine are due to immunological mechanisms of the 5HT system [122].

From an immunological point of view, some antidepressants are able to induce a TH-1-to-TH-2 shift; Maes and colleagues demonstrated the potency of three different antidepressants (sertraline, clomipramine, and trazodone) to significantly reduce the IFN-γ/IL-10 ratio in vitro. These three drugs significantly reduced IFN-γ production, sertraline and clomipramine and increased IL-10 production significantly [18].

Other researchers, however, observed a decrease of IL-6 during treatment of the serotonin reuptake inhibitor fluoxetine [123]. These very preliminary results point to different immune modulating effects of different antidepressants, but the immune effects of antidepressants and especially the effect on the cytokine production has not yet been studied systematically.

An interesting study showed a relationship between IL-6 production from the lymphocytes of depressed patients and the treatment response: patients producing low levels of IL-6 showed a better response to treatment with amitryptyline over six weeks compared to patients showing a high IL-6 production. During treatment, however, both groups turned to a normal IL-6 production. The production of TNF-α was high in responders to amitryptyline and turned to normal during therapy [123a].

With respect to the above-mentioned results, it can be speculated that amitryptyline might be especially efficient in patients with low IL-6 levels, which show a more TH-1-related immune response (melancholic type) similar to sertraline, clomipramine, and trazodone, while fluoxetine might be more efficient in patients with high IL-6 levels (nonmelancholic) with a monocyte/macrophage immune response.

Another role in immunotherapy has been suggested for the antidepressant rolipram [124]. Rolipram, a selective phosphodiesterase inhibitor, inhibits in vitro the production of activating cytokines, especially TNF-α and IFN-γ. Moreover, rolipram is effective in preventing, delaying, and reducing the clinical severity of EAE, the animal model of MS [124,125]. Rolipram, not yet marketed, has been shown to be safe and effective in the treatment of depression [126,127] and was proposed to be a potential therapy for MS [124].

The downregulating effects of antidepressants on inflammatory cytokines, associated with the findings of increased proinflammatory cytokines and cellular immune markers, which were found mainly in nonsuicidal, nonmelancholic patients suffering from MD, point out that this immune effect might be related to the therapeutic effects of antidepressants. Further therapeutic strategies for MD should take into account those immune find-

ings, including the different findings in the different clinical subgroups. From this point of view, developing immunotherapeutic strategies will be a future research tool in depression.

XI. ANXIETY AND IMMUNE FUNCTION

The impact of anxiety on the immune function has widely been studied at the subclinical model of stress and stress coping (i.e., the cognitive appraisal of stress stimuli). Type and efficacy of psychological defense and coping can reduce acute emotional arousal and endocrine stress responses to real-life stressors.

Several authors point out that acute stress and anxiety are associated with decreased immune function. College students at the start of their studies exhibited significantly lower lymphocyte proliferative responses to the mitogen concanavalin A and lower serum levels of IL-1. A more negative attribution style of adversive events was associated with reduced blastogenic T-cell responses and reduced serum levels of IFN-γ [128]. Students with higher anxiety scores during examination stress showed altered lymphocyte subsets [129]. In other studies, high scores of anxiety have been related to decreased lymphocyte response to mitogens [130,131]. However, lymphocyte proliferation tests are very unspecific immunological methods and the results vary widely without giving conclusive answers to the state of the immune system. The definitions of acute or chronic stress often vary widely. Paradigms of acute stress reach from very short-lasting stress, like bungee-jumping or parachuting, to stress lasting over hours and days; chronic stress may last for the duration of exams or over months and years for caregivers of relatives suffering from diseases such as Alzheimer's, as stress models from the literature show. Cum grano salis most of the studies point out that short-term stress is associated with an increase in immune function while long-term stress is associated with a decrease in immune function.

Anxiety is often associated with sympathetic activation. Acute psychological stress can induce short-term catecholamine increases that may result in lymphocytosis and leukocytosis [132]. In parallel, the increase of T-lymphocyte function and natural killer cells during acute stress might be mediated by sympathetic activation [133].

Another example are studies examining IgA concentration in the saliva. During the short-term stress model of competition between soccer coaches during a play, an increase of IgA concentration can be found [134] while dental students show a decrease of salivary IgA concentration during stress periods of the academic year [135]. In addition, air-traffic controllers had a significant increase in salivary IgA after 4 h of work [136].

Regarding the stress model of public speaking, persons who showed a high sympathetic reaction had increased NK cell number, while persons with a low sympathetic reaction showed no increase of NK cells [137]. Public speaking in particular appears to be a model for social phobia because a marked and persistent fear of a social performance situation in which the person is exposed to unfamiliar people is a key fear in social phobia. It seems that the acute stress paradigm might be a suitable model for the social phobia type of anxiety disorder.

The results of the stress paradigm studies, however, are not conclusive. This may be due to several methodological pitfalls. Besides different definitions of acute or chronic stress, the subjective appraisal of stress, personality traits, coping mechanisms, and cognitive function, as well as the important influence of eustress or distress, plays a role. Regarding the immunological parameters and mechanisms, older stress experiments often measured simplified immunological in vitro parameters that may not really reflect the influence

of stress on the complicated in vivo immunological state. On the other hand, those studies had an avant-garde function during the 1960s to 1980s.

XII. ANXIETY AND IMMUNE FUNCTION IN PATIENTS WITH GENERALIZED ANXIETY DISORDER

Patients with generalized anxiety disorder (GAD) suffer from a chronic disorder that lasts over weeks, months, or even years. Immune changes in those patients have only been studied marginally. Regarding the stress model, GAD might fit with the model of chronic stress. In patients with GAD, a significant decrease in the density of the peripheral benzodiazepine receptors on the circulating lymphocytes has been observed, which was largely normalized by effective treatment with chronically administered diazepam.

The results of clinical and experimental studies suggest that the peripheral benzodiazepine receptors play a prominent role in the cellular and humoral immune response. Acutely administered diazepam was without effect. These changes in the receptor density would appear to be specific for the anxiety disorder [121]. In addition to the direct action of benzodiazepine on peripheral benzodiazepine receptors located on immune cells, it is also possible that the immune system is indirectly affected by the increased synthesis of adrenal corticosteroids.

During anxiety and stress, an upregulation of the HPA axis has been widely described. On the other hand, cytokines such as IL-1, IL-6, or TNF-α also induce an upregulation of the HPA axis via stimulation of CRH in the hypothalamic region [138] (Fig. 3).

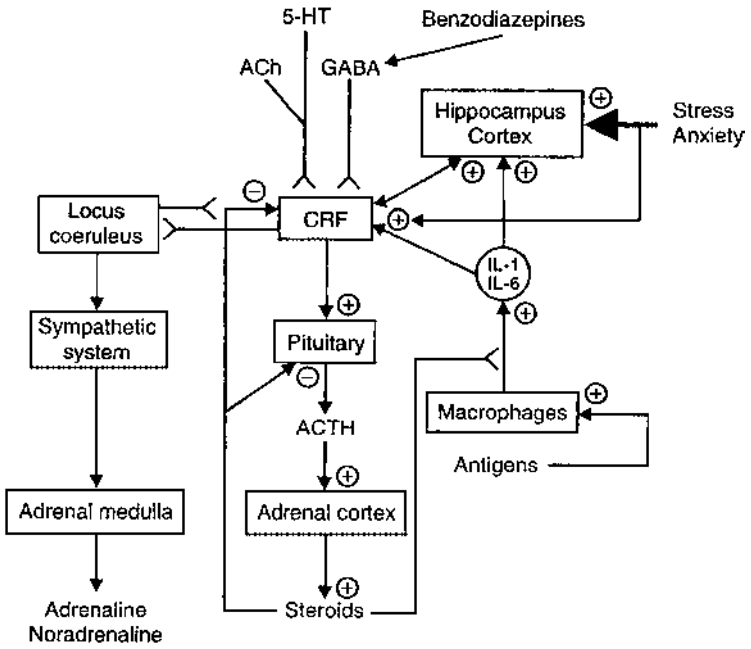

Figure 3 Diagram of changes in the endocrine and immune systems in stress and anxiety. ACh, acetylcholine; 5-HAT, 5-hydroxytryptamine; GABA, gamma-aminobutyric acid; CRF, corticotropin-releasing factor; IL-1, interleukin-1; +, stimulatory; −, inhibitory. (Adapted from Ref. 141.)

The results of clinical and experimental studies suggest that the peripheral benzodiazepine receptors play a prominent role in the cellular and humoral immune response. Anxiolytics that act on central and/or peripheral benzodiazepine receptors can modulate the immune system either indirectly by reducing the central secretion of CRH, the major stress hormone, by acting on central gamma-aminobutyric acid (GABA) receptors, or directly by stimulating the peripheral benzodiazepine receptors on immune cells.

XIII. IMMUNE CHANGES IN PANIC DISORDER

Panic disorder with or without agoraphobia is a syndrome in which severe anxiety, often somatic symptoms of the autonomous nervous system and a stress reaction, is the core syndrome of the disease. Panic attacks initiate severe stressful episodes and they are also often associated with severe depressive episodes. In panic disorder, as well as in GAD, the results of immunological studies are somewhat controversial. A major problem is interpreting the results, arising from the fact that several patients studied suffered from depression in addition to panic attacks. The heterogeneity of the clinical symptomatology therefore might have profoundly influenced the results.

Brambilla et al. [139] found a normal mitogen stimulation of T lymphocytes but an elevated adenocorticotrophic hormone (ACTH) concentration and a blunted cortisol response to dexamethasone. The administration of CRH, which was associated with a hypersecretion of ACTH and cortisol, did not suppress the proliferative response of the T lymphocytes. This lack of change in the proliferative response of T lymphocytes suggests that the secretion of IL-2, IL-6, and IFN-γ would be unchanged. However, there is evidence from other studies that female patients with panic disorders have a slight increase in the serum concentration of IL-2. A solution to this controversial finding could be the suggestion of a relative imbalance within the group of $CD4^+$ T lymphocytes. Both TH-1 and TH-2 cells show the $CD4^+$ surface marker, but secrete different cytokines (TH-1 cells secrete IL-2). The cytokine production of these cells is associated with immunological function.

Some studies described parallels between patients suffering from panic disorder and severe acute psychological stress. Patients suffering from panic disorder and patients with social phobia had increased $CD16^+$ (natural killer) cell numbers. Panic disorder patients also had increased numbers of $CD19^+$ cells (B lymphocytes), human HLA-DR-presenting cells, and more cells with the combination of HLA-DR and CD19 surface markers (B lymphocytes with $HLA\text{-}DR^+$ on their surface), an activation marker of the immune system. These data, although preliminary, suggest that subjects with panic disorder may have alterations in circulating lymphocyte profiles and that, especially in panic disorder, the B-cell system is activated [140]. B lymphocytes produce immunoglobulin including IgA. Similar to the findings in the acute stress model of soccer coaches, patients with "pure" panic disorder showed a significant increase in serum IgA concentration relative to a control population, while concentrations of IgG, IgM, and IgE were unchanged.

Nevertheless, a major problem in interpreting the data relating to the immune changes associated with panic disorder is the paucity of these studies, the heterogeneity of the patients studied, and the differences in the methods used to assess the immune parameters [141].

XIV. CONCLUSION

The current state regarding the immune system, depression, and anxiety disorders reflects a controversial picture. In depression, many psychoneuroimmunological studies have been

performed by several groups, especially during the last few years. The findings point to different immunological/inflammatory functions in different types of depression with respect to nonmelancholic or melancholic symptomatology and suicidality. These more conclusive results lead to preliminary therapeutic studies with immunomodulatory or anti-inflammatory agents that are on the way.

In anxiety disorders, the results are far more inconclusive and the role of immune changes needs to be further elucidated. Whether immunological changes are the result of an acute or chronic accompanying stress reaction or reflect specific alterations of anxiety disorders has to be the objective of further studies.

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Brain Imaging in Depression and Anxiety

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I. INTRODUCTION

Neuroimaging research in affective and anxiety disorders has progressed considerably over the last decade. Researchers have used findings in related fields such as neuropathology, endocrinology, and preclinical animal work to develop high-quality hypothesis-driven neuroimaging studies. Moreover, emphasis is now being put on the importance of such issues as increasing the size of studies, separation of heterogeneous groups, research in drug-free patients and the importance of interpreting functional studies in light of corresponding structural and neurochemical alterations. In the field of radiochemistry, new and highly selective radiotracers have increased the ability to image neurotransmitter function in the living human brain to the point that alterations of receptor density and synaptic neurotransmitter release are now routinely measured. In statistics, major advances have taken place in the voxel-based analysis of images, and increases in computational processing power and memory have increased the practical feasibility of many aspects of image manipulation and data analysis, particularly in functional imaging.

Impressive advances have been made in the development of imaging technology, particularly in improved scanner resolution and sensitivity. For example, brain structure can now be imaged with a resolution of less than 1 mm. Alterations in anatomically small regions on functional scans of relatively low resolution can now be identified with great accuracy by delineating these regions on high-resolution coregistered structural images. Moreover, functional neurobiological processes can now be investigated with a high degree of temporal and spatial resolution. A basic understanding of the various commonly

used imaging modalities is advantageous for interpretation of the results of many studies, and a brief overview is given below.

II. IMAGING MODALITIES: AN OVERVIEW

Brain imaging techniques reviewed here are classically divided into structural (shape and size of brain regions and lesions), functional (flow and metabolism), and molecular domains. In the current state of the art, structural imaging is performed with magnetic resonance imaging (MRI), functional imaging with functional magnetic resonance imaging (fMRI), and molecular imaging with magnetic resonance spectroscopy (MRS) or radionuclide-based techniques, including positron emission tomography (PET) and single photon emission computerized tomography (SPECT).

A. MRI

MRI provides high-resolution images of brain structure and is able to distinguish gray and white matter with unparalleled specificity. It has superior contrast and soft-tissue imaging capability, and has replaced x-ray CT in most psychiatric neuroscience research applications. Newer contrast agents, such as gadolinium, have improved the ability to differentiate certain pathological processes, such as tumors, from normal tissue.

B. fMRI

Functional MRI produces images related to blood volume or blood flow. Under physiological conditions, these measures correlate with regional metabolic activity. Local cerebral blood flow can be estimated by blood oxygenation level detection (BOLD). Regional brain metabolic activity results in increased local blood flow and the delivery of more oxygenated blood than is immediately metabolically necessary. This results in a local reduction of deoxyhemoglobin, which can be measured, because deoxyhemoglobin is paramagnetic whereas oxygenated hemoglobin is not. Functional MRI is being increasingly used to characterize the brain regions associated with a wide range of psychiatric symptoms, cognitive functions, and therapeutic interventions. Although the groundwork in this field was mostly done using SPECT and PET, fMRI has advantages over these radionuclide techniques. The temporal and spatial resolutions are superior and no radiation exposure is involved, permitting multiple measures per subject and the safe imaging of children.

Furthermore, the last 10 years have seen a tremendous development of MR-based methods for producing images of brain perfusion akin to those produced by PET and SPECT, and numerous studies have now shown that MRI measurements of regional cerebral blood volume (rCBV) and rCBF are possible (for review, see Ref. 1). However, perfusion MRI is still in an early stage of its development and further work is necessary to address numerous quantification problems. At present, PET and SPECT remain superior for the quantitative measurement of rCBF. Despite this, it can be expected that future advances in MR technology may reverse this balance and lead to the ability to measure more accurately rCBF and rCBV without the need for radiation exposure.

C. MRS

MRS is a brain imaging technique that permits the noninvasive measurement of a number of psychiatrically relevant endogenous and exogenous brain chemicals in a given volume (voxel) of brain tissue. A standard MRI machine, with some modifications, is used. Its technical background and use in psychiatric illness have recently been comprehensively

reviewed [2]. Most studies in psychiatry have used ^{1}H or ^{31}P MRS technology. The most common is ^{1}H MRS (proton MRS) because of its ability to detect compounds in lower concentrations within the brain, although MRS can only observe compounds present in at least micromolar quantities. Commonly measured compounds include those containing myo-inositol (Ino), N-acetyl-aspartate (NAA), cytosolic choline (Cho), and creatinine (Cr). Ino is a sugar that is involved in second-messenger systems through the phosphatidyl-inositol cycle. NAA is a cell marker whose concentration correlates with neuronal density [3]. It is considered to be a marker of neuronal fitness or viability. Increases have been interpreted to represent improved neuronal function, hypertrophy, or hyperplasia and decreases to represent decreased viability or neuronal loss. Cho is a precursor and metabolite of membrane phospholipids, second-messenger compounds, and acetylcholine. Abnormalities in the Cho resonance have been linked to abnormalities in myelination, cerebral oxidative metabolism, and alterations in intraneuronal signaling or in endocrine status. Cr reflects systemic energy use and storage and is generally held to be an internal standard in ratio analyses of neurometabolic change. It has been shown to be stable within individuals over the course of months. In addition, particular ^{1}H MRS editing techniques permit measurement of amino acid concentrations, including glutamate, glutamine, and gamma-amino butyric acid (GABA) [4,5].

Phosphorus (^{31}P) MRS is also frequently used to measure phosphomonoesters (PMEs) and phosphodiesters (PDEs), including sugars and phospholipid-associated metabolic and catabolic components of cell membranes, as well as energy storage metabolites such as phosphocreatine (PCr) and nucleotide phosphates. Of further relevance to psychiatry, MRS technology can also measure brain drug concentrations using, for example, ^{7}Li for lithium medication and ^{19}F to detect fluorine-containing psychotropic drugs [6].

D. Radionuclide Imaging

PET imaging is based on the administration of radioisotopes that emit positrons when they decay. When positrons encounter electrons, the two particles annihilate each other, releasing high-energy gamma rays that radiate in opposite directions. These are detected by coincidence counters in the PET camera and the three-dimensional pattern of emissions is reconstructed and sliced (tomographed) by computer into an image. Typically the half-lives of clinically useful radioisotopes are short (e.g., ^{15}O, 2 min; ^{13}N, 10 min; ^{11}C, 20 min; and ^{18}F, 110 min) and require an on-site cyclotron, or (in the case of ^{18}F) rapid delivery from a cyclotron elsewhere. The radioisotope-containing compounds typically used to measure regional brain activity include ^{11}C-glucose and ^{18}F-deoxyglucose (FDG) for regional glucose metabolism (rCMRglu) and ^{15}O-water ($H_2{}^{15}O$) for rCBF. As in BOLD fMRI, rCBF is used as a proxy of regional metabolic activity because, under physiological conditions, the two measures are correlated.

SPECT is based on compounds (e.g., ^{123}I, ^{99m}Tc) that emit a single gamma ray photon on disintegration. These have longer half-lives than PET isotopes and do not require an on-site cyclotron. Moreover, gamma cameras are widely available in departments of nuclear medicine and, because of its relative simplicity and lower cost, SPECT is widely used in clinical research for the measurement of rCBF. However, images have poorer spatial resolution than PET and studies are generally limited to two SPECT scans per subject. As mentioned above, with the advent of fMRI it is foreseeable that the role of PET and SPECT studies of flow and metabolism in psychiatry research will be greatly reduced in the future.

On the other hand, the ability of PET and SPECT to image specific biomolecules is unmatched by any other method currently available to clinical investigators. Studies of receptors, transporters, enzymes and other processes, such as transmitter release, clearly constitute the future of radionuclide technology, particularly PET, in psychiatric research. Studies of synaptic neurotransmitter release are most advanced in the case of dopamine (DA). They are usually performed by stimulating DA release with pharmacological challenge and measuring the reduction in binding of the radioligand that results from competition at the receptor between the radioligand and the increased local levels of the endogenous ligand (i.e., DA). These techniques have already yielded a number of fundamental observations.

E. Integration of Structural and Functional Imaging

In recent years the findings from studies using different imaging modalities have started to become informed by each other in a number of important ways. For instance, until recently, PET and SPECT studies comparing patient groups with healthy controls have tended to report regional differences in blood flow or metabolism between groups as representing enhanced or diminished function in these regions. However, as structural MRI reveals idiopathic volumetric alterations in many brain regions in affective and anxiety disorders, it has become evident that metabolic changes will need to be corrected for tissue volume. In some instances, this has led to important insights. For example, although baseline CBF and metabolism appear abnormally decreased in subgenual anterior cingulate during major depressive episodes (MDEs), computer simulations that correct PET data for the partial volume effect of reduced gray matter volume conclude that the "actual" metabolic activity in the remaining tissue is *increased* in patients with major depressive disorder (MDD) relative to controls [7].

This will also apply to studies in, for example, late-onset depression in which cerebrovascular disease is now believed to play an etiological role (see below). Atherosclerotic changes reduce radiotracer delivery to affected areas and impair the interpretability of blood flow images by altering the relationship between local perfusion and local metabolic activity. Moreover, associated tissue atrophy may lead to artifactual reductions in measured rCBF or metabolism due to the same partial volume effects as described above and reduce the apparent binding potential (BP) of neuroreceptor radioligands. Thus, images from such subjects cannot be interpreted in the same way as images from patients with early-onset depression or healthy controls. In this regard, MRS and PET techniques that can characterize the metabolic activity of ischemic tissue may prove useful. Moreover, in imaging studies of mood disorders, only unmedicated patients are likely to provide reliable data on underlying pathophysiology, and patients with early-onset MDD must be separated from those with late-onset MDD or MRI evidence of white matter abnormalities [8].

III. AFFECTIVE DISORDERS

A. Major Depressive Disorder

1. MRI

Structural imaging studies have revealed a relative dichotomy between neuroanatomical abnormalities seen in depression of early or midlife onset and depression of late onset (usually defined as onset after ~55 years of age).

Late-Onset Depression. The most widely replicated finding in the whole of structural imaging of affective disorders is an increased incidence of hyperintensities on T_2-weighted MRI in the deep and periventricular white matter of patients with late-onset depression [8–15]. White matter hyperintensities (WMH) are small areas where the signal intensity is high relative to the surrounding tissue. They are extremely rare in younger, healthy individuals and are usually associated with aging, hypertension, diabetes, cerebrovascular disorders, and a number of neurological abnormalities. Although their incidence appears to be modestly increased in early-onset depression [16], the incidence reaches 70 to 90% in late-onset depression. Moreover, in an elderly, nondemented population, the severity of WMH is positively correlated with both symptoms of depression and a history of past depression [9,17].

MRI diffusion tensor imaging (DTI) shows that hyperintensities damage the structure of brain tissue [18], and postmortem neuropathological examination of these areas reveals atherosclerosis and, when larger, infarction [19]. WMH would therefore appear to reflect the MRI correlate of cerebrovascular disease. Hyperintensities in subcortical gray matter have also been noted in the basal ganglia of patients with late-onset depression [20,21]. Hyperintensities are most common in frontal and striatal areas, and several studies report that these are the areas in which infarctions are correlated with depression following ischemic stroke [22,23]. However, the literature in this area is conflicting, and a recent systematic review offered no support for the hypothesis that the risk of depression after stroke is affected by the location of the brain lesion [24]. Despite this, the great majority of evidence would suggest that the cerebrovascular disease may play a role in the pathogenesis of late-onset MDD in patients with MRI hyperintensities [8]. This has led Steffens and Krishnan to propose the new mood disorder subtype of "vascular" depression [14].

Hyperintensities are associated with increased risk of psychotic features, cognitive impairment, and treatment-related adverse reactions such as delirium, which are also more prevalent in subjects with known cerebrovascular disease [8]. They are also associated with increased risk of suicide attempt [25], poor long-term outcome [26], as well as poor response to pharmacotherapy [27,28] and ECT [29]. Moreover, a recent study suggests that cerebrovascular disease (on the evidence of deep WMH) may also underlie the depressive symptoms (especially symptoms of impaired motivation, concentration, and decision making) often found in older individuals who are not clinically depressed, particularly in those with the apolipoprotein E-4 (APOE-4) allele [30].

However, in addition to high-intensity lesions, it is likely that atrophy represents a complementary pathway in late-life depression [17]. Such patients have greater cerebral sulcal and temporal sulcal atrophy, and larger sylvian fissures, lateral ventricles, third ventricles, and temporal horns [13]. Volumetric reductions are also found in cerebellum [31], frontal lobes [17,32], orbitofrontal cortex [33], basal ganglia [34], amygdala [35], and hippocampus [35,36]. Hippocampal size is significantly negatively correlated with total lifetime duration of depression, but not age, suggesting that repeated stress during recurrent depressive episodes may result in cumulative hippocampal injury as reflected in volume loss [36].

Early-Onset Depression. There is obvious overlap between the imaging finding in earlier onset (i.e., before ~55 years of age) and late-life depression. For example, even depressed adolescents are found to have smaller frontal lobes and enlarged lateral ventricles [37,38]. Moreover, many imaging studies of depression have included both younger and elderly patients. However, structural imaging findings are less consistent in younger

patients with MDD, and MDD in this population is likely to be heterogeneous with respect to etiology and pathophysiology. Because of this, a number of studies have enhanced their likelihood of finding neurobiological abnormalities by deliberately including only unmedicated subjects who also have a history of mood disorder in a first-degree relative (see Refs. 7, 8). Early-onset, familial mood disorders appear to be associated with a distinct pattern of gray matter reductions in limbic or paralimbic areas of the orbital and medical prefrontal cortex and the ventral temporal lobe [7,8,39,40]. At post-mortem examination, gray matter reduction in "subgenual" anterior cingulate (i.e., ventral to the genu of the corpus callosum) in familial affective disorder has been found to be associated with a reduction of glia, without an equivalent loss of neurons [41,42]. These structural findings compliment an extensive, and complicated, body of literature derived from functional imaging studies, mainly PET, in these regions. These will be addressed below.

Elevated levels of glucocorticoids in depression have been hypothesized to be associated with damage to the hippocampus, a brain area involved in learning and memory. In this model, chronic repeated episodes of depression may lead to progressive hippocampal atrophy over time, possibly increasing the risk for subsequent depressive relapse. The imaging literature would currently seem to support an association between depression and hippocampal size dependent on the cumulative duration and severity of MDD. Three recent studies report normal hippocampal volume in younger populations not characterized by severe, recurrent MDD [43–45]. However, reduced hippocampal volume has been replicated in euthymic patients with a history of severe and repeated depressions [36,46,47], and in those with severe, treatment-resistant depression [45,48]. This atrophy is correlated with longer cumulative duration of depression [46,49], does not appear to resolve over time [47], and a review of the subject tentatively concludes that it appears to be irreversible [50].

Studies of neurodegenerative diseases, striatal lesions, and functional imaging suggest that the striatum may be implicated in depression. However, the majority of recent MRI studies have failed to find reduced striatal volume in MDD. Earlier MRI studies found reduced caudate [51] and putamen [52] volumes, and this has seen more recent support [53]. However, four studies have failed to find an association [54–57]. This is in contrast to neurochemical and functional imaging studies that have found evidence for striatal abnormalities.

2. MRS

Three early ^{1}H and ^{31}P MRS studies measured spectra before and after ECT in depressed patients. However, the studies were mainly related to exploring the pathophysiology of lactate production and seizures and the results were equivocal [58–60]. Subsequent ^{31}P MRS studies have reported increased PDE levels in the basal ganglia and frontal lobes [61], associated with decreased levels of ATP [62], suggesting alterations of regional tissue energetics [2].

More recently, ^{1}H MRS has been used to investigate whether there is evidence of hippocampal damage following ECT on the basis that neuronal damage may show up as a reduction of NAA signal [63]. No changes were detected after ECT, suggesting that ECT is unlikely to induce hippocampal atrophy or cell death. The study also measured Cho levels in the right and left hippocampus of the depressed patients before ECT and compared them with a group of healthy controls and remitted depressed patients treated with amitriptyline. Cho levels were found to be lower bilaterally in the depressed patients

and increased following ECT, which corresponded with clinical response. The authors suggest that this represents increased membrane turnover as a result of ECT treatment. These findings are concordant with rat experiments in which repeated seizures of the hippocampal-parahippocampal circuits can cause mossy fiber sprouting without cell death [64]. The authors speculate that the Cho signal increase after ECT treatment might similarly reflect mossy fiber sprouting in hippocampal subfields.

These findings raise a number of issues. First, ^{1}H MRS measurements (Cho in particular) appear to be mood-state dependent. Depressive symptoms were associated with lowered Cho in hippocampus, but the diagnosis of depression was not sufficient to explain this finding, in that remitted depressive patients successfully treated with medication displayed Cho values similar to controls. Second, this study suggests that the Cho resonance might prove to be a more reliable marker of the severity and past number of episodes of depression, and a more sensitive index of the processes underlying remission and recurrence than the NAA signal. However, another study using ^{1}H MRS reports increased Cho levels in the medial temporal lobe structures of patients with treatment-resistant MDD [48], underlining the point that the association of clinical improvement with increasing Cho remains tentative at present and requires replication. Moreover, future studies will also need to exclude the confounding effects of current or very recent antidepressant medication.

In other brain regions, ^{1}H MRS has been used to investigate frontal lobes, anterior cingulate cortex, the orbitofrontal cortex, and the basal ganglia in MDD, and most results still require replication. In a preliminary study of the frontal lobes, mean Ino/Cr ratios were lower in patients with MDD than in controls, but the difference was not statistically significant [65]. Moreover, the depressed and control groups were not age- and sex-matched, and 15 of the 22 patients were taking a variety of antidepressant medications. However, a subgroup of 10 patients was age- and sex-matched to controls and the Ino/Cr was then significantly lower in the patients.

In the anterior cingulate cortex, no significant alterations in NAA, Cr, or Cho were found in MDD [66], and the authors concluded that the study supported an absence of neurodegenerative or membrane metabolic changes. However, reduced glutamate and glutamine were found, suggesting a possible role for altered glutamatergic neurotransmission within the anterior cingulate in the pathogenesis of MDD. In the orbitofrontal cortex of adolescents with MDD, increased Cho was found when compared to controls in the absence of structural differences [67]. In the basal ganglia, the Cho signal has been found to be altered in depressed patients [68–70] but the direction of the changes is controversial. However "true" response (as opposed to placebo response) to fluoxetine is reported to be associated with an increase in Cho [71].

A number of authors suggest that Cho has a key role in the integration of affective processing within prefrontal circuitry. One author has suggested that higher Cho may reflect greater myelin turnover [72], as the Cho peak includes both precursors and breakdown products of myelin. The issue of whether the Cho signal is a trait or state marker of depression is as yet unresolved, and whether increase over time in the Cho signal in cortical regions in healthy subjects is a harbinger of the onset of a depressive episode. In interpreting the cortical ^{1}H MRS data, regional and tissue differences of brain metabolites must be considered [73]. The variability of both NAA and Cho has to be shown to be dependent on region of interest, tissue differences (gray versus white matter), subtype of affective illness, age, and gender. Thus, in an attempt to reconcile the finding of lowered

Cho in hippocampus in severely depressed subjects about to embark on ECT [63] with the findings of elevated Cho/Cr and Cho/NAA in orbitofrontal regions [67], the tissue composition of voxels examined must be taken into account.

Several lines of evidence suggest that MDD is associated with dysfunction of gamma-amino butyric acid (GABA) systems. Using ^{1}H MRS, GABA levels were found to be highly significantly (52%) reduced in the occipital cortex of medication-free patients with MDD when compared to healthy control subjects with no history of mental illness [74].

Overall, the current limited evidence suggests altered membrane dynamics and abnormal cellular energetics in MDD. However, more studies in tightly defined, drug-free patient samples are required before this can be firmly concluded [2].

3. Regional Blood Flow and Metabolism

The literature on functional anatomical correlates of MDD is extensive and complex. Numerous alterations of rCBF and rCMRglu have been reported in PET and SPECT studies in regions known to be implicated in the generation or modulation of affective states. However, many studies are difficult to interpret as they have mixed patients from different age groups, studied small numbers of patients or failed to adequately account for the confounding effects of state-dependent differences, diagnostic heterogeneity, or current medication. Because of this, a simple catalogue of the findings from various studies can be unrewarding. Moreover, the interpretation of these findings requires an understanding of the normal function in these regions in various mood states as well as possible neuropathological and volumetric alterations in these regions between healthy subjects and patients with affective disorders. For many brain regions, these data are not yet known in humans and must be inferred from the results of animal studies, or studies in humans with regional brain lesions. Despite this, the number of high-quality functional imaging studies has now grown to the point where their findings can be integrated with data from structural, neuroreceptor, endocrine, lesion, and electrophysiological studies to develop hypotheses regarding the neural substrates of MDD. A number of such integrative and comprehensive reviews of this literature (see Refs. 7, 8, 75–78) have concluded that anatomical circuits involving orbital and medial prefrontal cortex, striatum, pallidum, medial thalamus, amygdala and hippocampus are implicated in the pathophysiology of MDD. These findings are summarized below, and the reader is referred to these reviews for details of individual studies.

The finding with the most consistent replication is of decreased rCBF and metabolism in the anterior cingulate cortex ventral to the genu of the corpus callosum (subgenual AC) in unipolar and bipolar depressed patients. This would appear to be at least partly accounted for by a corresponding left-lateralized volumetric reduction in this region and, when correction is made for this, the "actual" metabolism in the remaining tissue is found to be normal or increased during depression. This pattern also appears true for healthy, nondepressed subjects, in whom flow increases in the subgenual AC in experimentally induced sadness. It is also compatible with PET studies showing that effective antidepressant medication results in *decreased* metabolic activity in this region in MDD. Data for the rest of the AC are less consistent. In the pregenual AC, most studies find increased rCBF and rCMRglu in a depressive episode, but relationship with treatment response differs across studies. In the dorsal AC, rCBF is decreased in depression and its normal cognitive activation is attenuated during a depressive relapse triggered by acute tryptophan depletion.

In orbitofrontal cortex, ventrolateral prefrontal cortex and anterior insula, rCBF and metabolism are increased during depression and decrease with remission. However, this increase is mainly seen in less severely ill and treatment-responsive patients. More severe depression tends to be associated with normal or reduced activity in these regions. As increased activity in the orbital cortex during a depressive episode may represent an endogenous attempt to attenuate the abnormal mood, the lack of this increase in more severe depression may indicate that the orbital cortex is being prevented from exercising this effect, possibly by pathologically increased activity in interacting regions such as the amygdala or by abnormalities in the orbital cortex itself.

There is evidence to support both possibilities. Neuropathological abnormalities have been found in the posterior orbital cortex, including fewer glia and smaller neurons. In the amygdala, rCBF and metabolism are increased during depression in familial pure depressive disorder and bipolar disorder type II. These increases are positively correlated with the severity of depression and decrease to normal levels with successful antidepressant treatment. However, euthymic, remitted patients who are not on medication also show increased activity in the left amygdala and this may confer a susceptibility of depressive relapse. Electrophysiological, endocrine, and animal studies suggest that many of the behavioral, endocrine, and mood changes associated with depression could be related to pathologically increased amygdalar activity through its various reciprocal connections with other brain regions.

CBF and metabolism are also abnormally raised in the left medial thalamus, and decreased in the caudate during depression. Reductions in caudate volume may at least partially explain this reduction. However, following acute tryptophan depletion, those patients who experienced a depressive relapse had lower CBF in the caudate, suggesting that alterations of caudate function may be involved in depression.

Conversely, reduced activity during depression found in dorsolateral prefrontal cortex—an area related to executive rather than affective control—may represent the relative suppression of otherwise normal, nonessential, competing modalities.

4. Neuroreceptor Studies

Abnormalities in several neurotransmitter systems may be relevant to the pathophysiology of depression. The serotonin [5-hydroxytryptamine (5HT)] system has been the most extensively studied, in part because of the antidepressant effect of medications that inhibit the synaptic reuptake of serotonin, as well as a wealth of postmortem, preclinical, and clinical data suggesting that reduced serotonergic function may be implicated in depression. Theories involving subpopulations of 5HT receptors are most advanced for $5HT_{2A}$ and $5HT_{1A}$ receptors, and the availability of suitable PET radioligands has allowed the in vivo investigation of their putative abnormalities in depression.

Serotonin Transmission. The earliest PET study of $5HT_2$ receptors and depressive symptoms used [^{11}C]-N-methyl-spiperone to investigate binding in patients with poststroke depression, and reported increased binding [79]. Yet, it is not clear how this finding can be generalized to more common clinical presentations of depression. Another early study using 2-[^{123}I]-ketanserin and SPECT reported increased and asymmetrical cortical uptake of the tracer in depressed patients when compared to controls [80]. However, 2-[^{123}I]-ketanserin has significant limitations as a SPECT radiotracer because of high nonspecific binding.

Since then, five PET studies have used newer $5HT_2$ PET radiotracers, [^{18}F]-setoper-

one [81], and [^{18}F]-altanserin [82], to investigate cortical $5HT_{2A}$ receptor binding in drug-free depressed patients. Biver et al. [83], using [^{18}F]-altanserin, reported reduced tracer uptake in a region of the right hemisphere including the orbitofrontal cortex and the anterior insular cortex. However, one limitation of [^{18}F]-altanserin is that it produces radioactive lipophilic metabolites that probably cross the blood-brain barrier and contribute activity in the nondisplaceable compartment [84]. Two studies investigated midlife depression using [^{18}F]-setoperone and concluded that there is no major change or asymmetry in $5HT_{2A}$ receptors [85,86]. In both studies, the great majority of patients had been free of antidepressant medication for over 6 months. A fourth study supported these negative findings and reported no significant alteration in $5HT_{2A}$ receptor binding in an untreated group of patients with late-life depression without cognitive impairment [87]. Finally, the largest of the five studies [88] found a widespread reduction in $5HT_{2A}$ receptor BP and concluded that brain $5HT_{2A}$ receptors are decreased in patients with major depression. However, 40% of the patients in this study had been drug-free for only 2 weeks before scanning. This factor may be significant as the majority of antidepressants downregulate $5HT_{2A}$ receptors [86,89,90].

In summary, three studies reported no significant alteration in $5HT_{2A}$ receptor binding in major depression, and two studies found reduced $5HT_{2A}$ receptors. Differences between studies might stem from methodological issues, illness heterogeneity, and medication effects. None of the recent studies confirmed the earlier findings of increased binding [79,80]. Similarly, the increase in $5HT_{2A}$ receptors found in some, but not all, post-mortem studies of depressed suicide victims (for review, see Ref. 91) has not been confirmed by in vivo investigations. Therefore, there is currently no strong evidence supporting the hypothesis that depression per se is associated with marked alterations of $5HT_{2A}$ receptor density.

Two lines of evidence have implicated the $5HT_{1A}$ receptors in depression. The first is the finding that depressed patients have blunted neuroendocrine responses to $5HT_{1A}$ receptor agonists in vivo, and the second is the dense distribution of these receptors in the hippocampus. Recent theories have implicated interactions between stress, corticosteroids, growth factors, and hippocampal $5HT_{1A}$ receptors in depression [92–94]. Postmortem studies of $5HT_{1A}$ receptors in suicide and depression have been inconsistent, showing increased, decreased, and unchanged $5HT_{1A}$-receptor levels in various regions [95–99]. These discrepancies may reflect the possible confounding effects of suicidality, antemortem medications, differences between radioligands, and differences in the regulation of $5HT_{1A}$ receptors by corticosteroids and local levels of 5HT in different brain regions. The results of in vivo PET imaging of $5HT_{1A}$ receptors in depressed patients are therefore of interest.

Two PET studies have investigated $5HT_{1A}$ receptors in unmedicated depressed subjects using [*carbonyl*-^{11}C]WAY-100635 and both have reported reductions in receptor binding. The first study [100] found modest (approximately 10%), but significant, widespread reductions in BP in cortical regions including medial temporal cortex (hippocampus and amygdala) in a group of 15 men with major depression. Subsequently, Drevets et al. [92] reported reductions in the medial temporal cortex (27%) and raphe (41%) in a study that limited its primary hypothesis to these two regions. This group of subjects included both unipolar and bipolar depressed patients. All subjects had first-degree relatives with mood disorders. Interestingly, the differences found were largely accounted for by the subjects with bipolar disorder and those with uniplar depression who had relatives with bipolar disorder.

Additional studies are warranted to confirm these findings of generalized decrease in $5HT_{1A}$ receptors in depression. Since a major depressive episode is associated with hyperactivity of the HPA axis, and increased cortisol levels might be associated with $5HT_{1A}$-receptor downregulation [101–104], these findings might be secondary to the neuroendocrine dysregulation associated with depression.

Reductions in SERT levels in depressed patients have been reported in numerous post-mortem studies (for review, see Ref. 91). The first ligand used to image SERT in vivo was the SPECT radiotracer [^{123}I]β-CIT. β-CIT binds to both DAT and SERT with comparable affinity (Ki 1.4 and 2.4 nM for DAT and SERT, respectively) [105,106]. The lack of DAT versus SERT selectivity is not a problem for measuring DAT in the striatum, as the density of SERT in striatum is much lower than that of DAT [106]. However, in the midbrain, this proportion is reversed, and the β-CIT midbrain uptake mostly corresponds to SERT binding [107,108]. Studies on nonhuman primates and humans have shown that, in the midbrain, [^{123}I]β-CIT is selectively displaced by administration of SSRIs (but not by DAT selective drugs) [107,109]. [^{123}I]β-CIT has been extensively used in clinical studies both for striatal DAT [110–116] and midbrain SERT evaluation [116–119].

In depression, findings from two SPECT studies using [^{123}I]β-CIT were in agreement with postmortem results. A reduction in SERT binding was found in the midbrain in patients with unipolar depression [120], and in thalamus-hypothalamus in depressed patients with seasonal affective disorder [121].

A selective SERT radiotracer is required to investigate SERT density in other regions of the brain. The first PET radiotracer available to measure SERT in humans was [^{11}C]McN 5652 [122]. The usefulness of [^{11}C]McN 5652 as a PET tracer for SERT was validated in primates [123] and humans [124–126]. However, [^{11}C]McN 5652 has limitations, which include high nonspecific binding, poor signal-to-noise ratio, nonmeasurable free fraction in the plasma, and slow clearance from the brain [127]. Therefore, studies using [^{11}C]McN 5652 require long scanning time (up to 120 min), and this ligand can provide reliable quantification of SERT only in regions of relatively high SERT density (midbrain, thalamus, and striatum). More recently, compounds from the phenylamine class have emerged as promising targets for both SPECT and PET tracer development. [^{123}I]ADAM [128] is a highly selective SPECT imaging agent for SERT. Its ^{11}C-labeled counterpart, [^{11}C]ADAM, was recently reported [129]. Another compound in this series, [^{11}C]DASB, was recently introduced and has been evaluated in rats [130] and humans [131]. Thus, it is anticipated that, in the near future, several studies will be performed to evaluate SERT density with PET in patients with major depression. If the results obtained with [^{123}I]β-CIT are confirmed, the reduction in SERT density might provide a useful biomarker for this disorder.

Dopamine Transmission. The critical role of DA in brain reward systems, the reports of low cerebrospinal fluid homovanillic acid levels in depressed patients, the association of major depression with Parkinson's disease, and the enhancement of dopaminergic activity by several antidepressant treatments suggest that a deficiency of dopaminergic function might be associated with major depression (for review, see Refs. 132–135). Five studies compared striatal D_2 receptor availability with [^{123}I]IBZM in patients with major depression and control subjects. Two of the five studies reported higher [^{123}I]IBZM specific binding in the striatum of depressed subjects compared to controls [136,137], whereas three studies reported no change [138–140]. Amphetamine-induced DA release was also

assessed in patients with major depression and found to be unchanged [140]. Two studies examined [^{123}I]β-CIT striatal binding in patients with major depression and yielded conflicting results: one study reported normal levels of striatal DAT in patients with major depression [120], while the other one reported increased DAT levels [141]. Finally, [^{18F}]DOPA uptake in the left caudate was observed to be significantly lower in depressed patients with psychomotor retardation than in depressed patients with high impulsivity and in comparison subjects [142]. Thus, major depression per se does not appear to be consistently associated with alteration of the dopaminergic parameters at the level of the whole striatum. However, DA might play a role in the neurobiology underlying some clinical features of depression, such as psychomotor retardation.

B. Bipolar Disorder

Bipolar disorder (BPD) has been extensively investigated by imaging, particularly by structural MRI and MRS, and this literature has recently been reviewed by a number of authors [143,144].

1. MRI

The most consistent finding in MRI studies is a higher than expected incidence of white matter hyperintensities (WMH), particularly in periventricular regions, and there are several comprehensive reviews and meta-analyses of these structural studies in BPD [15,145–147]. The nature of WMH is discussed in the section of MRI findings in late-life MDD. In BPD, they appear to be present early in the course of the illness [148,149] and are found more frequently in patients of all ages, even children and adolescents [150,151]. Their exact etiology and relevance in BPD is currently uncertain, most patients do not have them, and they do not appear to be associated with cognitive deficits [152]. However, some (but not all) studies have reported that they are associated with a worse clinical course and outcome [15,146,153]. Moreover, a recent study has reported that birth season, illness outcome, and deep subcortical white matter lesions appear to be closely linked, raising the hypothesis that these lesions may be a marker of a toxic or infective insult in utero [154]. There is no clear association between WMHs and medications, such as lithium, which can alter lipid metabolism and lead to MR signal changes [145,155].

A number of studies have reported increased ventricular volume in BPD, particularly in lateral ventricles [156] or temporal horn [157]. One study found that the degree of enlargement was indistinguishable from that in schizophrenia [157]. However, most find no change [15,145], and there does not appear to be a consistent decrease in volume of temporal lobes [156] or gray matter in the brain regions surrounding the lateral and third ventricles [158].

Studies in most other regions have been inconsistent or require replication. Prefrontal cortical volumes were found to be smaller, and this reduction was correlated with attentional dysfunction in a group of patients hospitalized for a manic episode [159]. Hippocampal volume has been found to be both smaller [160,161] and normal [156,162]. The amygdala has been reported to be both smaller [163] and larger [162], and the thalamus has been found to be smaller [164]. Perhaps the most consistent volumetric finding is decreased cerebellar size, particularly in older patients and those who have had multiple affective episodes [15,165]. However, reduced cerebellar size is also found in other psychotic disorders including schizophrenia, and other factors may also contribute to altered cerebellar size, such as alcohol and lithium medication.

Studies have also found that the volume of the subgenual anterior cingulate is decreased in patients with familial affective disorder, including MDD and BPD [40,41,166]. Significantly decreased volume was not found in patients without a family history of affective disorder, nor in schizophrenics [40]. This region has been implicated in the control of affective state. This finding is consistent with abnormalities found in MRS [167,168], functional imaging [166], and postmortem studies [41] of the anterior cingulate region in BPD.

Several other recent studies are of interest, although they will require replication. Brambilla et al. report findings consistent with an exaggerated age-related gray matter decline in bipolar patients [169], and Sassi et al. find smaller pituitary volumes in BPD [170]. The authors suggest that this latter finding may reflect a dysfunctional HPA axis.

2. MRS

A recent meta-analysis of the ^{31}P MRS literature [171] concluded that phosphomonoester (PME) values are lower in euthymic bipolar patients than in healthy controls, and that bipolar patients have significantly higher PME values when depressed compared to when euthymic. The PME resonance by ^{31}P MRS is often taken to be a measure of precursors for membrane lipids, and this would support the idea of abnormalities of membrane phospholipid metabolism in BPD, which may reflect a dysregulation in brain-signal transduction systems.

Studies using ^{1}H MRS report various patterns of altered chemicals in the basal ganglia [172–175], thalamus [176], frontal lobes [177], dorsolateral prefrontal cortex (DLPFC) [178], and anterior cingulate [167,168]. Increased thalamic NAA was interpreted possibly to represent neuronal hypertrophy or hyperplasia, reduced glial cell density, or abnormal synaptic and dendritic pruning. Increased thalamic creatine may represent altered cellular energy metabolism [176]. Increased choline in the anterior cingulate was interpreted as consistent with impaired intraneuronal signaling mechanisms [167].

Moreover, acute lithium treatment is associated with a significant reduction of myo-inositol levels, and this action may underlie (or perhaps only trigger) its therapeutic effect [168,179]. Decreased NAA in the DLPFC may represent decreased neuronal density or neuronal dysfunction. Interestingly, lithium therapy recently has also been shown to increase brain NAA levels [180]. As NAA is considered to be a marker of neuronal viability or function, the authors suggest that some of lithium's long-term beneficial effects may be mediated by neurotrophic or neuroprotective processes. This hypothesis is supported by the finding that chronic lithium significantly increases total gray matter volume [181]. Also of interest is the recent finding that in a drug-free group of children with BPD, all subjects had elevated levels of glutamate/glutamine in both frontal lobes and basal ganglia relative to a control group [182]. This is in line with increasing evidence for an association between alterations of brain glutamatergic neurotransmission and the pathophysiology of affective disorders. It may also be consistent with a recent ^{1}H MRS study that reports significantly higher CSF glutamine concentrations in depressed patients [183]. The authors suggest that this may reflect an abnormality of the brain glial-neuronal glutamine/glutamate cycle associated with NMDA receptor systems in patients with depression.

3. fMRI

In line with the structural data related to the cerebellum (above), and current theories that support a role for the cerebellum in both cognitive and affective processes, Loeber et al. used dynamic susceptibility contrast MRI (DSC MRI) to measure cerebellar rCBV in three

study groups: schizophrenic patients, bipolar patients, and psychiatrically healthy controls [184]. In this technique, which uses MRI technology coupled with a contrast agent, blood volume is taken as a measure of the amount of active tissue in a region [1]. Bipolar patients were found to have mean values that were lower than controls and schizophrenic patients had mean values that were higher than controls. This difference was most significant in the tonsillar region and, importantly, this finding did not appear to be because of differences in tissue volume.

In another study, adults with BPD showed a reduction in DLPFC activation and an increase in amygdalar activation in response to viewing a fearful facial affect. No changes were found in these regions in a group of healthy controls. In addition, bipolar patients had an impaired ability to correctly identify fearful facial affect but not happy facial affect. The authors concluded that, in some patients with BPD, there may be a reduction of frontal cortical function that may be associated with affective, as well as attentional, processing deficits [185].

4. Blood Flow and Glucose Metabolism Studies

Most rCBF and CMRglu studies including bipolar depressed patients have also included subjects with unipolar depression. As mentioned in the section on MDD above, there is strong evidence for altered rCBF and CMRglu in patients with MDD. At present, results of studies comparing bipolar and unipolar patients show no clear consensus and are relatively few in number. Moreover, confounding effects of methodological differences, illness heterogeneity, state-trait differences, and treatment effects remain to be fully elucidated. During a depressive episode, unipolar depressed patients had relatively increased rCBF in the left frontal lobes, but no differences were found between controls and bipolar depressives [186]. Conversely, significant decreases in rCBF have been reported in prefrontal cortices, limbic system, and paralimbic areas in both depression groups [187]. Another study has reported increased metabolism in basal ganglia and thalamus during bipolar depression [188].

In mania, there are reports of increased CMRglu in the left amygdala [189], increased rCBF in superior anterior cingulate [190], increased rCBF in subgenual prefrontal cortex [166], and decreased rCBF in the orbitofrontal cortex during rest along with impaired activation in rostral and orbital prefrontal cortex during word generation [191]. A recent study has also reported posterior hypermetabolism in a group of rapid cycling, treatment-resistant patients [192].

5. Radioligand Studies

In comparison with major depressive disorder, only limited radioligand PET studies have been reported in patients with bipolar disorders. As discussed above, it may be significant that the findings of reduced $5HT_{1A}$ receptor binding in the medial temporal cortex and raphe of depressed patients [92] were largely accounted for by the subjects with bipolar disorder and those with unipolar depression who had relatives with bipolar disorder.

Because of the relationship between mania and psychosis, a number of PET studies have investigated the DA system in bipolar disorders. D_1 receptor binding in the frontal cortex was reported to be decreased in a study of 10 symptomatically heterogeneous, drug-free bipolar patients [193]. Increases in D_2-like (i.e., D_2, D_3, and D_4) receptor density in the striatum were found in seven psychotic patients with bipolar disorder when compared to 7 nonpsychotic patients with bipolar disorder and 24 control subjects. The authors concluded that an increase in D_2-like receptors is associated with the state of psychosis

rather than with a diagnosis of bipolar disorder [194]. As part of the same studies, Gjedde and Wong also reported findings consistent with an elevated concentration of synaptic DA in bipolar patients with psychosis, but not in nonpsychotic bipolar patients [195]. On the other hand, amphetamine-induced DA release was reported to be normal in euthymic patients with bipolar disorders [196].

In conclusion, few investigations have been reported using PET molecular imaging techniques in patients with bipolar disorders, and the findings reported so far might be related to clinical states (depression, mania with psychosis) rather than to the bipolar condition per se.

IV. ANXIETY DISORDERS

A. Imaging Studies of Anxiety in Healthy Human Subjects

A number of studies have investigated putative neurobiological differences between healthy subjects with differing levels of trait anxiety. A significant negative correlation was found betwen trait anxiety and cortical $5HT_{1A}$ receptor binding [197]. The authors report that this is consistent both with animal models that have shown higher anxiety in mice lacking $5HT_{1A}$ receptors and with clinical trials demonstrating anxiolytic properties of partial $5HT_{1A}$ agonists [198]. MRS studies have shown a high positive correlation between anxiety and chemical concentrations, particularly NAA levels, in orbitofrontal cortex [199,200].

Functional studies using $H_2{}^{15}O$ PET have also investigated rCBF changes associated with induced anxiety in healthy volunteers. However, there is no current uniformity of protocol and methods used to provoke anxiety have included intravenous cholecystokinin [201,202], anticipation of electric shock [203,204] and memory induction [205,206]. While there is considerable variability in the findings, a number of paralimbic-cortical regions are consistently identified, including medial prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex, anterior temporal cortex, parahippocampal gyrus, and the claustrum-insular-amygdala region. On the basis of these regions it has been suggested that sadness and anxiety are represented by segregated, but partially overlapping, corticolimbic pathways [206,207]. A major role has been proposed for selective dorsal cortical deactivations during sadness, and ventral cortical deactivations in anxiety [206].

This partial overlap of the pathways may have relevance to the clinical observation of high comorbidity of sadness and anxiety symptoms in depressive disorder and some anxiety disorders. Neuroimaging studies of patients with anxiety disorders will therefore be important to determine the relevance of the normal volunteer studies to the pathophysiology of clinical conditions. Initial studies suggest that thalamic and medial prefrontal regions may participate in aspects of normal emotion unrelated to its type, and that differing clinical anxiety disorders may share underlying neural mechanisms involving anterior cingulate, inferior and orbital prefrontal cortex, insular cortex, and midbrain [208,209].

B. Generalized Anxiety Disorder

In comparison with other anxiety disorders, relatively few imaging studies have been reported in generalized anxiety disorder (GAD). A preliminary MRI study in child and adolescent subjects with GAD found that right and total amygdala volumes were significantly larger. Other measures did not differ, including intracranial, cerebral gray and white

matter, temporal lobe, hippocampal, and basal ganglia volumes and measures of the mid-sagittal area of the corpus callosum [210].

One PET study reported lower absolute metabolic rates in basal ganglia and white matter in patients with GAD [211]. Relative metabolism was increased in the occipital lobe, right posterior temporal lobe, and the right precentral gyrus. Benzodiazepine (BZD) therapy resulted in decreased absolute metabolic rates for cortical surface, limbic system, and basal ganglia but was not associated with normalization of patterns of glucose metabolism.

Because of the anxiolytic and anxiogenic effects of agonists and inverse agonists at the central BZD receptor on the $GABA_A$ complex, abnormalities of BZD receptor function have been hypothesized in anxiety disorders. An initial SPECT study of GAD in drug-free female patients using [^{123}I]NNC13-8241 reported reduced binding in the left temporal lobe, and decreased heterogeneity of distribution [212]. However, this was not confirmed in a PET study using [^{11}C]-flumazenil, which found no differences in drug-free patients [213].

C. Panic Disorder

By comparison, patients with panic disorder have been more extensively studied. Structural MRI studies have found an increased incidence of focal atrophy or abnormal signal intensity in temporal lobes [214,215], particularly in the septo-hippocampal region [216]. A recent volumetric study found smaller temporal lobes bilaterally despite normal-sized hippocampi [217].

Early PET studies showed abnormal hemispheric asymmetries of parahippocampal rCBF, blood volume, oxygen metabolism, and abnormally high whole-brain metabolism in lactate-sensitive patients with panic disorder, but not in those who were lactate-insensitive [218,219]. Since then, a fairly consistent pattern has been reported in unmedicated patients of asymmetrically reduced rCBF or rCMRglu in hippocampal, parahippocampal and inferior frontal regions [220–223] that do not normalize with imipramine treatment [221].

Abnormalities in these regions in panic disorder are broadly supported by the results of receptor imaging studies of the BZD receptor. Three studies using [^{123}I]iomazenil SPECT have reported that binding is decreased in the lateral temporal region [224], decreased in the right prefrontal cortex [225], and increased in the right orbitofrontal cortex in BZD-naïve patients [226]. However, there were differences in methodology and control groups in these studies. A fourth [^{123}I]iomazenil SPECT, using a quantitative measurement of regional binding potential (BP), reported decreased binding in the left hippocampus and precuneus in panic disorder patients relative to controls. Interestingly, patients who had a panic attack at the time of the scan had a relative decrease in binding in the prefrontal cortex, suggesting that BZD receptor function in the prefrontal cortex may be involved in changes in state-related panic [227]. In support of these SPECT studies, a fully quantitative PET study using [^{11}C]flumazenil in medication-free patients found a global reduction in benzodiazepine receptor binding throughout the brain in patients with panic disorder compared with controls. The largest regional decreases were in the right orbitofrontal cortex and right insula [228].

These findings have been complimented by the results of ^{1}H MRS studies that show reduced total occipital cortex GABA concentration [229], as well as an exaggerated rise in brain lactate levels in response to lactate infusion [230–232] and hyperventilation [233] in patients compared to controls.

D. Phobias

1. Specific Phobias

Functional imaging studies in phobic anxiety have investigated alterations in rCBF or rCMRglu associated with the anxiety of being exposed to phobia-relevant material in subjects with specific phobias. However, there is great variability in the results of studies using PET, and no clear consensus can be derived. Several report increased rCBF in the secondary visual cortex and reduced rCBF in the hippocampus, orbitofrontal cortex, prefrontal cortex, posterior cingulate cortex, and temporal lobe [234,235]. Others report increases in rCBF in the anterior cingulate cortex, the insular cortex, the anterior temporal cortex, the somatosensory cortex, the posterior medial orbitofrontal cortex, and the thalamus [236]. Still others implicate the thalamus [237,238] or distinct neuronal pathways that also involve the amygdala and striatum [238]. However, the involvement of these areas does not appear to be exclusive to phobic anxiety and they may be involved in uncontrolled emotions in other conditions like post-traumatic stress disorder, panic disorder, and schizophrenia [238]. One study found reduced rCBF only in the primary visual cortex [239] and another study found no clear association between phobic anxiety and rCBF using SPECT [240].

2. Social Phobia (Social Anxiety Disorder)

Neurobiological mechanisms underlying social phobia, including neuroimaging findings, have recently been critically reviewed [241,242]. A consensus is that the field of social phobia research has until recently had a number of weaknesses, including lack of guiding theories, static comparisons between subject groups, analysis oblivious to individual variations [241], and neglect of neurodevelopmental processes and the functional interactions between neurotransmitters [242]. Both reviews provide theoretical frameworks and recommendations to guide future research in the field, including neuroimaging studies. There are few replicated neuroimaging studies to date in social phobia, but the data so far implicate basal ganglia structures, the amygdala, and a number of cortical regions.

A volumetric MRI study has found no statistically signifiant differences in total cerebral, caudate, putamen, and thalamic volumes between social phobia patients and normal control subjects. However, it did reveal an age-related reduction in putamen volumes in the social phobics that was greater than seen in controls [243].

The results of MRS studies have so far been inconsistent. A preliminary study reported decreased levels of Cho, Cr, and NAA in a number of cortical and subcortical regions including thalamus and caudate [244]. These changes were attributed to an alteration in the activty of the 5HT and/or DA systems in these brain areas. However, the use of signal-to-noise ratios and relatively poor resolution are limitations in this study. A repeat MRS study reported significantly lower NAA and higher Cho and Ino in cortical gray matter. Higher Ino levels were also observed in subcortical gray matter. These differences were not observed following clonazepam treatment [245]. These findings were interpreted as showing an alteration in phospholipase C activity. Phospholipase C is a second-messenger pathway associated with a number of different neurotransmitters, including noradrenaline, dopamine, and serotonin. While these data might point to altered neurotransmitter function in social phobia, there are other possible explanations [241].

A preliminary blood flow SPECT study found normal rCBF in subjects with social phobia who were not experiencing social anxiety at the time of tracer injection [246]. In another SPECT study, treatment with the SSRI citalopram led to significantly reduced

activity in the left temporal and frontal cortices and the left cingulate cortex [247]. However, changes in these regions are not exclusive to socially induced anxiety.

Two $H_2{}^{15}O$ PET studies of symptom provocation have been reported. In one, the changes specific to social phobia included increased rCBF in the right DLPFC and left parietal cortex [248]. In the other, rCBF was found to decrease in the social phobics and increase in the comparison subjects in the orbitofrontal and insular cortices and in the temporal lobe during public speaking [249]. The study concluded that the functional neuroanatomy of social phobia involves the activation of a phylogenetically older danger recognition system. Moreover, increased anxiety was accompanied by enhanced rCBF in the amygdaloid complex in social phobics relative to control subjects [249]. Functional abnormalities in the amygdala and hippocampus have also been found in two fMRI studies. In the first, the amygdala was selectively activated in social phobics during presentation of potentially fear-relevant stimuli, which in this case were neutral face stimuli [250]. Second, in a study of conditioned aversive stimuli, signal decreases were found in the amygdala and hippocampus in normal subjects, but an opposite increased activation was found in both regions in social phobics [251].

Radioligand studies give some support to the hypothesis that dopaminergic neurotransmission may be altered in social phobia, although they are not currently conclusive. Tiihonen et al., using SPECT and [^{123}I]β-CIT to label DAT in the striatum, reported that densities were markedly lower in patients with social phobia than in age- and gender-matched controls [252]. The authors suggested that the lowered DAT density might reflect an overall smaller number of dopaminergic synapses and neurons in the striatum. Another study using [^{123}I]IBZM reported a significant decrease in D_2 receptor BP in patients with social phobia compared to controls [253]. However, the interpretation of this report is difficult to reconcile with the report by Tiihonen et al. of decreased DAT binding, as decreased [^{123}I]IBZM binding potentials could also reflect increased levels of synaptic DA in the vicinity of D_2 receptors, altered affinity of D_2 receptors for DA, or some combination of these factors.

Of possible relevance to social phobia is the association between dopaminergic abnormalities and the personality trait of personal detachment. Studies using [^{11}C]raclopride report that personal detachment is related to low D_2 receptor density in the striatum [254]. However, the relationship is not evident on all measures of detachment [254,255]. Detachment was also found to be associated with low DAT binding in the putamen [256]. In view of the association between social phobia and low DAT and D_2 receptors [252,253], it has been argued that these neurobiological findings might underlie a commonality between detachment and social phobia [257].

Imaging findings in other neurotransmitter systems are relatively less advanced at present. In the 5HT system, a recent study using [^{11}C]McN 5652 failed to find marked alterations in SERT in patients with social phobia, despite an excellent response to SSRI treatment [258].

E. Obsessive-Compulsive Disorder

Imaging studies have been important in the development of current hypotheses regarding the pathophysiology of obsessive-compulsive disorder (OCD). These studies have recently been comprehensively reviewed and integrated with the basic science literature on the functional neuroanatomy of cortico-basal ganglia-thalamo-cortical circuits to present a theoretical model of brain mediation of OCD symptoms and response to treatment

[259,260]. This model suggests that OCD symptoms are mediated by hyperactivity in orbitofrontal-subcortical circuits, which may be due to an imbalance of tone between direct and indirect striato-pallidal pathways. Serotonergic drugs may improve OCD symptoms by changing the relative balance of tone through the indirect versus direct orbitofrontal-subcortical pathways, thereby decreasing activity in the overall circuit that exists in the symptomatic state.

Functional imaging studies show a highly consistent pattern in these circuits, particularly those involving the orbitofrontal and anterior cingulate cortices. Hyperactivity is found during neutral states [261–265], becomes accentuated during symptom provocation [266–269] or cognitive challenge [270], and attenuates with successful treatment [261,271–274]. Moreover, lower pretreatment metabolism in the orbitofrontal cortex has been found to predict greater improvement on SSRI medication [275]. However, different treatment modalities may have different predictive levels of pretreatment metabolism [276]. Functional abnormalities have also been replicated in lateral frontal cortex [266,268,277], medial temporal lobe structures including amygdala [268,278] and hippocampus [267,279], and in lateral temporal cortex [266].

OCD has been widely studied by volumetric MRI in an attempt to find structural correlations with the above functional abnormalities. Studies have shown significantly less total white matter and greater total neocortical volume [280–282] or increased gray matter density [283]. Reduced orbitofrontal and amygdala volumes have been found in some studies [284] but not in others [280]. A recent study in drug-naive children with OCD found increased thalamic volume, which declined significantly after paroxetine treatment to levels comparable with those of controls. Moreover, the decrease in thalamic volume was associated with reduction in OCD symptom severity [285]. The corpus striatum has been the most extensively studied region but, despite the large number of studies, the findings remain inconsistent. Some studies have found reduced caudate size [281,286,287], another found increased caudate size [288], and most found no difference [281,289–292]. This variability may possibly be explained by clinical heterogeneity (e.g., pediatric or adult subjects, the presence or absence of tics in the populations studied, age at onset, illness duration, gender, treatment status, and issues related to data analysis).

In contrast to the structural data, the results of MRS studies of NAA currently appear to be more consistent. As mentioned earlier in this chapter, NAA is considered to be a putative marker of neuronal viability and decreased levels suggest decreased neuronal density or viability. In studies of drug-free and drug-naive patients with OCD, decreased levels of NAA have been reported in corpus striatum [290,293], anterior cingulate [293], and thalamus [294], even in the absence of measurable volumetric reduction [290]. Interestingly, one study failed to find a difference in NAA in the caudate-putamen in a group of 12 patients, 10 of whom were taking clomipramine or haloperidol treatment [295]. The suggestion has therefore been made that MRS may provide a more sensitive measure of neuronal loss than volumetric MRI [290]. Moreover, MRS studies have demonstrated increased glutamate concentration in the caudate of treatment-naive children with OCD [296,297]. This declined significantly after paroxetine treatment to levels comparable with those of controls, and the decrease was associated with a decrease in OCD symptom severity [296]. Excessive glutamate levels are known to be neurotoxic, and the authors suggest that paroxetine treatment may be mediated by a serotonergically modulated reduction in caudate glutamate concentration.

Abnormalities of serotonergic neurotransmission have long been hypothesized in the pathophysiology of OCD on the basis of the therapeutic efficacy of medications that

selectively increase synaptic 5HT levels (including SSRIs and clomipramine) in nondepressed OCD patients, and the high level of comorbid depression in OCD. No neuroreceptor PET studies have so far been reported in OCD, but studies of the regional binding of $5HT_{1A}$ and $5HT_{2A}$ receptors and the SERT will be of great interest.

F. Post-Traumatic Stress Disorder

Imaging studies in post-traumatic stress disorder (PTSD) have recently been comprehensively reviewed by a number of authors [50,298–300]. Such studies are leading to rapid advances in our understanding of the pathogenesis and pathophysiology of this disorder.

Reduced hippocampal volume has been consistently demonstrated in adults with chronic PTSD, although the time course of this volume reduction and its relation to trauma remain to be elucidated. Radionuclide and fMRI studies have implicated functional abnormalities in a number of regions, including the anterior cingulate and amygdala. As a result of such studies, it has been hypothesized that symptoms of PTSD are mediated by a dysfunction of the anterior cingulate, along with a failure to inhibit amygdala activation and/or an intrinsic lower threshold of amygdala response to fearful stimuli. The model further proposes that hippocampal atrophy found in volumetric MRI studies may be a result of chronic hyperarousal symptoms mediated by amygdala activation [299]. There is a large body of literature on the damaging effects of chronically or repeatedly raised levels of glucocorticoids on the hippocampus. However, the role of glucocorticoids in PTSD—which often follows a single overwhelmingly stressful event—remains controversial [50]. Indeed there are no imaging data published at present to support the idea that hippocampal atrophy occurs in PTSD arising from a single trauma. These findings, and their relevance to the pathophysiology of PTSD, are discussed below.

Using MRI, children and adolescents with PTSD have been reported to have smaller intracranial and cerebral volumes [301,302], attenuation of frontal lobe asymmetry [302], and larger lateral ventricles [301] than matched controls. Brain volume was positively correlated with age of onset of trauma and negatively correlated with duration of abuse [301]. In this population, hippocampal volume appears to be normal [301,302]. However, well-controlled studies have consistently reported smaller hippocampal volumes in adult subjects with chronic PTSD following Vietnam combat experiences as adults [303,304] or following sexual or physical abuse in childhood [305,306]. It has been suggested [307] that left atrophy predominates in PTSD arising from childhood [305,306] and that right hippocampal atrophy predominates in PTSD arising from adulthood [303]. However, such a lateralized effect in adulthood is not consistent [304].

These findings have given rise to a number of important questions, particularly as to whether atrophy occurs prior to trauma, as a result of trauma, or as a result of the PTSD following trauma. The idea that small hippocampi may be present prior to PTSD has been significantly weakened by the results of two more recent longitudinal MRI studies, which show that hippocampal atrophy is not demonstrable in the first 6 months after trauma in adult subjects with PTSD following a single traumatic event [308] or in peripubertal children with maltreatment-related PTSD followed up for at least 2 years [309]. This leaves the question of whether atrophy is related to the trauma itself, or to the subsequent PTSD. However, the answer to this remains unclear. On the one hand, the recent longitudinal MRI findings would tend to argue against the idea that hippocampal atrophy arises from the trauma itself, leaving the suggestion that atrophy may arise as a consequence of the PTSD. On the other hand, however, the data of these studies will only become pertinent

if those with PTSD ultimately do demonstrate atrophy. Moreover, it has been pointed out [50] that in two of the studies cited above [304,305], atrophy may be related more to trauma itself than to a diagnosis of PTSD. Furthermore, in adults with PTSD related to abuse in childhood, hippocampal atrophy does not appear to correlate with time since trauma [306,310]. Distinguishing among those possibilities will require more data indicating when atrophy is first demonstrable (with respect to the trauma), whether it worsens with time after trauma, and the extent to which atrophy is a correlate of trauma itself, as opposed to PTSD [50].

Neurochemical studies using MRS have shown that hippocampal volume reduction is associated with reduced levels of NAA in PTSD [311]. However, a more recent study suggests that reduced NAA can be independent of hippocampal volume [312]. In this study, 18 male subjects with combat-related PTSD showed bilateral reductions of 23% in NAA in the hippocampus. Importantly, comorbid alcohol-related disorders were excluded if they occurred within the previous 5 years, as these have been associated with decrements in hippocampal volume on MRI. However, 10 of the 18 subjects had a lifetime history of major depression, and this may be of relevance as depression is itself known to be associated with hippocampal volume and chemistry, as discussed in the MDD section of this chapter. Unfortunately, no post hoc analyses of these subjects were reported. The authors interpret the finding of decreased NAA in the absence of volume loss to reflect their neuronal loss in the presence of gliosis and/or neuronal metabolic impairments. There were no alterations reported in the Cho resonance in this study. An earlier ^{1}H MRS study in PTSD examining hippocampal regions specifically reported that patients with PTSD had lower NAA in the right medial temporal lobe and lower Cho in the left medial temporal lobe compared to controls [313]. Because NAA is regarded as an indicator of neuronal density, this finding was interpreted (in line with structural MRI findings) to suggest that neuronal density of right-sided medial temporal structures in patients with combat-related PTSD may be decreased. Abnormalities in the region of the anterior cingulate have been consistently found in PTSD using radionuclide imaging (see below). This region was investigated by ^{1}H MRS [314] in 11 children and adolescents with PTSD secondary to maltreatment. NAA was significantly reduced suggesting (in line with the radionuclide studies) that anterior cingulate neuronal metabolism may be altered in childhood PTSD.

Functional imaging studies have implicated a number of regions in the symptomatology of PTSD. In a controlled study using fMRI, Rauch et al. studied combat veterans with PTSD and demonstrated exaggerated amygdala responses to masked-fearful versus masked-happy faces in these patients. The authors conclude that this represents evidence for exaggerated amygdala responses to general negative stimuli in PTSD [315]. A number of controlled SPECT and PET studies have investigated the alterations in rCBF or rCMRglu associated with experimentally provoked intrusive imagery and enhanced anxiety. The main groups studied have been Vietnam combat veterans [316–319], adults with a history of childhood sexual abuse [320,321], and victims of torture [322]. A particularly consistent finding in the PET studies is a relative failure to activate the anterior cingulate [317,320,321] during provoked PTSD symptoms. This is supported by the results of a recent fMRI study showing diminished response in rostral anterior cingulate cortex in the presence of emotionally relevant stimuli in Vietnam combat veterans with PTSD [323].

Other areas associated with symptom exposure include medial prefrontal cortex, posterior cingulate, visual association cortex and hippocampus, as well as middle and superior temporal, middle frontal, right orbitofrontal, occipital, parahippocampal, anterior temporal, and inferior frontal cortices. These have shown either increases or decreases in

perfusion depending on the study conditions and sample population. The severity of provoked imagery in chronic PTSD was recently found to be correlated with rCBF in a widely distributed group of regions including brainstem and areas associated with motor control, complex visual/spatial cues, and memory [324].

Any alterations of regional receptor populations in PTSD are currently unknown. However, PET quantification of 5HT and benzodiazepine receptor binding potential in anterior cingulate and medial temporal areas will be of particular interest.

V. CONCLUSION

Neuroimaging research has provided a number of insights into underlying pathophysiological processes in affective and anxiety disorders. However, only in late-onset MDD can it so far be said to have provided convincing evidence of etiological factors. It would appear that cerebrovascular disease may play a role in the pathogenesis of late-onset MDD in patients with MRI evidence of hyperintensities, and the new mood disorder subtype of vascular depression has been proposed. In depression of earlier onset, amygdalar activity appears to be pathologically increased, and abnormalities have been replicated in cortico-striatal-pallidal-thalamic circuits particularly implicating the orbital and medial prefrontal cortex. There also seems to be an association between depression and hippocampal size dependent on the cumulative duration and severity of MDD. In the 5HT system, reduced densities of $5HT_{1A}$ receptors and SERT have been replicated.

In other disorders, the integration of structural, neurochemical, and functional imaging findings is not so far advanced. However, this should not obscure the number of well-replicated and highly consistent patterns found in these disorders that are providing clues to underlying pathophysiological processes. In BPD, the most consistent findings are of a higher than expected incidence of white matter hyperintensities and abnormalities of membrane phospholipid metabolism. Dopaminergic neurotransmission is also dysregulated during psychosis, but this finding does not appear to be specific to BPD. In panic disorder, a fairly consistent pattern has been reported of reduced activity in hippocampal, parahippocampal and inferior frontal regions, along with reductions of BZD receptor binding. In OCD, there is a highly consistent pattern of increased activity in cortico-striatal-thalamic-cortical circuits involving the orbitofrontal and anterior cingulate cortices, which attenuates with successful treatment. Moreover, replicated MRS findings suggest reduced neuronal density in the striatum in OCD. Finally, in PTSD, reduced hippocampal volume has been consistently demonstrated in adults with chronic PTSD, along with relative failure to activate the anterior cingulate gyrus and relatively increased amygdalar activity.

Beyond these relatively well-replicated findings, it is interesting to note that a number of forebrain regions (orbitoprefrontal cortex, anterior cingulate, amygdala, hippocampus, and striatum) have been frequently implicated by imaging studies in the pathophysiology of anxiety and mood disorders. This anatomical overlap mirrors the frequent comorbidity of anxiety and mood disorders, but also illustrates the limitations of brain-imaging techniques. While these techniques might point toward regional alterations in shape, volume, metabolism, or concentration of specific biomolecules, the precise neuronal basis of these abnormalities remains to be described. So far, brain imaging has not contributed significantly to the elucidation of the etiology of these afflictions nor to the development of new medications.

To move beyond phenomenological descriptions is the challenge facing the study of these disorders by brain imaging. In the next decade, development of more specific

and dynamic biomolecular imaging probes, realistic and testable models for imaging regional and neuronal connectivity, and close collaboration with other fields of neuropsychiatric research (molecular biology, genetics and epidemiology, animal models, post-mortem studies, neurocognitive sciences) will be important factors contributing to the growth of brain-imaging techniques.

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14

Neurobiology of Anxiety and Depression

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I. INTRODUCTION

The current diagnostic nomenclature in psychiatry is based on phenomenology. Signs and symptoms, with defined time periods, are the predominant criteria for diagnoses, with the additional requirement of a threshold of distress/dysfunction. Anxiety and mood disorders are carved into several categories with precise criteria. This allows for diagnostic reliability. However, reliability does not necessarily equate with validity. Core components of an illness may not get the appropriate emphasis if they cannot be reliably measured. In addition, diagnostic labels are categorical, to the uninitiated, implying clear boundaries without overlap and coincidental comorbidity. Brain function and pathology do not fall into such simple categories. The reality is that clinical diagnoses have overlapping presentations and are heterogeneous with multiple etiopathological pathways. This heterogeneity may also be the basis of significant variance in treatment response as well as overlap in results from studies in neurobiology. Failure to recognize such limitations are potential impediments to the development of novel pharmacological as well as psychotherapeutic treatments. Starting from an understanding of brain functions may allow a new view of the neurobiology underlying anxiety and depressive disorders and, in a "reverse engineering" approach may guide us in improving the classification of anxiety and depressive disorders.

II. THE DIAGNOSTIC CONUNDRUM

There are several dimensions of symptoms subsumed under anxiety and depressive disorders. The disorders are largely a collection of symptoms that clinical medicine would

consider as syndromes. In major depressive disorder (MDD), these dimensions are emotional (sadness, anhedonia, anxiety), cognitive (impaired concentration and memory, specific cognitions such as guilt, worthlessness, and suicidal thoughts), endocrine manifestations (change in sleep and appetite, diurnal variation), somatic expressions (preoccupation with physical symptoms and health), as well as behavioral manifestations (motor retardation, inertia). Similarly, generalized anxiety disorder (GAD) has domains that are emotional (anxiety), cognitive (uncontrollable worry, poor concentration), somatic (muscle tension, autonomic symptoms, somatic preoccupation), and behavioral (irritability, restlessness).

Symptomatic overlap is present to a prominent degree among diagnostic categories in the anxiety and depressive syndromes. Thus anxiety as symptoms are the norm rather than the exception in mood disorders like MDD. Thus, the majority of patients with MDD experience anxiety at an emotional, cognitive, and somatic level, including paroxysmal anxiety as in panic attacks [1]. Similarly, anxiety disorders have dysphoria as a common symptom. It is hard for individuals preoccupied with intense anxiety to focus much on the experience of pleasure.

Part of the confusion might be that several overlapping terms are used to denote the key states of anxiety and dysphoria. Thus words like nervousness, fear, worry, apprehension, tension, irritability, agitation, and restlessness all capture some component of the rubric of anxiety. Similarly, dysphoria also includes sadness, anguish, dissatisfaction, rumination, and somatic preoccupation. As is evident, many of these terms can be used interchangeably in anxiety and depressive disorders. The difficulty is partly the result of the subjective component of these experiences, which do not have a linear relationship with the underlying biological processes that mediate their entry into conscious awareness.

An example of the diagnostic confusion is the criteria for GAD and dysthymia. Both require long-term symptoms (6 months for GAD and 2 years for dysthymia). GAD requires anxiety and cognitive worry as its core symptoms, associated with restlessness, fatigue, poor concentration, irritability, muscle tension, and sleep disturbance. These associated symptoms are also part of the criteria for dysthymia, which requires sadness and anhedonia as its core symptoms. However, many patients are unable to report whether anxiety or sadness at an emotional level are their core experiences and clinicians may have difficulty distinguishing the worry of GAD from depressive ruminations; this raises questions about the reliability of such distinctions. Such conceptual confusion has implications. Thus, recent genetic studies suggest that the genetic vulnerability for GAD and MDD is the same [2]. That may be so, or the unity of genetic factors may be the result of an inability to clinically separate the two conditions with validity.

Comorbidity of disorders may occur historically or concurrently. Anxiety disorders have high comorbidity with depressive disorders [3]. In a study of 109 patients with a primary diagnosis of GAD, 42% met current or past criteria for MDD [4]. In the Epidemiological Catchment Area study, 16.6% of subjects with social anxiety disorder had a history of MDD and 12.5% had dysthymia. Rasmussen and Eisen [5] reported that among 100 subjects with primary obsessive-compulsive disorder (OCD), 67% met lifetime and 31% met current criteria for MDD.

Among patients with MDD, there is a similar high representation of comorbidity with anxiety disorders. Schatzberg et al. [6] reported that 58% of their patients with a current diagnosis of MDD had a history of an anxiety disorder and 49% met current criteria for anxiety disorder. Similarly, the National Co-Morbidity Survey reported 58% of individuals with a lifetime episode of major depression also met criteria for an anxiety

disorder [7]. The anxiety disorder developed first in 68% of individuals, with major depression developing, on average, 11 years later. This period was shorter for GAD and panic disorder (average 1.5 years) and over a decade each for post-traumatic stress disorder (PTSD), simple, social, and agoraphobia [7]. This is consistent with data from the Epidemiological Catchment Area study, which found 47.2% of patients with lifetime MDD also had an anxiety disorder [8]. The anxiety disorder came first in 55% of individuals, and MDD in 18%. The average number of years between the phobic disorders and the onset of MDD was around a decade, but around a year for panic disorder and OCD. Thus, anxiety and depressive disorders are highly comorbid syndromes.

The distinction between those patients who have full syndromal comorbid anxiety disorder and MDD from those with only a single disorder has not been adequately addressed in the literature. A large proportion of studies performed by the pharmaceutical industry for regulatory purposes did not use structured interviews to examine comorbidity even though they were diagnostic categories in the exclusion criteria. Thus many of the pivotal and other studies performed without a stringent diagnostic emphasis are of little value in examining these issues.

III. NEUROCHEMISTRY OF ANXIETY AND DEPRESSION

A brief review of the studies that examined neurochemical aspects of individual anxiety and depressive disorders is provided below.

A. Generalized Anxiety Disorder

The diagnostic criteria for GAD have evolved with iterations of the DSM over the past two decades. Thus, the current knowledge of the neurobiology of GAD rests largely on studies that have used previous definitions. In addition, the overall number of studies is limited.

The study of noradrenergic receptor function has been implicated in GAD although not universally [9]. The blunting of the growth hormone response to clonidine challenge and yohimbine has been noted to evoke an attenuated MHPG response in GAD patients as compared to controls [10]. Peripheral platelet α_2 binding sites have also been reported to be reduced [11,12].

The function of the serotonin system has also been studied to a limited extent in GAD. In one study, the 5HT agonist meta-chlorophenylpiperazine (mCPP) has been shown to produce increased anxiety and hostility in GAD [13]. Reduced cerebrospinal fluid levels of 5HT [14] and reduced platelet paroxetine binding [15] have also been observed in GAD. Limited studies have also been done on the GABA/benzodiazepine receptor system. A lower number of peripheral benzodiazepine binding sites have been noted on platelets [16] and lymphocytes[17]. In two studies, treatment with benzodiazepine drugs produced an increase in the number of peripheral binding sites [16,18]. Reduced sensitivity of central benzodiazepine receptors has also been found in one study [19].

Lactate and CO_2 challenge studies have also been performed in GAD. Both lactate and CO_2 challenge produced higher rates of anxiety in GAD patients as compared to nonanxious controls, but rates of panic attacks were lower than those found in panic patients [20–23]. The function of the hypothalamic-pituitary-adrenal (HPA) axis was also assessed utilizing the dexamethasone suppression test in two studies. Both studies reported

elevated rates of dexamethasone nonsuppression [24,25]. A third study, however, did not reveal any evidence for increased baseline urinary cortisol in GAD [26].

B. Panic Disorder

There is evidence suggesting noradrenergic, serotonergic, respiratory, and peptidergic abnormalities in panic disorder. Panic patients have an increased sensitivity to β-adrenergic stimulation. Isoproterenol, a β-adrenergic receptor agonist, induces panic attacks in patients with panic disorder compared to nonanxious controls [27,28]. Central α_2-adrenergic receptor function is altered in panic disorder as growth hormone release by clonidine, an α_2-adrenergic receptor antagonist, is blunted in panic patients in the majority of studies [29–34]. Clonidine also disproportionately reduces serum levels of the noradrenergic metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in patients with panic disorder [29–31]. In addition, the α_2-adrenergic receptor antagonist yohimbine has produced significantly greater rises in plasma MHPG, elevations in systolic blood pressure, and elevated ratings of anxiety symptoms in patients with panic disorder as compared to controls [35–37].

Evidence suggests the involvement of the serotonin system in panic disorder. The serotonin reuptake inhibitors are effective in the treatment of panic disorder. Meta-chlorophenylpiperazine, a $5HT_{2C}$ agonist, produced significantly greater rates of panic and anxiety symptoms in patients with panic disorder compared to controls [38], although this was not replicated [39]. Different doses and routes of administration used in these two studies may explain the discrepancy. Studies using other direct serotonin agonists including ipsapirone, fenfluramine, and clomipramine have generally found evidence of abnormal serotonin receptor responses [40–43] although not universally [44]. Serotonergic stimulation may induce a state of high anticipatory anxiety that predisposes to a panic attack rather than directly triggering one [45,46]. Differences in the outcome of studies may also result from the impact of other variables like sex, seasonal factors, and age, all of which impact upon serotonergic function [47,48].

The prominence of respiratory symptoms like dyspnea in panic attacks has led to examination of the chemoreceptor function in panic disorder. Two techniques have been used—lactate infusion and carbon dioxide inhalation. Lactate infusion has triggered panic symptoms in a significantly greater number of patients with panic disorder than controls in several studies [21,49,50]. The lactate-infusion-induced panic attacks are similar to those experienced during naturally occurring panic [51] and are blocked by pharmacological agents effective in the treatment of panic disorder [19,52–55]. Inhalation of various concentrations (5–35%) of a carbon dioxide mixture have also induced panic attacks in a large proportion of patients with panic disorder compared to controls [22,56–63]. Carbon dioxide–induced panic is also subjectively similar to naturally occurring panic attacks [64] and is blocked by pharmacological agents effective in the treatment of panic disorder [65,66]. The precise mechanism by which these challenges induce panic attacks remains to be elucidated.

Neuropeptides also appear to play a role in the biology of anxiety, particularly panic attacks. The neuropeptides cholecystokinin (CCK) and neuropeptide Y have been demonstrated to modulate anxiety in animal models of anxiety [67,68]. CCK-related peptides have consistently elicited panic-like symptoms under a challenge paradigm in panic disorder patients and have done so in a dose-dependent fashion [69,70].

C. Obsessive-Compulsive Disorder

The efficacy of serotonergic drugs in OCD and the failure of noradrenergic medications have resulted in a focus on serotonin in neurochemical studies. The direct serotonin receptor agonist mCPP has been used in challenge studies to assess the function of serotonin systems in OCD. The results of these studies have been mixed, as some studies have shown mCPP to increase anxiety and obsessions in OCD patients as compared to normal controls [71,72], whereas other studies have failed to replicate these findings [73]. The discrepancy may be related to the differing doses and routes of administration used in the various studies.

Noradrenergic function has also been studied. Clonidine challenge has been shown to result in blunted GH release in some studies of OCD patients [74,75] but not in others [76].

D. Social Anxiety Disorder (Social Phobia)

Social anxiety disorder (SAD) is relatively poorly understood from a neurochemical perspective. This limited understanding may be further complicated by the fact that the generalized and discrete subtypes of this disorder may be significantly distinct and may require separate study [77].

Studies of SAD involve challenge studies designed to study the functioning of specific neurotransmitter systems. In studies of noradrenergic function, an abnormal blunting of growth hormone response to intravenous clonidine challenge was found in one study of SAD patients as compared to controls. The degree of blunting was found to be less than that seen in panic patients in the same study [78]. A follow-up study using oral clonidine challenge, however, failed to demonstrate any abnormality in growth hormone response [78]. A role for serotonin in the pathophysiology of SAD has been supported by the clinical effectiveness of SRI medications in the treatment of SAD. In studies investigating serotonergic function, SAD patients have shown increased anxiety relative to controls upon exposure to mCPP [79] and fenfluramine, with augmented cortisol responses also noted upon exposure to fenfluramine [78,80,81]. Dopamine receptor functioning has also recently been studied using SPECT functional neuroimaging scans. One study revealed a 20% reduction in striatal dopamine transporter sites. A study by Tiihonen et al. [82] as well as a second study revealed a similar magnitude of reduction in dopamine-2 receptor binding [4].

Challenge studies assessing chemoreceptor function have also been used to study the neurobiology of SAD. Three studies using 35% CO_2 have shown increased rates of CO_2-induced panic in SAD patients as compared to controls. Two studies showed panic induction rates lower than those seen in panic disorder patients [59,83], but the third study revealed similar rates to panic patients [84]. A single lactate challenge test has been performed and revealed similar responses in SAD patients and controls [85].

E. Post-Traumatic Stress Disorder

There have been significant recent advances in the study of the neurochemistry post-traumatic stress disorder in adults. Alterations in catecholamine function have been noted in adult PTSD patients. Peripheral catecholamine levels are increased in PTSD patients under basal [86] and stimulated conditions [87]. Administration of yohimbine produces

both an increase in PTSD symptoms and serum levels of MHPG in trauma survivors with PTSD [88].

The function of the HPA axis in PTSD has also been the subject of a number of studies. Lower plasma cortisol levels and/or 24-h urinary cortisol levels have been found in combat veterans with PTSD [89], Holocaust survivors with PTSD [90], and in the high-risk group of adult children of Holocaust survivors [91] (rather than the intuitively expected elevation of cortisol levels in the face of stress). Another study did, however, find elevated 24-h urinary cortisol levels in female survivors of childhood sexual abuse with PTSD [92]. Initially, these findings of low cortisol levels were explained as reflecting a chronic adaptation to stress [93]. More recent findings, however, have called that explanation into question. One study has shown cerebrospinal fluid levels of corticotropin-releasing factor (CRF) to be elevated in PTSD patients as compared to controls [94]. This raises the possibility of a disconnection between CRF and cortisol release in PTSD. A recent longitudinal study has shown that motor vehicle accident victims who went on to develop PTSD had a significantly lower cortisol response immediately following the trauma than did patients who went on to develop major depression. The response of those who did not develop a psychiatric disorder was found to be between the other two groups [86]. A second study demonstrated that women with a prior history of rape or assault had relatively lower cortisol levels immediately after rape than did women without such a history [95].

Combat veterans with PTSD also have greater cortisol suppression following dexamethasone administration than do controls [90,96,97]. This finding has more recently been demonstrated in adult women survivors of childhood sexual abuse [98] and in adolescents who developed PTSD following an earthquake [99]. This finding appears to be related to enhanced sensitivity of glucocorticoid receptor [100].

F. Major Depressive Disorder

The neurochemical explorations in clinical depression have focused largely on major depressive disorder. The studies were initially based on the fortuitous findings of therapeutic benefits of pharmacological agents subsequently labeled as antidepressants. These were initially monoamine oxidase inhibitors and tricyclic antidepressants and subsequently the new generation of serotonergic medications, particularly the inhibitors of the serotonin transporter. Using a reverse engineering method, the mechanism of action of antidepressants was thought to be related to the pathophysiology of the disorder.

Functional measures of presynaptic activity were performed by measuring neurotransmitters and their metabolites in the CSF and other body fluids. Peripheral measures included platelet studies. Reactivity of these systems was also measured by pharmacological challenge paradigms. Neurotransmitter depletion methods that reversed the antidepressant effects of antidepressants using dietary or pharmacological manipulations were also performed.

The antihypertensive medication reserpine was observed clinically to induce a state of depression indistinguishable from idiopathic MDD. Reserpine depleted monoamines and so the catcholamine hypothesis of MDD postulated that a reduction in activity of the norepinephrine system was the basis of depression and the therapeutic efficacy of antidepressants was based on its reversal. MHPG is a metabolite of NE and a measure of NE levels. Roughly 20% is derived from the CNS pool [101], but no consistent pattern is recognized in studies of unipolar patients. Clonidine challenges measure α_2-adrenergic stimulation, resulting in secretion of growth hormone (GH) from the pituitary through

postsynaptic effects. Studies have generally reported blunting of this response in MDD, both during the depressive episode as well as following successful treatment, suggesting it is a trait marker for MDD. Alpha-methyl-para-tyrosine (AMPT) inhibits tyrosine hydroxylase, the rate-limiting enzyme for the production of norepinephrine. AMPT-induced depletion of NE in MDD resulted in a relapse of depressive symptoms in those on a noradrenergic antidepressant but not a serotonergic one.

The serotonergic hypothesis was based initially on the benefit of trazadone and subsequently the serotonin reuptake inhibitors (SSRIs). Several studies, though not all, have reported CSF levels of 5HIAA decreased in MDD, particularly in those who were significantly suicidal and impulsive. Intravenous tryptophan increases serotonin and serum prolactin and this has been reported to be blunted in MDD. Fenfluramine also increases prolactin through its presynaptic effects, with blunting reported in MDD. Depleting tryptophan, the amino acid precursor of serotonin, induces relapse of depressive symptoms in those on a serotonergic antidepressant, but not untreated MDD patients.

IV. FUNCTIONAL ANATOMY OF ANXIETY AND DEPRESSION

Preclinical studies in the neurosciences provide insights into neural circuits that underlie motivational systems from which emotional and behavioral responses are derived [102,103]. The neural circuitry associated with emotional processing is complex but overlaps significantly with the classical limbic system as described by Papez. Thus, there is a significant overlap between the anatomical structures that are correlated with the depressive and anxiety disorders, and the symptomatic overlap may be a function of the overlap of neurocircuits.

Structures in the basal forebrain are critical hubs for the emotional experiences at the core of anxiety and pleasure. Neuroanatomical models of fear and anxiety have focused on the central role played by the extended amygdala. Pleasurable or hedonic responses have been associated with the so-called reward pathways in the brain, including structures like the ventral tegmental area (VTA) and the nucleus accumbens. These motivational systems are closely and reciprocally connected to the executive systems mediated by the prefrontal cortex. Under typical conditions, there is a flexible shift in the activity of these systems with the potential for willful choice of affective or cognitive responses driving behavioral output. In pathological states such as anxiety and depressive disorders, the interrelationships between these systems are altered such that there is diminished executive control, which is easily overridden by more automatic, noncognitively driven responses programmed through the more phylogenetically primitive structures.

Systems mediating positive emotions like pleasure may be awry in depressive disorders. These have been best mapped primarily in studies examining substance use disorders, but their relevance in depression is increasingly being recognized. The clinical diagnosis of major depression requires sadness and the reduced ability to experience pleasure, also termed anhedonia. Pleasurable or hedonic responses have been associated with structures like the VTA, nucleus accumbens, and medial prefrontal cortex [104,105]. The rewarding (hedonic) system is a preservative one and responds to appetitive stimulation. In particular, the VTA/nucleus accumbens circuit is implicated in reward-driven behavior (e.g., operant conditioning). Neurochemical studies most strongly link dopamine to this circuit, but other neurotransmitters are associated with reward, with mounting evidence for both serotonin and norepinephrine. Evidence for state-dependent dysregulation of these circuits in MDD has been reported and may predict treatment responsiveness [106].

Fear and anxiety are often used interchangeably, although fear focuses on physical threat while anxiety response may be seen as predicting the threat. The amygdala is an almond-shaped structure consisting of several cell groups, part of the limbic system, critical for processing emotional experiences. Highly processed sensory information from various areas of the cortex reaches the amygdala through its lateral and basolateral nuclei. These nuclei then project to the central nucleus of the amygdala, which then projects to hypothalamic and brainstem target areas, which mediate the specific signs and symptoms of fear and anxiety.

Considerable evidence supports the hypothesis that the amygdala and its efferent projections represent a central fear/anxiety system involved in both the expression and the acquisition of conditioned fear. Different parts of the amygdala and closely related structures may be involved in different types of fear and anxiety. Three such forms of anxiety can be distinguished. An unconditioned threat results in the fear response. If this is paired with a neutral stimulus, that becomes a cued trigger for the fear response, called conditioned fear. Such unconditioned and conditioned fear elicited by explicit cues appears to be processed through the central nucleus of the amygdala.

Contextual fear refers to the fear noted when there is exposure to the environment (or context) where the fear conditioning occurred. Contextual fear may be closer to the concept of anxiety because the context is associated with the threat, although this association may or may not be consciously connected. Such anxiety appears to be processed via circuitry that involves not only the amygdala but also the hippocampus and the bed nucleus of the stria terminalis. The role of the hippocampus in processing spatial information and declarative (conscious) memory may aid in its role in contextual fear/anxiety.

Avoidance of aversive experiences like the triggering of the fear response is an additional component of the anxiety response. Such avoidant conditioning may be influenced by the medial prefrontal cortex, which is reciprocally connected to the amygdala. The extinction of conditioned avoidance is prolonged by damage to the medial prefrontal cortex. The prefrontal cortex may in fact allow for "cognitive" control of conditioned fear and provide the circuitry critical for the benefit in cognitive-behavioral therapies.

V. NEUROIMAGING STUDIES

The different anxiety disorders may share a common functional anatomy, but may also have additional and unique circuits involved in their expression.

The functional anatomy of panic disorder has been explored using neuroimaging techniques. PET studies have revealed that panic patients susceptible to lactate-induced panic have increased right parahippocampal blood flow and oxygen metabolism and abnormally high whole-brain oxygen metabolism in the nonpanic state [107,108]. A SPECT imaging study utilizing a substantially different methodology revealed decreased hippocampal blood flow in lactate-sensitive panic patients [109]. Another SPECT study revealed reduced regional cerebral blood flow in the frontal lobes of panic patients after yohimbine administration as compared to controls [110].

Neuroimaging and EEG studies have also been conducted in SAD. A PET study revealed changes in regional cerebral blood flow, including increased blood flow in the right dorsolateral prefrontal cortex and the left parietal cortex when SAD subjects were provoked with a script of fear-evoking autobiographical material [111] when compared to findings in controls under the same conditions noted in an earlier study [112]. Two studies using magnetic resonance spectroscopy (MRS) have found abnormal choline me-

tabolite levels in SAD patients [113,114]. An additional SPECT study in generalized SAD failed to reveal evidence of a focal abnormality in cerebral blood flow [115]. A recent EEG study comparing SAD patients and controls showed SAD patients to have marked selective activation of right frontal alpha power in anticipation of and immediately after a public speaking task [116].

Neuroimaging studies have also demonstrated significant changes in brain morphology in PTSD patients as compared to controls. The primary finding based on MRI studies has been reduced hippocampal volume in combat veterans and adult sexual abuse survivors with PTSD [94,98,117,118]. The laterality of findings has varied between studies. A recent functional neuroimaging study using PET demonstrated that recollection and imagery of traumatic events was accompanied by increased regional cerebral blood flow in anterior paralimbic regions. These changes were demonstrated in trauma-exposed individuals both with and without PTSD, but the patterns of changes were significantly different between the two groups [119]. One MRI study comparing maltreated but medically healthy children and adolescents with PTSD and matched controls has been reported. This study demonstrated significantly smaller intracranial and cerebral volumes in the PTSD group as compared to controls. However, contrary to adult studies, hippocampal volumes were not decreased in the PTSD group [120].

Obsessive compulsive disorder, appears to involve basal ganglia structures based on imaging studies. Abnormalities in basal ganglia–frontal cortex circuits, specifically the ventral prefrontal cortex–striatal circuits, have been implicated in the pathophysiology of OCD. Neuroimaging studies have demonstrated changes in adult OCD patients compared to controls. Functional neuroimaging studies have demonstrated increased metabolic rates in the ventral prefrontal cortex [121–125]. Functional imaging studies of adults with childhood-onset OCD have also shown that metabolic activity in the ventral prefrontal cortex and striatum correlate with OCD symptom severity and response to treatment [125,126]. Structural neuroimaging studies have in general yielded mixed results [127]. One recent study, however, showed several significant differences between OCD patients and controls including significantly smaller orbital frontal and amygdala volumes, as well as a lack of the normal hemispheric asymmetry of the hippocampus–amygdala complex in OCD patients [128].

Other studies have sought to identify specific brain circuits involved in OCD. These studies have focused on the prefrontal cortex which, based on animal models and clinical neuropsychological studies, appears to be primarily responsible for the inhibition of the repetitive, ritualistic thoughts and behaviors that characterize OCD [129–135]. A recent study utilized oculomotor tests to assess prefrontal cortex functioning in 18 children and adolescents with OCD and 18 matched controls. The OCD patients were not depressed and were medication naive. The OCD patients showed a significantly higher number of failures in the prefrontal cortex function of response suppression. The difference between the patient and control groups was particularly high among the younger patients and controls [136]. This finding replicated a similar study of medicated adult OCD patients [137]. An earlier study showed an increase in a number of neurological soft-sign abnormalities in pediatric OCD patients as compared to controls [138].

Neuroimaging technologies have also been used in the study of childhood OCD. An MRI study of treatment naive 7 to 17-year-old OCD patients did not reveal any differences in total prefrontal cortical gray or white matter volumes as compared to controls. This study also studied the morphology of the striatum specifically. The examination of the striatum was motivated by previous adult functional brain-imaging studies showing

differences in OCD patients in this brain region. Pediatric OCD patients had significantly smaller striatal volumes than controls and these findings correlated inversely with obsessive, but not compulsive, symptom severity. The putamen and caudate subcomponents of the striatum were also specifically measured and the putamen, but not the caudate, volumes were significantly smaller [139]. A follow-up study of 21 pediatric OCD patients and matched controls showed significantly larger ventral prefrontal cortical (VPFC) anterior cingulate volumes in OCD patients as compared to controls, but no significant differences in the posterior cingulate cortex or the dorsolateral prefrontal cortex [127]. It is noteworthy that a previous PET study demonstrated that the VPFC and its target fields in the striatum play key roles in inhibiting context-inappropriate responses to sensory stimuli in healthy human subjects [140]. An additional recent study examined the effect of SRI treatment on a specific brain region implicated in the pathophysiology of OCD. Magnetic resonance spectroscopy examinations of the left caudate in 11 psychotropic-naive children and 11 controls showed significantly greater caudate glutamatergic concentrations in the OCD patients, which declined to levels comparable to controls after 12 weeks of treatment with paroxetine [141].

VI. ETIOPATHOGENIC STUDIES IN OCD

Recent findings in the neurobiology of pediatric OCD may usher in a major paradigm shift in the study of the etiopathogenesis of a number of psychiatric diseases. A series of studies have explored the apparent link between the immune reaction to certain infectious agents and the development of OCD and tic disorders in children. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) is a relatively new diagnostic construct that refers to children and adolescents who develop and have repeated exacerbations of obsessive-compulsive disorder and/or tic disorders following group A beta hemolytic streptococcal infections. The proposed pathophysiology involves group A beta-hemolytic streptococcal bacteria triggering the production of antibodies that cross-react with the basal ganglia of genetically susceptible hosts, leading to the development of OCD and/or tics [142].

Several recent studies have supported this proposed link. Two MRI studies found significantly larger basal ganglia volumes in subjects meeting working diagnostic criteria for PANDAS as compared to matched controls [143,144]. IgG antibodies have also been noted in blood samples of a subgroup of children with PANDAS similar to the antibodies found in patients with Sydenham's chorea [145]. Also, the nonhuman lymphocyte antigen DR-positive cell surface marker D8/17, found in over 90% of patients with Sydenham's chorea [146] has also been detected in 85% of a group of patients with PANDAS [147].

Evidence hinting at the possibility that immunological mechanisms may be involved in other pediatric anxiety disorders has been noted in other studies. A retrospective review of childhood risk factors for psychiatric disorders in adolescence and early adulthood found a specific link between illness in the first year of life, particularly with high fever, and adolescent anxiety disorders [148]. An earlier study also noted an association between allergic symptoms, particularly hay fever, and inhibited temperament in young children [149].

VII. MOLECULAR STUDIES

The serotonin transporter in humans is coded in a single gene on the 17th human chromosome. A promoter region is genetically polymorphic with a long allele and a short allele

version. The majority of individuals appear to have the short allele version, which results in the reduced expression and function of the serotonin transporter. These individuals have a slightly greater propensity toward anxiety measured on personality tests [150]. An fMRI study reported that individuals with one or two copies of the short allele also demonstrated an increase in the amygdala response to fearful faces compared to individuals with the long allele [151]. The majority of studies in MDD report a decrease in serotonin transporter binding compared to normal controls and this normalizes with successful antidepressant treatment [152]. Pharmacogenomic studies will allow the classification of individuals with anxiety and depressive disorders into biologically homogeneous groups, which may produce greater precision in predicting side effects and response to treatment.

VIII. CONCLUSION

Given the high prevalence of anxiety and depressive disorders, their chronicity and societal burden, it is critical to elucidate the multiple neurobiological underpinnings of pathological anxiety and depression. Such knowledge will provide the foundation for the next generation of advances in pharmacological as well as other treatments.

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15

Intracellular Signaling Transduction Dysregulation in Depression and Possible Future Targets for Antidepressant Therapy: Beyond the Serotonin Hypothesis

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I. THE MONOAMINE HYPOTHESIS OF DEPRESSION

The monoamine hypothesis of depression was coined over 30 years ago [1,2]. This hypothesis proposes that there is an underlying biological basis for depression, namely a deficiency of the monoamine neurotransmitters norepinephrine, serotonin, and/or dopamine in the central nervous system [3]. With this theory in mind, various classes of antidepressants have been developed that act to increase levels of monoamines within the synaptic cleft, either by inhibition of their degradation or by blockade of their reuptake. Newer antidepressants that elevate serotonin, norepinephrine, and/or dopamine levels in the brain have been shown to effectively alleviate the symptoms of depression. Although substantial evidence exists to support a role of monoamine systems in the mechanism of action of antidepressants, intensive investigation has failed to find conclusive affirmation of a primary dysfunction of a specific monoamine system in patients with major depressive disorders [4–6].

A. Problems with the Monoamine Hypothesis and with Antidepressant Action on the Monoaminergic System

There are several major issues that have not been addressed by the monoamine hypothesis. These problems mainly concern the action of medication acting on serotonergic and noradrenergic systems.

1. Efficacy

Antidepressive medication, especially the newest generations of drugs including selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs) and selective serotonin/norepinephrine reuptake inhibitors (sSNRIs), appear to be effective in approximately 60% of the patients suffering from major depression [7]. However, even though the first tricyclic agent (TCA) was introduced more than 30 years ago, the newest selective SRIs or NRIs fail to demonstrate an enhanced efficacy compared to the older TCAs [8,9], despite the fact that they are better tolerated and do not show the serious cognitive, cardiac, and other somatic side effects commonly observed after long-term treatment with TCAs [10–17].

2. Selectivity

Although it is clear that SSRIs, NRIs, or NaSRIs act through the stimulation of serotonergic and noradrenergic systems, there is still confusion regarding the specific cellular or molecular targets responsible for their therapeutic action, which appears to include neurotransmitter transporters, specific receptors, intracellular proteins, several enzymes, and transcription factors [18–22]. Moreover, various lines of evidence indicate that during chronic administration of these agents, the selectivity of the therapy dissipates. In fact, after several weeks of continuous administration, highly selective drugs such as SSRIs or NRIs, which stimulate specific neurotransmitter systems, may even influence the activity of other neural systems not directly associated with the initial therapeutic target [23–27]. An intriguing possibility is that it might be their aspecificity (modulation of the activity of a broader range of brain areas), rather than their selectivity, which is mainly responsible for their antidepressant effects.

3. Mode of Action

Another important question to be addressed concerns the mechanisms by which antidepressants cause therapeutic effects. In contrast to antidepressants acting through the potentiation of monoaminergic transmission (TCAs, SSRIs, NRIs, and NaSRIs), other effective antidepressants act by enhancing serotonin reuptake (an opposite mechanism compared to SSRIs, e.g., tianeptine) [28,29] or by the inhibition and/or stimulation of selective enzymes and transcription factors that are not directly linked to serotonin or norepinephrine metabolism or signaling transduction pathways (such as lithium and valproate) [30,31].

4. Delayed Onset of the Therapeutic Effect

While the adverse effects of antidepressants are manifested within hours or days following the beginning of the administration, the therapeutic action is delayed and can take several weeks or even months to appear, causing considerable problems with the compliance of the patients [32–34].

5. Monoamine Depletion

Experimental monoamine depletion exacerbates depressive symptoms only in depressed patients that have previously responded successfully to treatment while monoamine depletion fails to induce the same effects in medication-free symptomatic patients or in healthy subjects. The failure to precipitate depressive symptoms implies that a serotonergic and/or noradrenergic dysfunction is unlikely to be the cause of depression, although it can be used to explain the mechanism by which antidepressants act [5,6,35,36].

While the monoaminergic systems appear to be crucial in the therapeutic effects of antidepressants, only fragmentary evidence seems to support a primary role of monoamine deficiencies in the etiology of depression. Several factors might be involved in the development of depression, but the dysfunction of the monoaminergic system may represent only one of the possible consequences of this disease. The biogenic amine neurotransmitter systems are extensively distributed within the limbic–cortical system, the neural network involved in the modulation of cognition and emotions but also implicated in the regulation of other functions as sleep, appetite, arousal, cardiovascular regulation, sexual, and endocrine activity [37–40]. It is not surprising that clinical research strategies over the years have identified dysregulation of noradrenergic, dopaminergic, serotonergic, and cholinergic systems as well as the hypothalamic–pituitary–adrenal axis (HPA) in depressed patients [41–46]. The behavioral and physiological manifestations of the illness are complex and undoubtedly mediated by a network of interconnected neurotransmitter pathways. Possibly the abnormal activity of one or more key components of this complex neural network may disrupt its ability to respond to incoming sensory stimuli with appropriate emotional responses. Depression appears to be a syndrome that originates from a heterogeneous pathology with several different etiological causes and, possibly, one consequence, that is an abnormal modulation of the limbic–cortical function leading to mood and cognitive impairments [46,47]. A breakthrough in the understanding of this heterogeneous disease and its treatment calls for a clear comprehension of the factors and mechanisms that could lead to limbic–cortical functional dysregulation. The discovery of novel antidepressants does not represent an easy task, however, primarily because of our poor understanding of the pathophysiology of this illness. Nevertheless, alternative but more speculative mechanisms may prove to be more successful in the long run.

II. STRESS AND BRAIN (DYS)FUNCTIONS

Depression is a complex disease in which several different etiological causes, including environmental risk factors such as stressful life events (SLEs), and genetic risk factors (vulnerability or predisposition), interact in various, complex manners [48]. Clinical studies have confirmed the importance of SLEs in the development of mood disorders: a strong and significant correlation has repeatedly been demonstrated between SLEs and the precipitation of depression, especially in women [49–51]. In addition, Kendler and colleagues have calculated that the association between SLEs and major depression that is causal is approximately 75% [51]. Although it is difficult to attribute a numerical value to the effect that SLEs play in the occurrence of depression, it appears that environmental factors may override genetic influences and induce the onset of depression in subjects independent of their genetic background, that is, with low or without vulnerability and predisposition [49,51].

The brain appears to respond to stress in a complex but orchestrated manner. Most

likely, it is the loss of the organized response that plays a crucial role in the development of psychiatric disorders. Preclinical studies suggest that stress can promote long-term changes in multiple neurotransmitter systems and brain structures implicated in the etiology of depression [52–54]. It has also been hypothesized that neurobiological changes associated with SLEs may confer vulnerability for the development of affective and other psychiatric disorders. A leading hypothesis to explain the influence of SLEs in the development of depression is that psychological stressors play a greater role in the initial stage than in the subsequent episode of depressive disorders: the strength of the relationship between SLEs and major depression progressively declines with an increasing number of previous depressive episodes (this hypothesis is also known as *kindling hypothesis*) [55–57]. SLEs have been found to be strongly associated with subsequent episodes of depression and their effect appears to be greater than other etiological factors including genetic vulnerabilities. Furthermore, the depressogenic effects of SLEs seem to be concentrated in the period immediately after event occurrence. However, although environmental risk factors play a major role in the occurrence of major depression, several other investigations have found that genetic influences cannot be forgotten. Two possible models might explain the relationship between these two main risk factors: the *additive* and the *genetic control of sensitivity to the environment model*. In the *additive model*, the increase in risk associated with the exposure to SLEs is similar for individuals with low-risk and high-risk genotypes; this model predicts that the impact of SLEs and genetic factors on liability to major depression are independent. In the *genetic control of sensitivity to the environment model*, the increase in risk of depression associated with the exposure to SLEs is greater for those with a high genetic risk than for those with a low genetic risk; genes have an impact on the risk of depression in part by altering the individual's sensitivity to the depression-inducing effect of SLEs [49]. Clinical data appear to be consistent with the *genetic control of sensitivity to the environment model* [50].

Another important question related to the relationship between SLEs and depression is the impact of multiple SLEs [58]. Also in this case, three plausible hypotheses can be articulated to explain that relationship. The simplest or *additive model* proposes that the impact of SLEs is independent of the occurrence of other events. Multiple SLEs might be positively interactive if the depressogenic effect of a SLE increases when it co-occurs with other SLEs. A *positively interactive model* suggests a reservoir of "coping ability" that might withstand the impact of one event but could be overwhelmed by multiple events. A *negatively interactive model* suggests a threshold for stress that, if exceeded, has no additional impact on depressive risk. Harris and Brown concluded that their work, which focuses on severe SLEs, tends to best support the *negatively interactive model*. Given one severe SLE, they found little or no increased risk for depression given additional severe SLEs [59]. Kendler and associates, who examined multiple SLEs occurring together in the same month, support the *positively interactive model*: the impact of increasing numbers of SLEs on risk for a depressive onset is significantly more than predicted under an additive model [58].

Although the association between stressful events, brain abnormalities, and the occurrence of depression appears to be consistent, much less is known about the mechanisms by which stress can induce changes in the CNS and lead to limbic–cortical defects. Stress, especially when prolonged, appears to affect brain activity and structure, and induce alterations at cellular and molecular levels. Changes at the molecular level include stress-induced modifications of protein expression and activity leading to alteration of the expres-

sion of specific genes, while changes at the cellular level include stress-induced dendritic remodeling and/or atrophy, reduction of neurogenesis, and neuronal death.

A. Stress-Induced Modification of Protein Expression

Advances in molecular biology have enhanced our insights in the mechanisms that are responsible for the deleterious effects of stress on cellular activity and the relationships between intracellular defects and the development of depression. In the last few years, several studies have begun to characterize the effect of stress and antidepressant treatments beyond the neurotransmitter and receptor level. This work has demonstrated that there are many intracellular pathways that could mediate the action of antidepressant treatments [18,60]. One such pathway, which appears to be a common target for medication that stimulates serotonergic and noradrenergic systems, is the cAMP signal transduction cascade [61,62]. Long-term use of antidepressants results in a sustained activation of the cAMP system in specific brain regions, including increased function and expression of the transcription factor cAMP/calcium response element-binding protein (CREB) in the hippocampus and neocortex [63,64]. Moreover, the time course for CREB induction is consistent with that for the therapeutic actions of antidepressant treatments (10–21 days) [63,65]. Importantly, the cAMP system and CREB are involved in the modulation of the expression of selective genes that play an important role in neural plasticity and emotional modulation, including tyrosine hydroxylase (the rate-limiting enzyme in biosynthesis of catecholamine neurotransmitters), brain-derived neurotrophic factor (BDNF), and TrkB receptor genes [66–68]. An intriguing possibility is that stress could possibly precipitate depression mainly through dysregulation of BDNF synthesis and release. Several preclinical studies have reported that chronic stress can induce a strong decrease of BDNF expression in several brain regions including the hippocampus [69,70]. Stress-induced glucocorticoid expression may augment this effect [71]. In our laboratory, we have investigated the effect of prolonged stress on phospho-CREB activity. Interestingly, 3 weeks of unpredictable and uncontrollable stress significantly downregulated phospho-CREB in several limbic and cortical regions of the rat brain. This effect was particularly significant in the frontal cortex (prefrontal cortex and anterior cingulate) and in the thalamus (paraventricular thalamic nucleus), two regions characterized by a dense serotonergic innervation (Fig. 1a,b). Thus, our data appear to substantiate previous observations about the effect of sustained stress on BDNF expression and may provide information about the way in which chronic stress may influence the activity of the serotonergic system and induce selective dysfunctions in limbic–cortical regions involved in the modulation of emotional responses and in the pathogenesis of depression, through its effects on phospho-CREB and/or BDNF expression.

B. Stress-Induced Modification of Brain Structure and Function

The adult brain appears to possess a high degree of plasticity and flexibility. Remodeling of synaptic contacts, growth, and branching of dendrites are only a few examples of such plasticity necessary for adaptation to the continuously changing environment. The dynamic process is based on the capability of neuronal systems, brain nuclei, single neurons, synapses, and receptors to adapt to alterations in the internal and/or external environment by modifying specific structure and functions [72]. In the past few years, neurogenesis has been reported in the adult brain of rats, tree shrews, macaques, and humans demonstrat-

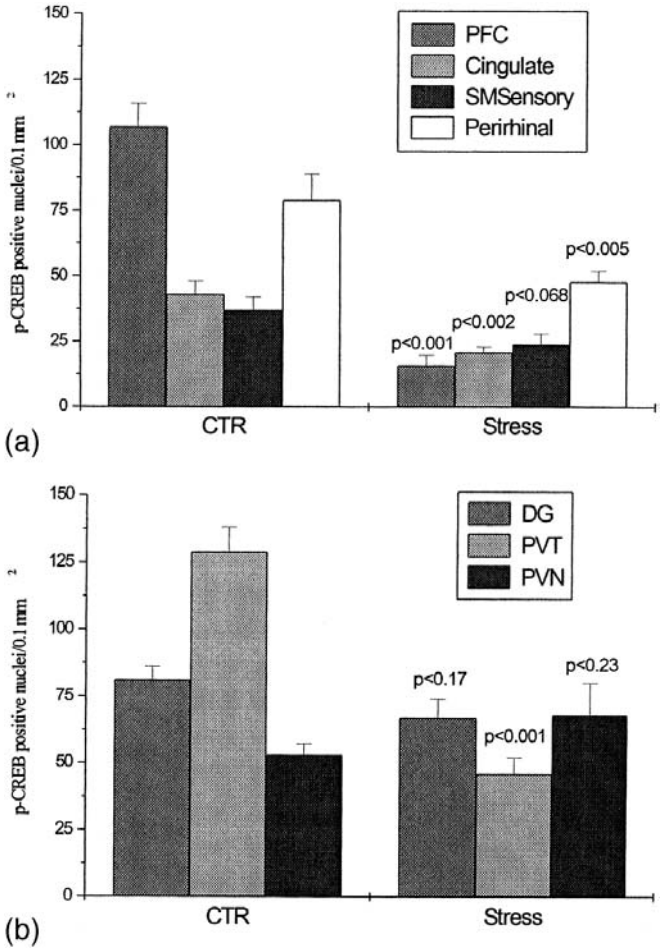

Figure 1 Phospho-CREB expression in cortical (a) and subcortical regions (b) following chronic stressful stimulation in male rats. Phospho-CREB expression was quantified in several cortical (PFC, cingulate, somatosensory and perirhinal cortex) and subcortical regions (dentate gyrus, paraventricular thalamus and paraventricular hypothalamus) in rats killed 2 h following the last stimulation. The greater decrease of phospho-CREB expression was detected in the PFC and in the thalamus ($p < 1e\text{-}7$ and $p < 1e\text{-}5$).

ing that adult-generated neurons are a feature common to the mammalian brain [73,74]. The newly generated neurons protrude axons and appear to establish functional connections with surrounding neurons, indicating that they are capable of integration into existing neuronal circuitry in the hippocampus and cerebral cortex. These brain regions have been extensively studied for several reasons, including involvement in stress-induced neural defects and the etiology of depression. The hippocampus is an especially plastic and vulnerable area that controls the HPA activity and, in turn, is affected by stress hormones [52]. It also responds to gonadal, thyroid, and adrenal hormones, which modulate changes in synapse formation and dendritic structure and regulate dentate gyrus volume during development and in adult life [75,76].

The structural plasticity of the brain is affected by stress [77,78]. Repeated stress causes atrophy and, in severe cases, death of hippocampal neurons. Moreover, chronic stress strongly reduces dendrite growth and branching [53]. Both acute and chronic stress has been shown to suppress neurogenesis, especially in the adult brain [73]. These forms of hippocampal structural plasticity are relevant for normal functioning of the hippocampus and subsequent mental health as a number of psychiatric disorders are characterized by selective hippocampal defects. Atrophy of the hippocampus has also been observed in patients suffering from recurrent major depression [79–81]. Thus, although mood disorders have traditionally been conceptualized as neurochemical disorders, there is now considerable literature demonstrating that these psychiatric disorders are also associated with significant reductions in regional central nervous system (CNS) volume and cell numbers. The structural changes observed in depression, however, do not appear to be limited to the hippocampus. Several recent postmortem studies of the prefrontal cortex have also demonstrated a reduction in volume and cell number in depressed patients [82–84]. In addition, unexpected reductions in the glial cell number and density were also observed [84–86]. Furthermore, in the prefrontal cortex (PFC), a histological study of area sg24 located in the subgenual PFC found striking reductions in glial cell number in patients with familial major depression (24% reduction) and manic depressive illness (41% reduction), as compared with control subjects [46]. Together, these findings provide convincing evidence that decreased regional CNS volume, caused by reduction in cell numbers and neurogenesis, and structural changes may be involved in the development of depression, at least in a subset of depressed patients.

Depression, therefore, seems to be a multifactorial disorder, for which the risk of development is influenced by several factors, including genetic as well as environmental elements. In the absence of assessed SLEs, the risk of the onset of depression is influenced by genetic factors, but even in the absence of high genetic risk, SLEs continue to be significant predictors of the onset of major depression. Clinical evidence suggests that the role of environmental factors may even overcome the effects of genetic factors in the onset of depression. Nevertheless, even though it is clear that several factors are involved in the precipitation of depression, less is known about the manner and mechanisms in which these factors, individually or combined, interact to induce changes in brain structure and function that eventually contribute to increased vulnerability for the development of affective disorders. Defining the pathway by which stressful stimuli interact with genetic predisposition in order to induce brain defects appears to be fundamental for both the prevention and the treatment of depression.

C. Regulation of Gene Expression: Coupling Extracellular Signals with Selective Gene Expression

Alterations of the internal or external environments cause rapid changes in gene expression in the brain. Extracellular stimuli that trigger long-term phenotypic changes in neurons, such as differentiation of a precursor cell or synaptic strengthening, elicit changes in gene expression. These stimuli cause changes in gene expression by activating intracellular pathways that propagate the initial signal from the plasma membrane to the nucleus. These pathways are composed of protein kinase cascades that culminate in the phosphorylation and activation of critical transcription factors, including CREB and FOS [87,88] (Fig. 2).

FOS was one of the first transcriptional factors to be identified. It is codified by the proto-oncogene c-fos and belongs to the family of immediate early genes (IEGs). IEGs

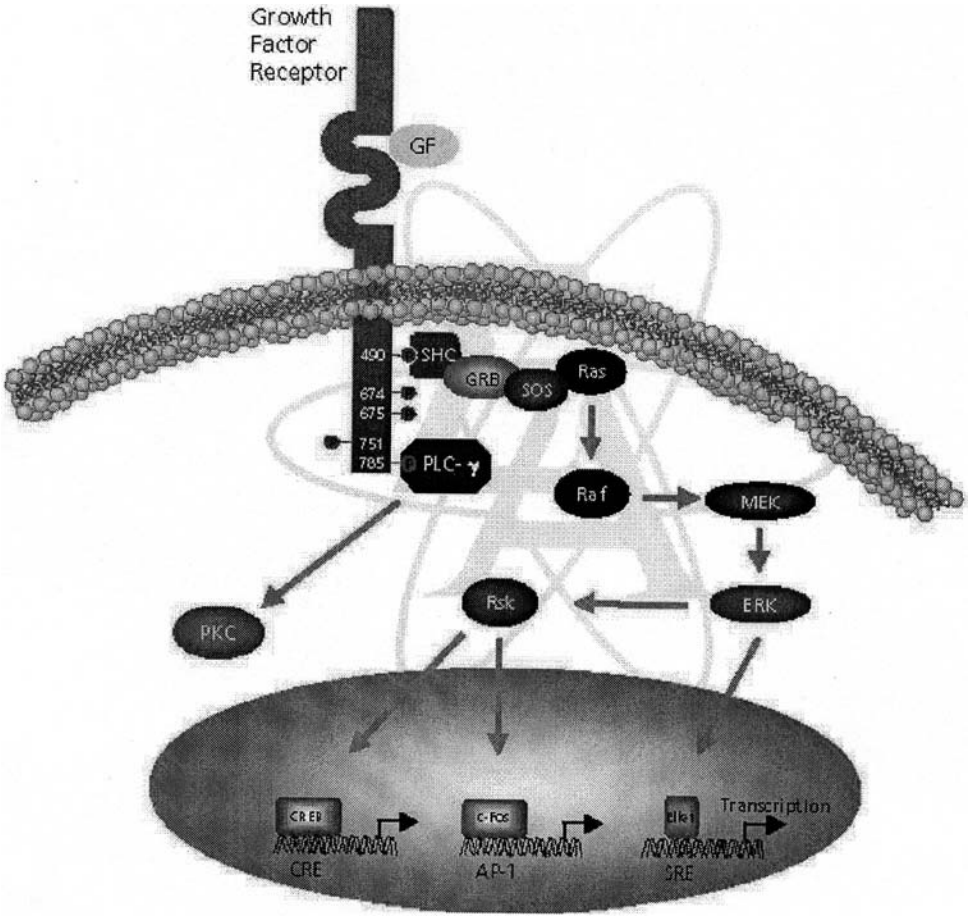

Figure 2 Transcription factor regulation (CREB and c-Fos). The transcription factor CREB binds to the cAMP response element (CRE) and activates gene transcription in response to a wide variety of extracellular signals (including growth factors, hormones, and neurotransmitters). Transcriptional activation of CREB is controlled through phosphorylation at Ser^{133} by ERK1 and ERK2. The transcriptional activity of the proto-oncogene c-Fos has been implicated in cell growth, differentiation, and development. Fos is induced by many stimuli, ranging from mitogens to pharmacological agents. c-Fos has been shown to be associated with another proto-oncogene, c-Jun, and together they bind to the AP-1 binding site to regulate gene transcription. (Reprinted with permission from Sigma-Aldrich Chimie B.V., The Netherlands.)

are transcribed in a very rapid phase immediately after individual cells perceive extracellular stimuli and play important roles in signal transduction and transcriptional regulation in normal cells [89]. Importantly, FOS has long been used as a marker of neuronal activation following various stimuli in living animals. In fact, when animals are subjected to stressors, the c-fos gene is immediately expressed in discrete brain areas, especially in the limbic and HPA axis systems. Although FOS is constitutively expressed in many brain areas, its levels rapidly and transiently rise in those regions activated by new stimuli,

peaking after 2 h before returning to the normal expression levels. This provides detailed maps of the activation patterns of selective neural circuits in response to specific extracellular stimuli [90,91]. IEGs code for transcription factors that bind to regulatory regions of target genes to regulate their transcription. FOS, in particular, forms heterodimers with members of the Jun family of IEGs and its activity is dependent on new protein synthesis. This FOS-Jun complex is referred to as AP-1 and binds to AP-1 binding sites of target genes modulating their expression [92].

CREB is perhaps the best characterized, stimulus-induced transcription factor. It was originally identified as a target of the cAMP signaling pathway, but recent studies have revealed that this transcription factor is also a target of other signaling cascades activated by a diverse array of stimuli. Contrary to FOS, CREB-mediated gene expression is not directly dependent on new protein synthesis but on its phosphorylation instead. In fact, only the phosphorylated CREB (phospho-CREB) is able to induce gene expression. Thus, all signaling pathways that activate CREB lead to the phosphorylation of a particular residue, the serine 133 (Ser133). CREB phosphorylation at residue 133 appears to have multiple roles. Since non-phosphorylated-CREB has also been found in the cytoplasm, phosphorylation at Ser133 might induce translocation of the cytoplasmatic CREB to the nucleus. In the nucleus, phosphorylation at Ser133 might affect the ability of CREB to dimerize and might promote its binding to the cAMP-responsive element (CRE). Finally, phosphorylation at Ser133 might lead to transcriptional activation by promoting interaction with components of the transcription machinery [93]. Phospho-CREB is involved in the regulation of the expression of several genes including somatostatin, tyrosine hydroxylase, and BDNF. Interestingly, CREB phosphorylation also appears to trigger the transcription of c-fos [94].

III. A MOLECULAR AND CELLULAR THEORY OF DEPRESSION: THE STRESS-BDNF HYPOTHESIS

A. The Neurotrophin Transduction Pathways

Neurotrophins mediate a broad range of neuronal functions, including synaptic plasticity, survival, differentiation, growth, and apoptosis. The biological activities of neurotrophins are mediated by two structurally distinct classes of cell surface receptors, the Trk tyrosine kinase receptors (TrkA, TrkB, and TrkC receptor types), and the p75 neurotrophin receptor (p75NTR) [95,96]. In particular, nerve growth factor (NGF) binds TrkA; BDNF and neurotrophin-4/5 bind to TrkB; neurotrophin-3 binds primarily TrkC: the p75NTR binds all neurotrophins at least with low affinity. While Trk receptors transmit positive signals such as enhanced survival, p75NTR transmits both positive and negative signals. The signals generated by these two neurotrophin receptors can either augment or oppose each other. Trk and p75NTR receptors thus exist in a paradoxical relationship, each acting to suppress or enhance the other's actions [97].

The activation of neurotrophin receptors by their extracellular ligands triggers a complex sequence of intracellular events that begin with receptor autophosphorylation, followed by the activation of several downstream phosphorylation cascades that finally lead to the stimulation and/or inhibition of the expression of selective genes (Fig. 3). The first intracellular neurotrophin-activated signaling protein shown to mediate survival in neurons was the small GTP-binding protein Ras. This protein is responsible for the 40 to 60% of neurotrophin-mediated neuronal/cellular survival even though it does not act di-

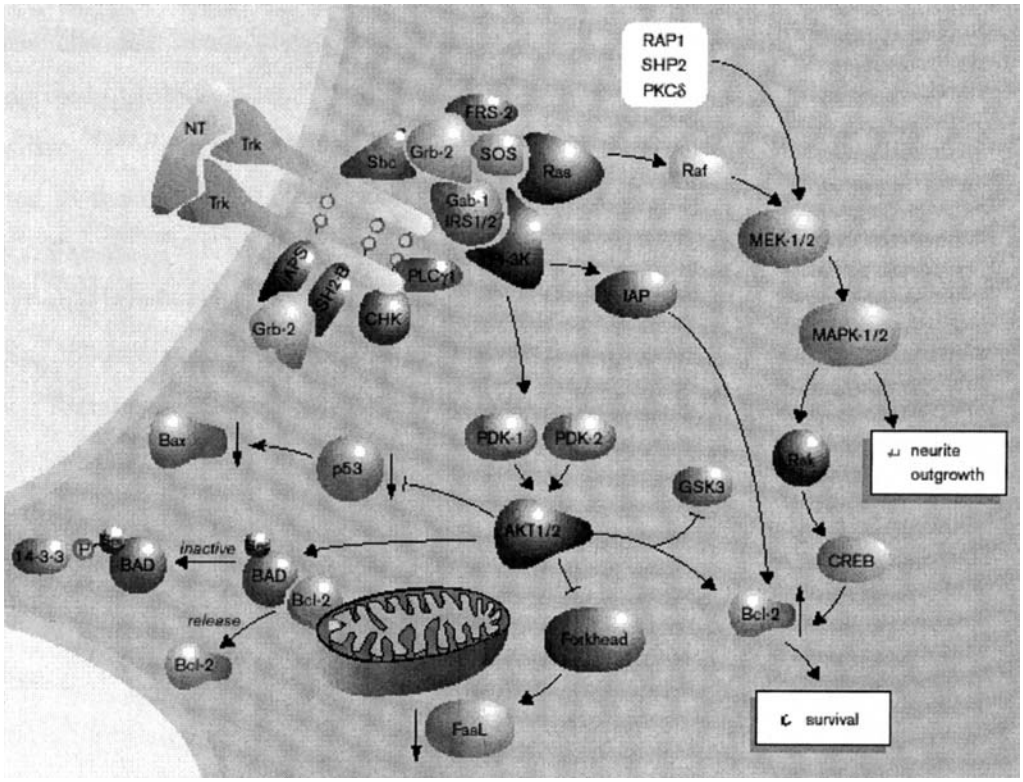

Figure 3 Trk signaling pathways regulating survival and neurite growth in neuronal cells. Neurotrophin binding to Trks stimulates receptor transphosphorylation, resulting in the recruitment of a series of signaling proteins to docking sites on the receptor. These proteins include Ras and Raf, which participate in activating MAPK. Trks also activate PI-3K through Ras. PI-3K activity stimulates the activities of Akt. The targets of PI-3K/Akt antiapoptotic activity, including BAD, GSK-3, Bcl-2, and the p53 cell death pathway. (Reprinted from Ref. xx.)

rectly to promote this. Rather, Ras functions by translating and directing neurotrophin-initiated signals into multiple signal transduction pathways. Recent data indicate that two of these signaling cascades, phosphatidylinositol-3-kinase/protein kinase B (PI-3K/Akt) and mitogen-activated extracellular kinase/mitogen-activated protein kinase (MEK1/ERKs), are the major effectors of neurotrophin-activated survival [97].

The PI-3 kinase enzymes are present in all cell types, and their activities have been shown to be necessary for many different regulatory cell pathways. The array of different functional roles attributed to these enzymes includes a role in mitogenesis as well as in terminally differentiated cells, suggesting that there are many complexities both in the regulation of the enzymes and in their downstream targets. In neurons, the Ras/PI-3K/Akt route is recognized as the main mediator of the neurotrophin-mediated protective effect. A pivotal role in this survival pathway is played by the protein kinase B (or Akt-1) [98,99]. Several studies have reported the involvement of this enzyme in neurotrophin-related protective activity. Its effects may involve a direct inhibition of the activity of p53, Bad (an inhibitor of the Bcl-2 anti-apoptotic protein), an indirect suppression of GSK-

3 apoptotic activity, or the blockade of the function of the primary neuronal apoptotic pathway [99–101]. Most likely, Akt-1 mediates cell survival at a number of levels, depending upon the cell type, target availability, and requirements for transcriptional or post-transcriptional events to suppress apoptosis.

The second main intracellular cascade involved in the transduction of neurotrophin signals is the MEK1/ERKs pathway [102,103]. Recent data suggest that this cascade might play a critical role in suppressing neuronal apoptosis triggered by cellular damage and its main function may be to protect neurons from death due to injury or toxicity [104]. MEK1/ERKs induce survival by stimulating the activity or expression of antiapoptotic proteins, including Bcl-2 and CREB. Nerve growth factor (NGF), through this cascade, potently increases Bcl-2 levels in sympathetic neurons, which in turn protect these and other neurons from apoptotic cell death [105]. CREB activity is also required for Bcl-2 expression and survival by NGF, suggesting a MEK1/ERKs-CREB-Bcl-2 survival pathway [106]. The activation of CREB by survival factors is likely to be due to phosphorylation at Ser133 by multiple kinases, including MAP kinases and Akt-1 [107] (Table 1).

Thus, neurotrophins regulate neuronal survival and apoptosis at several levels, which requires the orchestrated activity of different receptors, enzymes, and transcription factors. The functional cross-talk between all of these effectors appears to be crucial in determining the final result of their activity.

B. The Neurotrophin Hypothesis of Synaptic Plasticity

The neurotrophin (NT) family of molecules is a set of gene products that have multiple functions at different stages of development and at different locations in the CNS. They play a fundamental role in neuronal differentiation and survival, axon pathfinding, and synaptic plasticity [108,109]. Repetitive neuronal activity enhances the expression, secretion, and/or actions of the synapse to modify synaptic transmission and connectivity, thereby providing a connection between neuronal activity and synaptic plasticity. The expression of these proteins is linked to activity-dependent plasticity: neurotrophin genes are regulated by neuronal activity. BDNF is one of the members of the NT family. Its expression and secretion is upregulated by electrical activity, but other NTs may remain in the cytoplasm under resting conditions to be released in response to depolarization [110]. BDNF has been shown to increase the length and complexity of the dendritic trees in cortical neurons, morphological changes requiring neuronal activity. Neuronal activity appears to enhance BDNF-induced potentiation of synaptic activity. It seems that presynaptic depolarization elevates the activity of the cAMP system, which in turn facilitates the transduction of BDNF signaling. Both BDNF and synaptic plasticity have been reported to activate the calcium-calmodulin-dependent pathway that leads to CREB phosphorylation [111].

C. Neurotrophin Signaling Pathway Members, Survival, and Antidepressants

Additional evidence about the role of the neurotrophin signaling pathway in the etiology and treatment of depression is provided by the large number of studies reporting alterations in the expression of one or more of the members of this cascade as a consequence of antidepressant therapies. The neurotrophin intracellular cascade appears, in fact, to be a common target of antidepressants independent of their pharmacological profile.

Table 1 Enzymes and Protein Involved in the Transduction of Neurotrophic Signals and in Their Cellular Actions

Protein/enzyme	Function	Refs.
Ras	MAPK kinase kinase kinase	221
MEK1	MAPK kinase 1: it is responsible for the dual phosphorylation that activates ERK1 and 2	221
ERK1 and 2	MAP kinases: they are involved in the transduction of neurotrophin signals and play important roles in the adult brain modulating synaptic plasticity and neuronal survival. They are activated by MEK1: phospho-ERKs can phosphorylate a broad variety of proteins and transcription factors including microtubule-associated proteins, CREB and FOS.	221
PI-3-Ks	The PI 3-kinase activities have been shown to be necessary for many different cell regulatory pathways. Their functions are crucial during mitogenesis but also in terminally differentiated cells. In neurons, they have a fundamental role in the neurotrophin-mediated survival.	98,222
protein kinase B (Akt-1)	Serine/threonine kinase downstream from PI-3K. This enzyme plays a pivotal role in the modulation of survival in neurons regulating the activity and expression of several pro- and anti-apoptotic proteins, including bcl-2, p-53 and GSK-3-β.	98,99,222
P53	p53-protein functions include maintenance of DNA stability, control of mitochondria integrity and regulation of apoptosis. p53-mediated apoptosis may involve multiple mechanisms, including the interference with the growth factor–mediated survival signals. The inhibition of p53 functions and expression appears to prevent apoptosis.	223
Bcl-2	bcl-2 proteins are involved in the regulation of cell death. Many of these proteins show widespread expression and are also expressed in the nervous system in developing and adult organisms. A physiological role for bcl-2 in neuron survival has been shown: these proteins have been shown to protect neurons from a wide array of toxic insults.	113
GSK-3	Glycogen synthase kinase-3-β (GSK-3-β) is a serine/threonine protein kinase ubiquitously expressed and involved in several neuronal functions including cytoskeleton stability and apoptosis. GSK-3-β has been shown to be the target of several intracellular cascades. PKA and Akt have been reported to phosphorylate GSK-3-β inhibiting its proapoptotic activity.	224,225

1. Mood-Stabilizing Drug: Lithium

For almost half a century, lithium has been the most widely used treatment for bipolar depressive disorder, but the mechanisms by which this cation exerts its long-term beneficial effects are not yet clear. Lithium has a variety of benefits in the treatment of mood disorders, including acute antimanic and antidepressant effects, antidepressant-potentiating effects, and long-term prophylactic effects. It is unlikely that any single biochemical action can mediate all of lithium's clinical effects [31]. It has recently been

demonstrated that lithium-mediated stabilizing effects on bipolar depression might be mediated by key enzymes and transcription factors of the neurotrophin intracellular signaling cascade, including GSK-3-β, ERKs and bcl-2 [112]. The latter is an inhibitor of both apoptotic and necrotic cell death and an enhancer of neurite outgrowth, axonal growth rate, and regeneration in the mammalian CNS [113]. Several cellular mechanisms are involved in the protective effects of bcl-2, including inhibition of caspase activation and enhancement of antioxidant effects, mitochondrial calcium reuptake, and release of calcium and cytochrome C from mitochondria [114–116]. Lithium appears to robustly increase the level of bcl-2 both *in vitro* and *in vivo* and recent studies have shown that both phosphatidylinositol-3-kinase and MAPK pathways are involved in this action. MAPK cascade activation, in particular, does so through CREB involvement. In this manner, lithium might enhance hippocampal neurogenesis and exert the neuroprotective effects reported to be fundamental for antidepressant activity [117]. Other targets of lithium are GSK-3-β and p53/capsase cascades. Lithium, at therapeutic concentrations, is a potent inhibitor of the GSK-3-β [118] enzyme involved in the maintenance of cytoskeletal integrity via tau and synapsin I, as well as phosphorylation of c-jun and β-catenin that mediate long-term effects via alteration of gene transcription [119,120]. In addition, lithium has been shown to increase basal transcription factor binding to AP-1 and CRE sites [121]. These lithium-induced changes in transcription factor binding activity may be an effect of the inhibition of GSK-3-β, which, in turn, phosphorylates transcription factors such as c-Jun, inhibiting its DNA binding capacity [122]. Moreover, lithium affects the expression of p53, another protein involved in the regulation of apoptosis [123]. It is well established that p53 positively regulates Bax but negatively regulates bcl-2 expression [124,125]. Since accumulation of p53 in the nucleus is a critical intermediate step for many signaling processes that culminate in cell death, attenuation of p53 activation by lithium may be a mechanism by which this cation supports neuronal survival. To date, lithium is the only medication that produces robust increases in the level of bcl-2 in the frontal cortex and hippocampus *in vivo*. Evidence of lithium's neuroprotective effects, as well as the growing appreciation that mood disorders are associated with cell loss and/or atrophy, suggests that these effects on neuroplasticity may be critical aspects for long-term treatment of mood disorders [126].

2. Serotonin and/or Norepinephrine Reuptake Inhibitors

cAMP-mediated regulation of gene transcription has been implicated in the activity of antidepressant drugs acting on serotonin and/or norepinephrine neurotransmitter systems in which CREB appears to be the main effector in the cascade. Chronic antidepressant administration increases CRE-mediated gene expression and CREB phosphorylation in a region- and drug-specific manner [63,65]. The most consistent effects were observed in the amygdala, hippocampus, and cerebral cortex [64]. More importantly, induction of CRE-mediated gene expression and CREB phosphorylation were not observed in response to acute antidepressant treatment, which is consistent with the time course of the therapeutic action of these drugs [63]. Thus, antidepressant induction of CREB phosphorylation has been found in brain regions that are thought to play a role in the regulation of emotion and responses to stress. Previous studies have demonstrated that the amygdala mediates some of the behavioral actions of antidepressants and that neurochemical adaptations to antidepressants are observed in this limbic area [64]. The amygdala plays a significant role in fear conditioning and conditioned avoidance behavior [127,128]. The possibility that CREB influences the function of this limbic area is supported by recent observations

that illustrate that overexpression of CREB in the amygdala alters long-term memory of fearful conditions [129,130]. It is also possible that neurochemical alterations in the amygdala could contribute to the displaced emotion often observed in depressed patients. Chronic antidepressant treatment also results in neurochemical and cellular adaptations in the cerebral cortex and hippocampus [63,131]. Importantly, CREB's involvement in the mode of action of antidepressant drugs is supported by the fact that chronic antidepressant treatment increases the expression and function of CREB in these aforementioned brain regions. In addition, it has been found that SSRIs and NRIs increase the expression of BDNF in the hippocampus [131]. The possibility that BDNF induction involves CREB is supported by recent reports that show that the promoter of the BDNF gene contains a CRE. Thus, upregulation of CREB and BDNF, induced by antidepressant treatment, could act to oppose the damaging effects of stress on hippocampal neurons or facilitate the recovery from previous insults. A role for CREB in the pathophysiology of depression and in the antidepressants' mechanism of action is supported by a recent study demonstrating that CREB levels are decreased in the cerebral cortex of depressed patients and increased in patients receiving antidepressant medication prior to death [18].

3. Serotonin Reuptake Enhancer: Tianeptine

Tianeptine is a novel antidepressant agent, both structurally (modified tricyclic) and in terms of its pharmacodynamic profile. Unlike other antidepressant agents, tianeptine stimulates the uptake of serotonin [132]. In patients with major depression without melancholia or psychotic features or with depressed bipolar disorder or dysthymic disorder, the antidepressant efficacy of short-term (4 weeks to 3 months) tianeptine therapy appears to be similar to that of amitriptyline, imipramine, and fluoxetine [28,133]. The hippocampus, most likely, is an important target for tianeptine's therapeutic action. The human hippocampus undergoes atrophy in the aftermath of traumatic stress, recurrent depression, and Cushing's syndrome [79,134–136]. Prolonged psychological stress in monkeys is associated with loss of hippocampal neurons [137], whereas repeated psychological stress in primitive primates and in rats causes hippocampal CA3 pyramidal neurons to undergo dendritic atrophy [138,139]. The atrophy is only seen in the apical dendritic tree and comprises a reduction in length and branching and must involve alterations in the apical dendritic cytoskeleton [140]. Three factors play a role in hippocampal damage. These include glucocorticoid hormones, which potentiate damage produced by other insults, endogenous excitatory amino acids, and serotonin [141,142]. Serotonin is released by stressors and plays a role in the actions of stress on nerve cells. It appears that stressors activate the release of excitatory amino acids from mossy fiber synapses and promote serotonin release and adrenal steroid secretion that concur to enhance the effect of stressful events in the hippocampus. Interestingly, tianeptine treatment prevents stress-induced atrophy of CA3 pyramidal neurons, whereas neither fluoxetine nor desipramine has such effects. Tianeptine treatment also prevents the stress-induced learning impairment. The molecular mechanisms by which tianeptine prevents stress-mediated dendritic atrophy is not yet fully understood, however [29].

4. Electroconvulsive Therapy

Although electroconvulsive therapy (ECT) has been used for the treatment of depression for several decades, the neurobiological effect of this antidepressant treatment has not been determined. The majority of studies investigating the effects of ECT have focused on the monoamine neurotransmitter systems. However, alterations of the synaptic levels

of serotonin and norepinephrine, or adaptations of the receptors for these neurotransmitters, cannot account for the delayed action of antidepressants [66]. These results have generated a novel hypothesis stating that the pathophysiology and treatment of depression and the mechanisms of ECT action may result from alterations in the expression and function of neurotrophin factors [19]. The expression of BDNF and TrkB are affected by short- and long-term electroconvulsive shock (ECS) treatment as ECS treatment results in a rapid and dramatic induction of BDNF mRNA expression in cortical and limbic brain regions. This increase in BDNF mRNA is most pronounced in the hippocampal dentate gyrus and cerebral cortex, areas frequently implicated in the etiology of depression. The expression of TrkB mRNA is also increased in the same brain regions in response to a single ECS treatment [131,143]. Long-term ECS treatment results in a larger and longer-lasting (>18 h) induction of BDNF mRNA [131]. The induction of BDNF in response to ECS treatment most likely involves the activation of multiple neurotransmitter systems and intracellular signal transduction pathways. A prominent role appears to be played by the cAMP/calcium system and by the transcription factor CREB [19]. CREB-mediated induction of BDNF expression is supported by the finding that ECS, as well as antidepressant drugs, increase CREB function and expression. Moreover, the time course of expression and cellular distribution of CREB is similar for antidepressant and ECS-induced expression of BDNF and TrkB. Furthermore, upregulation of BDNF expression suggests an involvement of synaptic plasticity and alterations of neuronal structure in the mode of action of long-term ECS treatment [131]. These structural alterations could occur at several levels, including changes in length and complexity of the presynaptic processes and subtle alterations of the synapse architecture. Repeated, in contrast to single, ECS treatment significantly increases hippocampal granule cell sprouting. This effect is long lasting and can be observed up to 6 months following the last ECS treatment. Sprouting of granule cells could result in an altered functioning of these neurons leading to an enhanced responsiveness to stimuli. It is also possible that the neuronal sprouting and enhanced responsiveness represent adaptations to reverse the atrophy of hippocampal neurons or to protect these neurons from further stress-induced damage [19].

IV. STRESS-INDUCED BRAIN OVERLOAD

Preclinical studies suggest that stress can promote long-term changes in multiple neurotransmitter systems and brain structures implicated in the etiology of depression. It has also been well established that both anxiety disorders and depression are diseases that are characterized by a higher prevalence in women than in men. In our laboratory, we have investigated the response of several limbic regions to chronic stress in male and female rats using the IEG c-fos protein as a marker of neuronal activation (Fig. 4). Prolonged, but not acute, stress induced pronounced sex-related differences in the pattern of activation in several "higher" limbic regions, including the frontal cortex (Fig. 4a), the amygdala (Fig. 4b), the hippocampus (Fig. 4c), and the thalamus. In particular, chronically stressed females showed a decreased limbic activation while an increased regional activity was found in male rats. The pattern of limbic activity found in chronically conditioned females may demonstrate a different female susceptibility to the effects of stress as well as a transition from a physiological adaptation to an emotional circuitry malfunction. Important indications for understanding the differential response to prolonged stress came from the observation of a significant difference in limbic activity in basal conditions. Nonstressed females showed an overall higher limbic FOS expression and, consequently, a higher

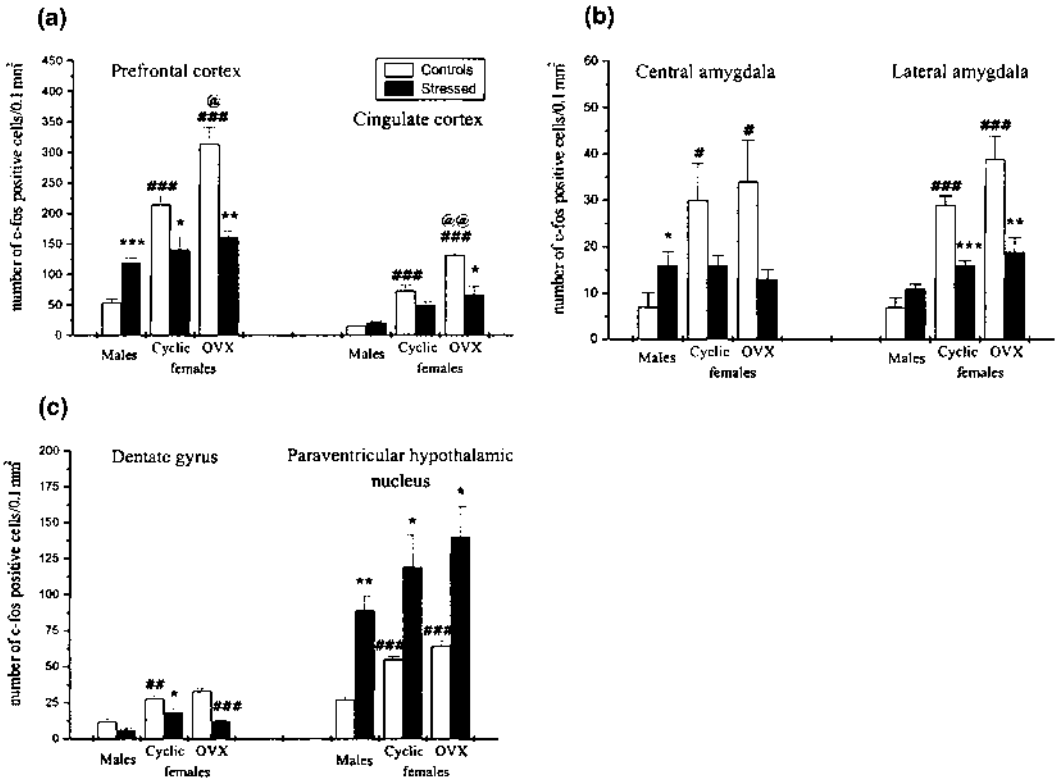

Figure 4 Effect of a chronic stressful challenge on FOS density in the (a) frontal cortex, (b) amygdala, (c) hippocampus and hypothalamus. The symbol * expresses the comparison of FOS density between control and stressed rats (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$), killed 2 h following the last stimulation. The symbol # expresses the comparison of the basal c-fos expression between control male and control female rats, both cyclic and ovariectomized (# = $p < 0.05$; ## = $p < 0.01$; ### = $p < 0.001$). The symbol @ expresses the comparison of the basal FOS expression between control cyclic and ovariectomized female rat (@ = $p < 0.05$; @@ = $p < 0.01$).

limbic activity than males (5–7 times higher), especially in the frontal regions. Interestingly, these differences are not limited to experimental animals, since several clinical studies also reported gender-related diversities in brain activity, with women exhibiting higher values than men [144–146]. Functional brain imaging techniques have illustrated sex-related differences in global as well as in the regional brain activity and reports of differential activation in the frontal lobes have been particularly prevalent [147]. Esposito and coworkers [148] reported substantial gender-related differences in the frontal lobe rCBF during performance of a variety of cognitive tasks as women demonstrated a significantly higher activation [148]. This higher level of neural activity in female rats may reflect larger cognitive abilities in basal conditions but reduced abilities following a chronic stressful challenge, as demonstrated by the decreased limbic activation and behavioral changes. No gender-related differences were found after acute conditioning, however. Of interest is the fact that these sex-related differences were particularly evident in the

frontal cortex (PFC and AC). Therefore, our results appear to be in line with those of Esposito, who reported a higher level of regional activity especially in frontal areas and during activation paradigms in women [148]. It has been well established that gender determines cognitive and learning abilities [149–151]. Therefore, these results appear to be in accordance with our data, as nonstressed male rats showed a lower FOS density than females but an increased FOS expression after chronic stress. Conversely, the basal neural activity in female rats was higher in nonstressed animals and decreased after chronic stress conditioning. This differential FOS response together with the abnormal behavioral response could be an illustration of distinctive susceptibilities between sexes for enduring effects of stress on brain activity [152]. Male rats appear better able to cope with stressful situations, whereas females suffer more from the effects of stress especially when it becomes prolonged. Thus, the decreased FOS expression after chronic stress training in female rats could represent the histological evidence of selective impairment of cognitive functions caused by reduction of limbic regional activity, particularly evident in the frontal cortical regions. Hypothetically, the brain may support only a limited level of activity. Women do show a higher vulnerability to mood disorders and this could be related to their higher basal limbic activity. This "overload threshold" hypothesis is supported by George and coworkers [146], who investigated neural activation during the induction of sadness. They found that men and women deemed themselves equally sad during the experiment, while the left prefrontal cortex of both sexes was equally activated. Nevertheless, women showed an eight times greater blood flow in the anterior limbic regions than men. George speculated that hyperactivity of the anterior limbic system in women experiencing sadness could, in time, exhaust that particular region and lead to the hypoactivity seen there during clinical depression [146]. Stress-induced limbic hyperactivity, subsequent overload, and a prolonged hypoactivity may not be enough to induce brain dysfunctions. As stated before, the brain responds to stress in a complex yet orchestrated manner that requires a high grade of plasticity and consequently a large availability of BDNF. BDNF release, however, is activity-dependent. Stress initially induces an increase in limbic activity: during this phase there is a massive release of BDNF that allows the brain to respond to stress with dynamic structural changes.

At this point there are two possibilities.

1. Prolonged and persistent stress is a cause of neuronal hyperactivity associated with massive release, and eventually exhaustion of BDNF supplies. This leads to a stress-induced deficiency of BDNF and reduction of neuronal plasticity.
2. Sustained stress gradually increases the activity of specific limbic areas and leads to their hyperactivity (earlier in females because of the higher basal activity closer to the overload threshold). Prolonged neuronal hyperactivity causes the exhaustion of the brain region and, consequently, hypoactivity (overload). At this point, the brain region, because of the reduction of BDNF synthesis and release (BDNF release is reported to be activity-dependent), is unable to face the effects of stress with the plastic changes that it needs. Thus, reduction of BDNF availability might increase the vulnerability of the brain to subsequent insults and may be the prelude to selective limbic defects because of the brain's inability to deal with the effects of stress with an adequate structural plasticity.

This second possibility is supported by our results regarding the effects of chronic stress on phospho-ERK1/2 (Fig. 5a,b) and phospho-CREB expression in male rats (Fig. 1a,b). After 3 weeks of exposure to a chronic unpredictable and uncontrollable stressor,

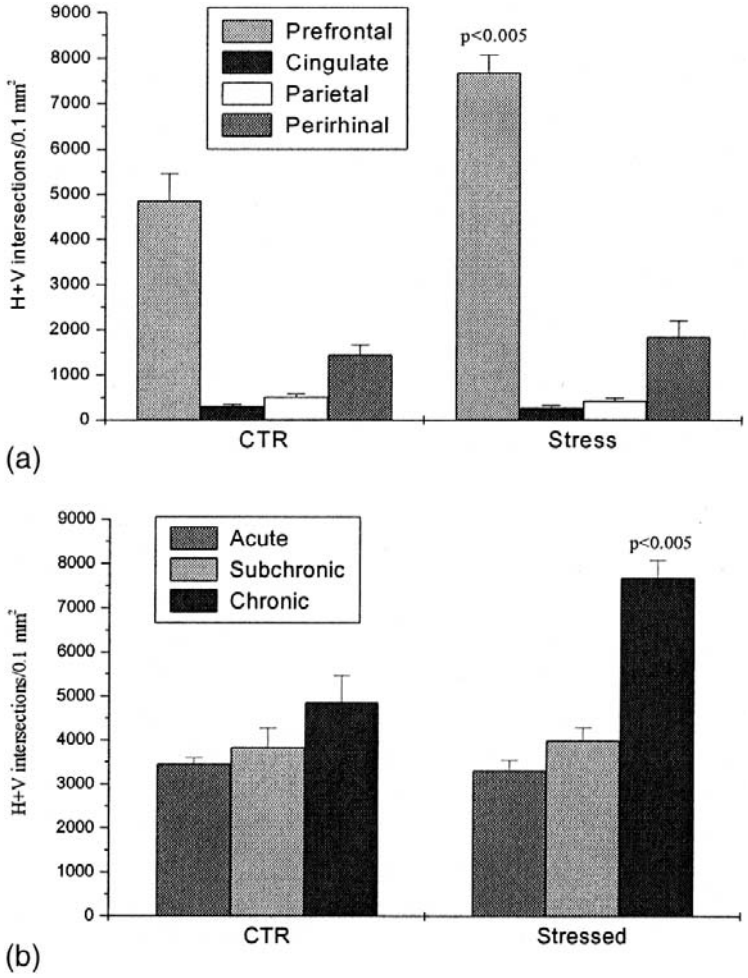

Figure 5 Phospho-ERK1/2-positive cortical dendrites after chronic stress in male rats (a). Phospho-ERK1/2-positive dendrites were counted in four cortical regions including PFC, cingulate, somatosensory, and perirhinal cortex. The number of p-ERK1/2-stained dendrites was quantified as the number of horizontal and vertical intersections between positive dendrites and the imaginary detection grid present in the quantification field, using a computerized image analysis system (Leica). The resulting data were reported as the number of positive H + V intersections/0.1mm^2. Phospho-ERK1/2 accumulation was selectively restricted to the PFC ($p < 0.005$). No other cortical regions showed an increase of activated MAPKs. Phospho-ERK1/2-positive prefrontocortical dendrites of male rats exposed to acute (3 days), subchronic (10 days), and chronic stressful conditioning (21 days) and killed 2 h following the last stimulation (b). A significant "accumulation" of phospho-ERK1/2 in the PFC was found only after chronic ($p < 0.005$) but not after acute or subchronic stress.

rats showed an accumulation of phospho-ERK1/2 in the dendrites of the higher layers (II and III) of the PFC (prelimbic and infralimbic regions) (Fig. 5a,b). This accumulation was significant only after chronic ($p < 0.005$) not acute (3 days) or subchronic (10 days) conditioning (Fig. 5b). Chronically stressed rats also demonstrated a global reduction of cortical phospho-CREB expression that was particularly significant in the PFC ($p \ll 0.001$) (Fig. 1a). CREB is an important nuclear transcription factor of the BDNF/MAPK signaling cascade and ERKs play a fundamental role in its phosphorylation. In turn, phospho-CREB is involved in the regulation of the BDNF gene expression. Thus, this uncoordinated response (increased presence of phospho-ERKs and decreased phospho-CREB expression) might be interpreted as a paradox.

The BDNF/MAPK cascade, in order to operate properly, requires a balanced activity between the members of the kinase and phosphatase systems and that, most likely, is assured by the availability of BDNF. MAPK phosphorylation states must be carefully regulated, as a perturbed ERK signaling has been related to neuronal dysfunctions and death [153]. ERK cascade, as seen above, plays a pivotal role in the transduction of neurotrophin signals but recent studies have also identified it as an important pathway involved in the regulation of cytoskeletal organization in neurons [154,155]. ERKs, through the phosphorylation of several key components of the cytoskeletal apparatus including neurofilaments and microtubule-associated proteins, control several important neuronal functions such as axonal transport, microtubule stability, complexity of the dendritic tree, number of synaptic contacts, and neurotransmitter release [156]. A balanced activity of this intracellular pathway appears to be crucial for proper neuronal activity and maintenance of cell structure. Stress influences neuronal activity; acute stress increases neuronal activation and enhances learning and memory, possibly to the stimulation of BDNF release and synaptic plasticity. Indirect evidence of this action is represented by the improvement of learning shown by animals facing acute stress situations [157]. The increased release of BDNF in response to acute stress might be related to the enhancement of neuronal activity. The synthesis and release of these proteins is activity-dependent and acute stress appears to enhance neural activity [111]. On the other hand, persistent stress shows deleterious effects on learning and memory, probably because of its negative effects on brain plasticity [53,138,158,159]. BDNF mediates synaptic plasticity and chronic stress has been reported to reduce its expression and, consequently, neural plasticity [69,160]. Therefore, it is possible that during acute and subchronic stress conditioning the brain is still able to cope with stress because of the availability of neurotrophins that assure sufficient plasticity. However, when stress becomes chronic, the reduction of BDNF availability and the consequent loss of neural plasticity reduce the ability of the brain to face the deleterious effects of stress.

During the acute stress phase, increased neuronal activity and BDNF release lead to the persistent activation of the MAPK/ERK cascade. BDNF not only increases the activity of the kinase system, but also modulates the phosphatase system since only a balanced activity of both systems guarantees the proper transduction of neurotrophin signals. As a consequence of persistent stress conditions and exhaustion of BDNF supplies, the release of neurotrophin ceases. The subsequent BDNF deficiency might particularly influence the activity and synthesis of new phosphatases and this defect in the phosphatase system leads to a permanent activation of ERKs [154,161]. ERK hyperactivity and accumulation may involve selective and vulnerable neuronal subpopulations. In turn, this situation might have nuclear and cytoplasmic consequences including defects in the phosphorylation of transcription factors and alteration of cytoskeletal stability (dendritic atrophy and

altered neurotransmitter release). Chronically stressed rats showed an abnormal reduction of cortical phospho-CREB expression indicating that, even if overexpressed, ERKs are not able to phosphorylate CREB. This consequence is particularly important because CREB is involved in the regulation of the expression of several genes, including the one coding for BDNF. Thus, the defect in CREB phosphorylation might lead to the reduced synthesis of this neurotrophin. In addition, several studies have reported that the reduction of phosphatase activity due to an unbalanced kinase/phosphatase system leads to dysregulation of the pattern of synapsin and synaptophysin expression [155,162]. A consequence of such dysregulation is a decrease in the complexity and extent of neurite arborization, loss of synapses, and neurotransmitter release [162]. Both nuclear and cytoplasmic abnormalities could act together and lead to a reduction in BDNF release (because of decreased synthesis, through a CREB-mediated mechanism, and decreased release, through a synapsin-mediated mechanism). It is less important which mechanism plays the main role in the chronic stress-mediated dysfunction: the final consequence remains the same, that is a reduction of synaptic and brain plasticity that reduces the ability of the brain to deal with the effects of stress with plastic changes as it requires. In turn, this inability could represent the prelude to the development of limbic defects.

V. FUTURE OPPORTUNITIES IN THE TREATMENT OF DEPRESSION

Whether or not chronic stress, through its action on neurotrophin expression, is one of the main pathological factors involved in the etiology of depression, this hypothesis provides new targets to improve the efficacy and/or reduce the delay and side effects typical of antidepressants today. These alternative approaches are summarized below, followed by an in-depth discussion of each perspective.

1. Removal or reduction of stress. Reduction of stress refers not only to the reduction of external stressors (psychological or physical stressors), but also to the physiological and/or pathological changes induced by their acute or prolonged exposition (such as increased blood pressure and cortisol levels, changes in releasing factors and/or releasing hormones). The normalization of the physiological parameters and restoration of body homeostasis might enhance the efficacy of antidepressant treatments.
2. BDNF replacement therapy.
3. Direct stimulation or inhibition of selective kinases or phosphatases involved in the neurotrophin transduction system (fluoxetine stimulates the expression of a gene that codifies for a MAPK-phosphatase).
4. Estrogen replacement therapy. Prevention of stress-induced limbic overload.

A. Removal or Reduction of the Stressor or Its Effects on Body Homeostasis

Stress plays a crucial role in the precipitation of depression but it might also be involved in determining the therapeutic effects of antidepressants by reducing or delaying their activity. The persistence of stressors may thwart, reduce, or slow down the onset of the antidepressant action. Therefore, an important measure to enhance the efficacy of antidepressant therapies may consist of removing the psychological or physical stressors and restoring the internal homeostasis. The alteration of several physiological parameters is reported in response to persistent stress and depression, as follows.

1. Cardiovascular Abnormalities

Stress and depression have been reported to induce important cardiovascular events that may reduce the therapeutic effects of antidepressant therapies. Several studies have investigated the effects of acute and chronic stress on cardiovascular function, finding a strong causal relation between stress and atherosclerosis, elevation of arterial blood pressure and heart rate, neurohormonal arousal, coagulation abnormalities, and impairment of coronary endothelial integrity. Interestingly, mental stress induces coronary vasoconstriction that leads to a reduction of blood flow and, consequently, oxygen supply [163]. Thus, a normalization of cardiovascular parameters might be necessary before beginning antidepressant therapy in order to assure improved therapeutic efficacy.

2. Neuroendocrine Abnormalities

Hypothalamic–Pituitary–Thyroid (HPT) Axis. Several abnormalities in the HPT axis have been reported in depressed patients [164]. Changes in thyroid status are involved in many cases of clinical depression. Virtually 100% of patients with severe hypothyroidism also suffer from depression [165]. A hallmark of depression is the finding of elevated serum T_4 or free T_4 without changes in T_3. Reductions in T_4 levels accompany a beneficial response to antidepressant medication. Moreover, thyroid status strongly influences both noradrenergic and serotonergic neurotransmission [166]. The changes seen in the HPT axis might explain or might be explained by a central monoamine deficiency. The normalization of the HPT axis in conjunction with the antidepressant therapy may improve its efficacy and/or reduce the subsequent relapse of depression.

Hypothalamic–Pituitary–Adrenal Axis. Hypercortisolemia, resulting from an increased release of glucocorticoid, is perhaps the main and most investigated effect of stress, represented by the increased release of glucocorticoids. The secretion of corticosteroid hormones (predominantly cortisol in humans) is the most important component of the response to stress. A common feature observed in depression is the increased secretion of this hormone throughout the day. This change is seen in approximately 50% of patients with major depression [167–169]. Glucocorticoids can have a broad range of effects in the brain, particularly in the hippocampus [78]. A permanent hypercortisolemia is thought to trigger neuronal and dendritic atrophy, neuronal death, and increase the vulnerability of this limbic region to subsequent insults [170]. High glucocorticoid levels might also reduce the effects of antidepressant treatment. In fact, it appears that glucocorticoids suppress BDNF expression at the mRNA and protein level. Activated mineralocorticoid and glucocorticoid receptors seem, in fact, to repress transcriptional activity of the BDNF promoter site specifically via interaction with other transcription factors [171]. As mentioned earlier, neurogenesis continues postnatally and into adulthood in the brains of many animals, including humans. This is particularly prominent in the dentate gyrus of the hippocampal formation. One factor that potently suppresses adult neurogenesis is stress, probably through the increased glucocorticoid levels [74]. Thus, high plasma levels of glucocorticoids through their effect on neurogenesis and/or BDNF synthesis may not only be an important factor in precipitating episodes of depression but also may be a crucial factor involved in determining the success of antidepressant treatment. Hypercortisolemia may thwart, reduce, or slow down the onset of the antidepressant action. Several options to reduce stress-induced hypercortisolemia are now available, including selective corticocotropin-releasing hormone (CRH) receptor 1 antagonists (R121919) [172], cortisol biosynthesis inhibitors (ketoconazole) [173,174], and selective glucocorticoid receptor antago-

nists (spironolactone) [175,176]. Clinical studies have demonstrated that these treatments effectively reduce plasma cortisol levels and improve several signs of anxiety and depression.

Restoration of internal body homeostasis by suppressing the physiological/pathological alterations induced by stress may denote the first fundamental step toward a more effective treatment of depression and a reduction in the number of treatment-resistant patients. In fact, disrupted body homeostasis may constitute the major destabilizing factor leading to an alteration of brain homeostasis and subsequently to a limbic defect.

B. BDNF Therapy

Neurotrophins play a crucial role in the maintenance, survival, and selective vulnerability of various neuronal populations within the normal and diseased brain. Recent evidence suggests that neurotrophic factors may protect mature neurons from neuronal atrophy in the degenerating human brain. Furthermore, it has been proposed that the pathogenesis of several human disorders, including Alzheimer's and Huntington's disease, as well as depression, may be due to an alteration in neurotrophic factors and/or Trk receptor levels. Thus, the use of neurotrophic factors as therapeutic agents designates a novel approach aimed at restoring and maintaining neuronal function in the central nervous system.

A promising treatment to remedy these psychiatric and neurological diseases is offered by BDNF replacement therapy. The potential of BDNF replacement therapy, however, is limited by the fact that it is impossible to administer the BDNF protein peripherally, because of the very high rate of removal of this neurotrophic factor from the circulation and the absence of transport through the brain capillary wall, which makes up the blood-brain barrier (BBB). These limitations have made invasive application strategies necessary. Several options to administer BDNF directly to the brain are now available, including intracerebroventricular administration [177], adenovirus-mediated transfer of the BDNF gene [178], and BDNF-secreting neural stem cell lines [179]. A promising and noninvasive alternative that allows the administration of BDNF peripherally consists of a molecular reformulation of BDNF that incorporates polyethylene glycol (PEG) to optimize plasma pharmacokinetics and links pegylated BDNF to the OX26 mAb, which undergoes receptor-mediated transport through the BBB *in vivo* [180]. In the absence of pegylation modification, BDNF is rapidly removed from the blood stream with a half-life of approximately 5 min. This chimeric peptide shows two very important advantages compared to native BDNF: a higher stability in the blood stream and the ability to cross the BBB and reach the brain compartment in a therapeutic concentration. Thus, this might provide a useful alternative for noninvasive neurotrophin delivery to the brain. Since more than 90% of the total body TrkB receptor is located in the central nervous system and less than 10% in peripheral tissue, peripheral administration of BDNF may be accompanied by a minimal number of side effects [180].

C. Direct Kinase Activity Targeting

BDNF therapy may represent a promising and efficient way to modulate brain activity, improve neuronal functions, and enhance plasticity and survival of neural cells through stimulation and/or inhibition of several enzymes of the neurotrophin signal transduction pathways. A possible alternative approach would be the selective modulation of the activity of individual proteins involved in the transduction of neurotrophin signals including ERK1 and 2, Akt-1, GSK-3-β, p53, and bcl-2.

Although numerous studies have associated ERK activation with neuronal plasticity and survival [102], ERK members do not appear to act universally to promote these fundamental functions. Recently, several reports have shown that prolonged ERK activation can contribute to neuronal death [153]. It is possible that the precise kinetics of ERK activation will ultimately dictate whether the activated kinases participate in a cell-death-promoting or cell-survival pathway. The selective "accumulation" of phospho-ERK1/2 that we found in prefrontocortical dendrites might illustrate a stress-induced defect in ERK signaling regulation in specific neuronal subpopulations. This increased number of cortical ERK-positive dendrites was not accompanied by a corresponding increase of ERK-positive cell nuclei (data not shown), however, supporting a persistent, and possibly uncontrolled, ERK phosphorylation. Several studies have reported a prolonged ERK cascade activation in cortical neurons after glutamate exposure that appeared to lead to excitotoxic degeneration or apoptosis [153]. A defect in the system involved in the modulation of ERK activity might lead to their excessive and persistent activation, which, in turn, leads to the hyperphosphorylation of cytoskeletal proteins, including microtubule-associated proteins and neurofilaments [156] and, consequently, to the weakening of the dendritic structure, especially in the synaptic terminals where these proteins are particularly abundant. If dysregulation of ERK activity represents a possible mechanism by which chronic stress induces prefrontal dysfunctions, the reduction of ERK hyperphosphorylation, through the activity of specific protein phosphatases involved in their dephosphorylation or through the administration of inhibitors of selective downstream kinases responsible for both phosphorylation and activation, might prevent the development of stress-related neuronal defects and/or facilitate the recovery. The inhibition of ERK phosphorylation, by using selective MEK1 and 2 (the kinases responsible for the activation of ERK1 and 2) inhibitors PD98059 and U0126, has been reported to reduce microtubule-associated protein hyperphosphorylation (and consequently disorganization of the cytoskeleton), neuronal degeneration, and apoptosis in neurons [153,181].

A second important neurotrophin signal transduction pathway that appears to play a crucial role in the modulation of neuronal function while providing important targets for an antidepressant therapy is the PI-3-K/Akt cascade. This cascade actually includes several members that play a crucial role in modulating neuronal functions involved in the therapeutic action of antidepressants, including Akt-1, GSK-3-β, and bcl-2. The importance of this cascade in neuronal survival has been confirmed by recent data regarding the effects of lithium on the brain. Chronic treatment with this bivalent cation has been reported to enhance neurogenesis and survival mainly through the activation of the serine/threonine Akt-1 [99,182], increased expression of the cytoprotective bcl-2 [112,117,123], and inhibition of the GSK-3-β [183]. Activation of Akt-1 seems to protect neurons against a wide range of insults including glutamate excitotoxicity, and its activity appears to be performed through different mechanisms. In fact, it has been reported that enhanced Akt-1 activation leads to the inactivation of several key proteins including BAD (a proapoptotic member of the bcl-2 family), p53, or GSK-3-β [99–101]. Akt-1, in fact, has been shown to phosphorylate and inactivate GSK-3-β and the inhibition of this serine/threonine kinase has been proposed as a mechanism by which neurons become resistant to apoptotic stimuli [98,184]. However, in spite of the large number of studies concerning the crucial role played by members of neurotrophin signaling transduction pathways in neuronal survival and their importance in the therapeutic effects of antidepressants, no drugs that selectively stimulate it have yet been developed. These drugs might offer a valid alternative to traditional antidepressants because of their selective intracellular targets, which allow therapeu-

tic action focused on specific brain regions, to reduce the latency period preceding the onset of therapeutic effects, and to limit the side effects associated with traditional antidepressant treatments. In addition, targeting selective intracellular proteins may also offer an effective prophylactic tool to prevent stress-induced brain overload or neuronal defects. In fact, whether prolonged stress can lead to neuronal dysfunction through the alteration of the activity of vulnerable enzymes, the administration of selective drugs able to modulate their activity and restore their original functions may reduce the depressogenic effect of SLEs and prevent the development of depression. These "second-messenger controllers" might be administered for a short interval immediately after serious SLEs and this may help subjects to overcome the series of events responsible for the deleterious consequences of chronic stress on brain function and prevent the precipitation of depression.

D. Estrogen and Neural Plasticity

For decades estrogen has been referred to only as a "sex" or "female" hormone. In recent years, however, estrogen has been shown to exert effects on the structure and function of the brain during development as well as in adulthood. Numerous studies from human and animal models suggest that estrogen has many positive effects on neural tissue, including stimulation of dendritogenesis, axon growth, and neurotransmitter synthesis [185–189]. It may also be beneficial in preserving cognitive function, preventing or retarding postmenopausal depression, and Alzheimer's disease and in facilitating recovery from brain injuries [190–192].

The classic estrogen receptors were considered ligand-induced transcription enhancers. This means that the regulation of gene expression by steroid hormones is mediated through receptor proteins that bind DNA sequence and then interact with the transcriptional machinery to initiate transcription [193]. To date, two estrogen receptor (ER) types have been characterized: ER-α and ER-β [194,195]. However, the classic mechanism of estrogen action, which is mediated by intracellular ER-α and ER-β, inadequately explains the extensive range of estrogen's actions. Recent studies suggest the presence of a new type of estrogen receptor that appears to be associated with the cell membrane [196,197]. Increasing evidence documents that this putative membrane-associated estrogen receptor can mediate extracellular signals in both an estrogen-dependent and -independent manner, through growth factor signaling pathways [198]. Neurons in the forebrain regions of both sexes coexpress estrogen and neurotrophin receptors and are also the sites of estrogen and neurotrophin synthesis [199,200]. Thus, estrogen and neurotrophins may influence each other's action by regulating receptor and/or ligand availability through reciprocal regulation at gene transcription level. Another possible consequence of this coexpression is the sharing of similar, if not overlapping, sequences of intracellular biochemical events through convergence or cross-coupling of their signaling pathways [201]. Cross-coupling of converging estrogen and neurotrophin signaling pathways may lead to similar nuclear endpoints, which result in the regulation of the same broad array of genes involved in dendritogenesis and neurite growth, including β-tubulin, MAP-2, tau-microtubule-associated protein, and GAP-43 [202–205]. A strong candidate for the transduction of estrogen signals is the MEK-ERK pathway, also involved in the transduction of neurotrophin signals. Estrogen has been shown to induce ERK activation in various cell models. In explants of the cerebral cortex, estrogen elicited a rapid and sustained activation of ERK1 and ERK2, an effect that requires MEK activation [206]. However, whether the

ERK pathway is the major intracellular cascade involved in the transduction of estrogen signals remains unclear. Other signaling substrates that could act either in parallel or by converging with the MAPK cascade might be activated by estrogen as well, including the cAMP, Ca^{2+} channels, and protein kinase A and C in neuronal cells [207–209]. In addition, immunohistochemical studies have shown that estrogen treatment in ovariectomized rats can induce a long-lasting increase of phospho-CREB; more importantly, this effect was post-transcriptional and did not arise from *de novo* RNA protein synthesis [210].

Thus, the effects of estrogen can no longer be restricted to those related to reproductive function. Cross-coupling of estrogen and neutrophin signaling pathways may be required for estrogen and the neurotrophins to elicit their neuroprotective effects, especially during development. Mediation of estrogen's actions through interaction with locally synthesized growth factors, their receptors, and their signaling cascades in the brain may represent the mechanism by which estrogen exerts its effects. Estrogen and neurotrophins, acting in concert, may not only have important developmental roles but may also decrease the vulnerability of target neurons to the consequences of neurodegenerative disease processes and enhance the compensatory response [211].

E. Estrogen-Replacement Therapy

Although depression is classified as an affective disorder, modulation of mood and emotion is one of the higher functions that is affected during the course of the disease. Another common alteration that can be observed in depressed patients is the impairment of cognitive functions, such as decreased concentration and memory loss. In addition, recent studies suggest that mild cognitive impairment might serve as an early marker of Alzheimer disease. Thus, the abnormalities observed in depression may predict the subsequent development of other serious illnesses.

The central nervous system is an important target for the steroid hormones. During the climacteric period the rapid decline of gonadal steroids causes neuroendocrine changes in different areas of the brain. The failure of gonadal hormone production causes specific symptoms due to the central nervous system derangement. Physical and psychological changes occur, the latter of which include anxiety, irritability, and depression [212]. Regional cerebral blood flow (rCBF) is diminished in hypoestrogen, with a pattern resembling that of depression and Alzheimer patients. Interestingly, menopausal women are often subjected to progressive cognitive decline while depression has been described as high as 35% during perimenopause [213]. In addition, some investigators have postulated that if decreasing estrogen levels have short-term effects on cognition during the perimenopausal period, then long-term estrogen deficiency may play a role in more significant cognitive impairments such as the development of dementia in some women. Therefore, it has been proposed that exogenous replacement of estrogen may prevent the cognitive decline in postmenopausal women [214–216], as well as the development of postmenopausal depression, while the onset of Alzheimer's disease may also be delayed [217,218].

Considerable evidence has been accumulated that suggests that estrogen modulates CNS function. These include increases in dendritic spine density and synaptic excitability, enhancement of cholinergic neurotransmission, and effects on other neurotransmitter systems including serotonin, norepinephrine, and dopamine [75,185–189]. In addition, estrogen also affects cerebrovasculature by increasing cardiac and cerebral blow flow [214,219]. Importantly, a decreased cerebral blood flow in specific limbic-cortical regions has been reported in depression and its normalization after antidepressant therapy is con-

sidered an important predictor for clinical recovery. Interestingly, brain perfusion abnormalities might not be responsible only for the emotional dysfunctions associated with depression but also for the cognitive impairment frequently observed to accompany depression. Estrogen-replacement therapy has been shown to reverse the detrimental blood flow changes following menopause and increase the relative rCBF in several limbic-cortical regions including hippocampus, parahippocampal gyrus, and middle temporal lobe [220]. There are also several reports of improved attention, reaction time, enhanced memory, and overall cognitive abilities associated with ERT in postmenopausal women [214–216]. The neurobiological mechanism through which ERT improves cognitive abilities is not completely understood. It may indirectly affect rCBF through its effects on the cerebrovasculature enhancing the perfusion of selective regions involved in attention and memory processes or directly by affecting intracellular pathways that modulate hippocampal function and in turn memory performance [220].

Although ERT alone cannot be considered as an antidepressant therapy, it might represent a useful augmentation treatment in combination with traditional antidepressant therapy. Estrogen is a potent trophic factor for the CNS and it may help the brain to oppose age-related plasticity reduction. Furthermore, ERT may help to prevent postmenopausal depression and age-related cognitive impairments, while also constituting an effective measure in the prevention of Alzheimer's disease in women.

VI. CONCLUSION

Depression appears to be a heterogeneous disorder in which several different etiological causes interact leading to an abnormal modulation of the limbic-cortical activity. A breakthrough in the understanding of this disease and its treatment calls for clear comprehension of the factors and mechanisms that could induce the functional dysregulation that characterizes it. Although the monoamine hypothesis of depression was introduced and accepted for more than 30 years as a valid explanation for the etiology of depression, intensive investigation has failed to find conclusive affirmation of a primary dysfunction of a specific monoamine system in depressed patients. A crucial role in the development of depression appears to be played by chronic stressful events. The brain responds to stress in a complex but orchestrated manner and it is most likely the loss of this organized response that plays a crucial role in the development of psychiatric disorders. Thus, the prevention of the deleterious effects of stress might represent a valid instrument to avoid the onset of the disease. Stress can promote long-term changes in the body and brain homeostasis that might constitute the major destabilizing factor that leads to an alteration of brain function and subsequently creates the basis for limbic-cortical defects and development of depression. The treatment of depression is mainly based on the use of serotonin and/or noradrenaline reuptake inhibitors. However, they offer a limited efficacy, delayed therapeutic action, and several side effects. New options in the treatment of depression are beginning to be available that may prove to be more successful in the long run, including BDNF replacement therapy to enhance brain plasticity and second-messenger controllers to prevent the detrimental effects of stress. Intracellular signal transduction cascades appear to offer a broad variety of interesting targets for future antidepressant therapy. The only doubts concern the selectivity of this intracellular approach. However, several clinical investigations have shown that intracellular alterations responsible for abnormal neural activity are limited to specific regions and not to the total brain. Thus, even the systemic administration of second-messenger controllers may modulate and target only the activity of those areas

showing a specific defect, making intracellular targeting a very effective and selective therapy. Another important advantage of intracellular targeting is the possibility of using second-messenger controllers as a prophylactic measure, which would not only help treat the disease but also prevent that sequence of intracellular events that leads to the development of depression. Finally, additional help for women comes from the neuroprotective effects of estrogen replacement therapy that may enhance the therapeutic action of antidepressants.

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Norepinephrine in Depression and Anxiety

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I. INTRODUCTION

Interest in the role of noradrenaline (NA) function in affective and anxiety disorders began a short time after it was discovered that antidepressant drugs potently interacted with NA neurons and receptors. In the shadow of the momentous discovery that basal ganglia dopamine (DA) dysfunction was associated with Parkinson's disease [1,2], early theorists hypothesized that similar relationships would be found for NA in depression and/or anxiety states [3,4]. It was proposed that diminished NA content [3,4] or function [5,6] caused depression and that increased NA content or function caused either mania [3] or severe anxiety or panic attacks [7].

In the 30 years that followed the development of these early hypotheses, researchers enthusiastically and systematically searched for the hypothesized deficiency or excess of NA or NA function in a variety of psychiatric patients. Initial studies of NA and its metabolites in blood, cerebrospinal fluid (CSF), and urine were followed by neuroendocrine challenge studies, autopsy studies, and studies of NA receptors on platelets and leukocytes [8]. However, in spite of the accumulation of a massive database on NA measures in humans, evidence of global NA dysfunction in patients with major depression, anxiety disorders, or mania has not been consistently identified.

The work of the past 30 years was not without benefit. Much has been learned about the NA system. Our understanding of the neuroanatomy, neurophysiology, neuropharmacology, and molecular biology of the NA system has grown tremendously. This knowledge

suggests a complex role for NA in a variety of cognitive and behavioral functions involved in mood and anxiety disorders, as well as several other major mental disorders, degenerative disorders, and normal states.

Much of the confusion about the role of NA function in mental disorders arises because of our relatively limited understanding of brain function and the relative ease with which large modulatory neurotransmitter systems like NA could be altered with relatively safe drugs. This led investigators to focus on these systems rather than attempting to understand the regulation and function of NA's target brain structures. For example, we have numerous drugs capable of increasing or decreasing brain NA, but few, if any, capable of selectively activating or deactivating specific target brain areas such as the prefrontal cortex, amygdala, or hippocampus. This has sometimes led to the erroneous conclusion that behaviors mediated by these terminal brain structures and nuclei are "NA behaviors."

This chapter will review the current state of knowledge about the function of the NA system and its possible role in mood and anxiety disorders and the therapeutic effects of certain medications.

II. THE NA SYSTEM

A. Anatomy

NA neurons are defined by the intracellular presence of NA and NA synthetic enzymes. They are localized in both the peripheral nervous system (PNS) as well as the central nervous system (CNS). In the PNS, NA is the major neurotransmitter in postganglionic neurons. Peripheral sympathetic neurons innervate the adrenal medulla, regulating the release of NA and epinephrine directly into the bloodstream as part of the "fight-or-flight" response to severe stress. NA neurons in the CNS form several distinct nuclei located primarily in the brainstem that project rostrally to almost all areas of the mid and forebrain, dorsoventrally to the cerebellum, and caudally to the lumbar segments of the spinal cord in a monosynaptic fashion (9). About half of all CNS NA cell bodies originate from the locus coeruleus (LC) in the dorsal pons [10,11]. It was first thought that LC axons branched to innervate all areas of cortex. Recent data suggest that LC neurons are not uniform and demonstrate a laminar distribution and at least four distinct cell types [12]. Additionally, there is evidence suggesting topographic innervation of target brain areas. The significance of this is not fully understood. The vast majority of rostrally projecting NA fibers do not cross over to the other hemisphere.

The axons of the LC form five major bundles. The dorsal bundle contains the majority of fibers projecting to the cortex [13]. Within the cortex, NA neurons primarily innervate layers I, IV, and V. While there is considerable heterogeneity, layer I tends to contain a dense plexus of fibers while layers IV and V have moderately dense, short oblique fibers, and layer VI tends to exhibit long fibers that are oriented anterior to posterior. Few NA fibers are found in white matter.

Not all NA-containing nerve terminals in the cortex make synaptic contact with the local cortical neurons; rather some of these neurons release NA in a manner similar to that through which hormones are secreted and thus have generalized effects on other CNS regions [14]. There is a natural loss of LC neurons with aging [15]; recent studies suggesting that this loss can range from 30 to 40% of cell bodies [12].

B. Biochemistry

NA was one of the first neurotransmitters to be discovered. A brief review of this history is of interest because it highlights how relatively young the field of neuroscience actually is. Prior to 1900, neurons were thought to communicate exclusively by passive current flow. In 1901, Langley noted that extracts from the adrenal gland had similar effects as stimulation of sympathetic nerves [16]. Elliot subsequently hypothesized that an adrenalin-like substance might be secreted by sympathetic nerve terminals and this substance chemically stimulated the effector organ [17]. The theory of chemical neurotransmission gained significant ground when Otto Loewi (1921) [18] showed that when the superfusion of one heart whose rate had been decreased by electrical stimulation was transferred to a second, unstimulated heart, its rate also decreased. Subsequently, Cannon and coworkers established the existence of an epinephrine-like substance ("sympathin") that they claimed was the chemical transmitter released by sympathetic nerves [19]. Nine years later, von Euler (1946) [20] established that the sympathetic neurotransmitter was demethylated epinephrine (norepinephrine).

The synthesis of NA and DA are dependent on availability of the precursor amino acid tyrosine. Tyrosine, one of eight large neutral amino acids (LNAA), is competitively transported across the blood-brain barrier from the serum to the cerebrospinal fluid by a LNAA carrier. In NA neurons, tyrosine is converted to L-dihydroxy-phenylalanine (L-DOPA) by the rate-limiting enzyme tyrosine hydroxylase. L-DOPA is then rapidly decarboxylated to DA and DA is metabolized to NA by the enzyme dopamine beta-hydroxylase.

Vesicular NA is transported to nerve terminals, where release into the synaptic cleft occurs during neuronal firing in a calcium-dependent process. Synaptic NA is rapidly taken back up into presynaptic terminals by the NA transporter (NAT) or into DA terminals by the DA transporter (DAT). Vesicular NA is then either transported back into vesicles or metabolized intraneuronally by monoamine oxidase A, producing 3,4-dihydroxyphenylglycol (DHPG) or 3,4-dihydroxymandelic acid (DHMA). These compounds are then metabolized further into the primary NA metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG) or 3-methoxy-4-hydroxy-mandelic acid (VMA).

C. Physiology and Pharmacology

NA neurons, particularly those making up the LC, are activated in a variety of stressful and aversive conditions and novel environments [21–23]. The activity of LC neurons is modulated by several different neurotransmitter systems. The LC receives inhibitory input from the serotonin (5HT), endogenous opioid, gama-amino-butyric-acid (GABA), DA, and glycine systems and excitatory input from the corticotropin releasing factor (CRF), methylxanthine, glutamate, substance P, and muscarinic cholinergic systems [11,24]. LC neurons may utilize electrotonic coupling, a process that could explain the coordinated firing characteristic of these cells. Another well-characterized regulatory mechanism is through an autoinhibitory presynaptic α_2-adrenoreceptor that decreases the firing rate of LC neurons.

NA mediates its effects on target neurons via receptors traditionally classified as being either of the α- or β-adrenergic subtype. Recent pharmacological, radioligand binding, and molecular biology data have led to a classification of adrenergic receptors into three groups based on the G-protein coupling. The three groups are the β-adrenergic sub-

type (Gs coupled; β_1, β_2), the α_2-adrenergic subtype (Gi coupled; α_{2a}, α_{2b}, α_{2c}), and the α_1-adrenergic subtype (Gx coupled; α_{1a}, α_{1b}) [25].

Studies of the NA system have implicated alterations in β-, α_1-, and α_2-adrenoreceptor binding after long-term antidepressant treatment. Long-term treatment with tricyclic antidepressants, monoamine oxidase inhibitors (MAOIs), trazodone, iprindole, and ECT (but not with some selective 5HT reuptake inhibitors, or bupropion, mianserin, and nomifensine) decrease β-adrenoreceptor binding in laboratory animals [26]. Increases in α_1-adrenoreceptor binding have also been reported for many antidepressants, and decreases in α_2-adrenoreceptor binding have been reported for some antidepressants. These findings have been much less consistent than for β-adrenoreceptors [5,27].

Electrophysiological studies in laboratory animals find that MAOIs produce an immediate decrease in the firing rate of the LC and this decrease persists with long-term treatment [28]. This suggests that the changes in LC firing rate are not sufficient for an antidepressant response to MAOIs. Sustained administration of citalopram or paroxetine gradually decreased (14–21 days) while sustained administration of desipramine rapidly (2 days) decreased LC firing [29]. Unlike the gradual return of firing rate of 5HT neurons during long-term antidepressant treatment, both 5HT and NA drugs caused a sustained reduction of LC firing, highlighting an important temporal and qualitative difference in how 5HT and NA neurons differ in their long-term response to antidepressant drugs [29].

D. NA and Neuroplasticity

NA and other monoamine neurotransmitters have both short- and long-term effects on postsynaptic neurons. The long-term effects have increasingly become the focus of attention. It is now well established that NA causes changes in the long-term expression of a variety of gene products [30]. Changes in synaptic levels of NA or 5HT have been hypothesized to result in adaptive changes in neurons that may underlie the behavioral effects of stress and the therapeutic effects of antidepressant drugs [31,32].

Studies have shown that a variety of antidepressants increase signal transduction through second-messenger systems. Long-term antidepressant treatment increased the coupling of the second-messenger adenylyl cyclase to the stimulatory G-protein Gs [33], increased levels of cAMP-dependent protein kinase in limbic brain regions [34,35], and increased the expression of the cAMP response element binding protein (CREB) [36]. While the precise mechanism by which NA increases signal transduction is not fully understood, one important result is to increase intracellular pathways leading to modification of the rate of gene expression, including the production of brain-derived neurotrophic factor (BDNF) [32]. Increased BDNF has been hypothesized to be a possible final common pathway for how NA- and 5HT-enhancing drugs mediate their therapeutic effects in depression [31]. The increase in BDNF and signal transduction mediated by NA could be explained by effects of NA on either β_1- or α_1-adrenergic receptors [32].

E. Function of LC

NA plays a critical role in modulation of the function of the prefrontal cortex (PFC). Both NA and DA systems densely innervate the PFC and these neurotransmitters have the capacity to modulate PFC function. The PFC is thought to be involved in the process of using working memory to regulate behavior and attention, allowing for inhibition of inappropriate responses [37]. For example, depletion of NA and DA markedly impair

working memory [38,39]. Lesions of the NA system also impair several other functions of the PFC, including maintenance of sustained [40] and shifting attention [41]. Deficits induced by depletion or lesions of the PFC can be reversed by administration of drugs that stimulate NA receptors, especially the α_2-adrenergic receptors on PFC neurons [42].

Physiologically the locus coeruleus is exquisitely sensitive to novel stimuli. It appears to modulate levels of arousal and has been postulated to be involved in opiate and alcohol withdrawal states, as well as anxiety disorders and depression [7,11]. Recent work suggests that the LC is primarily involved in the process of selective attention, especially in regard to novel stimuli [43,11]. NA decreases spontaneous neuronal activity while augmenting activity evoked by afferent stimulation, increasing the signal-to-noise ratio in target areas [43].

The NA system also has an effect on the acquisition of new memories [44]. Recall for details of a vignette that includes emotionally arousing material is significantly greater than for an emotionally neutral vignette [44]. This effect can be blocked by propranolol [45], a β-adrenergic antagonist that crosses the blood-brain barrier, but not by nadolol [46], a β-adrenergic antagonist that does not enter the CNS. These and other data have led to the hypothesis that NA modulates the amygdala in such a way as to strengthen memory consolidation and fear conditioning, playing an essential role in conveying emotional significance to prior experience [44,47].

III. ASSESSMENT OF THE NA SYSTEM IN HUMANS: ROLE IN DEPRESSION AND ANXIETY

Direct assessment of the role of NA in emotion, behavior, and mental illness has suffered because methods do not exist that allow direct measurement of CNS NA function in living humans. Many strategies have been used over the past 40 years to try to determine CNS NA function. These include measurement of NA and/or NA metabolites in urine, blood, and CSF, challenge studies, and neuroimaging studies. Several of these strategies have been suggested to be a reliable measure of central NA function; however, none has proven to be adequate.

While research into the role of NA in depression and anxiety dates back to the 1960s, several factors led to a relative reduction in interest in NA during the late 1980s and 1990s. These included the introduction of selective 5HT reuptake inhibitors (SSRIs); the failure to find evidence of a NA deficiency in depressed or anxious patients; and the disappointing results of studies of NA receptor agonists and antagonists as treatment for anxiety disorders and major depression.

The reawakening of interest in the role of NA in depression and antidepressant effects and the clinical development of selective NA reuptake inhibitors (NRI)s was fueled in part by results from neurotransmitter depletion studies [24,48–52]. These studies showed that the therapeutic effects of SSRIs could be transiently reversed by rapid depletion of 5HT but not by depletion of NA [24,50,53]. Conversely, the therapeutic effects of the NRI (desipramine) could be transiently reversed by depletion of NA but not by depletion of 5HT [50,53].

In spite of the growing confidence in the therapeutic effects of selective NRIs for treatment of depression and some anxiety disorders, the precise role of NA in the pathophysiology of depression and anxiety remains unclear. The following sections will review data from human studies aimed at addressing these questions.

A. Urine, Plasma, and CSF NA and NA Metabolites

As the metabolic pathways for NA were delineated, it was logical to measure urine, plasma or CSF levels of NA or its primary metabolite, MHPG [54]. Studies did show that there were moderate correlations between CSF, plasma, and urine measures of NA [55]. Additionally, manic patients and depressed patients with "high" anxiety had elevated CSF NA compared with "low" anxiety depressed patients and neurological controls [56]. However, there is more variability in NA than in MHPG in the CSF and the origin of NA is more likely to derive from spinal neurons than from cells originating in the locus coeruleus and other rostral projecting NA neurons [54,56]. Movement or exercise also affects NA and MHPG, making potentially small methodological differences very important when assessing these markers [57].

Concentrations of MHPG have been measured in the urine, plasma, and CSF of patients with depression, but no consensus has been reached as to whether there is a correlation between levels and depressive symptoms. For example, increased, decreased, and unchanged urinary concentrations of MHPG have been reported in patients with depression [58–62].

While it is clear that NA and MHPG in CSF, blood, and urine derive from NA neurons, it remains unclear to what extent and under which circumstances they may provide useful information about the status of brain NA systems of interest in regard to mood and anxiety disorders.

B. Platelets and Lymphocytes

Many studies have investigated NA receptors on platelets and lymphocytes, hoping that abnormalities inherent to the NA system might be similarly expressed in these peripheral systems. As in the CNS, presynaptic α_2-adrenoceptors on peripheral NA neurons also play an important physiological role in the regulation of NA release [63]. Some differences in NA measures between depressed patients and controls have been described. In addition, an increase in the affinity and density of α_2-adrenoceptors on isolated human platelets from depressed patients has also been reported [64].

Peripheral α_2-adrenergic receptors located on blood platelets have been extensively investigated with a variety of radiolabeled α_2-adrenergic receptor agonist and antagonist probes. Platelet α_2-adrenergic receptor number as measured by ^{3}H-clonidine [65], ^{3}H-dihydroergocryptine [66], ^{3}H-epinephrine, and ^{3}H-para-aminoclonidine [64] have been reported to be increased compared to healthy subjects. However, other studies utilizing ^{3}H-clonidine [67], ^{3}H-dihydroergocryptine [68], ^{3}H-yohimbine, and ^{3}H-rauwolscine [69] have revealed no significant difference between healthy subjects and depressed patients.

Studies of peripheral α_2-adrenergic receptor responsiveness have been used to assess the above conflicting results in receptor number. However, these studies have also been contradictory, with some studies noting no difference between patients and controls and other studies noting a blunted cyclic adenosine monophosphate (cAMP) response [69,70]. Antidepressant drug treatment and electroconvulsive therapy are associated with a decrease in the density of α_2-adrenoceptors and in the affinity of ligands at these receptors in platelets from depressed patients [71].

β-Adrenergic receptors are present on blood lymphocytes. Measurement of these receptors has also yielded mixed results with some authors reporting decreased numbers [72] and others reporting no difference [73,74] between depressed patients and healthy subjects. On the other hand, the cyclic adenosine monophosphate (cAMP) response to

stimulation of β-adrenergic receptors by β-adrenergic agonists has led to consistent reports of a blunted response in depressed patients when compared to healthy subjects [72,74, 75–77].

C. Postmortem Studies

A variety of studies have assessed the LC and markers for NA function in the postmortem brains of patients with depression or those having committed suicide. These studies have measured neuron density, NA receptor binding, as well as levels of NA synthetic enzymes. While allowing for direct measurement, autopsy studies are inherently limited because of the difficulty in obtaining reliable information relevant to diagnosis (including comorbid diagnoses), use of medications, levels of stress or distress prior to or during death, and use of alcohol or illicit drugs. In particular, many studies of suicide victims have included patients with a wide variety of known axis I diagnoses (including schizophrenia) as well as individuals with no known psychiatric diagnosis [78].

Some studies using postmortem tissue indicate that the density and affinity of the α_2-adrenoceptors (autoreceptors) are increased in the frontal cortex [79–81], and LC [82] of suicide victims. However, this finding has not been replicated in other studies [83]. A recent study assessing cerebral blood flow during performance of cognitive testing with [^{15}O]H_2O positron emission tomography (PET) before and after intravenous clonidine infusion in six depressed women compared with six matched controls [84]. While the study failed to identify differences in plasma growth hormone response to clonidine, the depressed patients showed an increase in blood flow in the right superior prefrontal cortex during clonidine, while the controls showed a decrease [84]. The authors interpreted this finding as consistent with the prior studies showing greater density and affinity of α_2-receptors in this region.

A variety of other measures also suggest differences in the LC of patients with depression or suicide. Direct assessment of the LC shows that some individuals with depression or suicide have decreased neuronal density compared with controls [12,85,86]. Tyrosine hydroxylase activity has been reported as increased [78], although another study reported a decrease in activity [87]. Reduced binding of radiolabeled nisoxetine, a ligand for the NA reuptake transporter, has been reported in the postmortem locus coeruleus tissue from suicide victims and patients with depression, compared with control subjects [88].

D. Challenge Studies

1. Neuroendocrine Challenges

As detailed knowledge of the regulation pituitary hormones by CNS monoamine neurotransmitter systems emerged, it was logical to attempt to gain an understanding of the function of CNS monoamine systems by measuring changes in plasma levels of pituitary hormones. Because measurement of static levels of most compounds is inherently associated with greater variability within as well as between subjects, neuroendocrine challenge strategies evolved as a method by which dynamic changes could be assessed.

While there was considerable optimism in the 1980s that the neuroendocrine challenge strategy might uncover the biological origin of mood and anxiety disorders, as the decade wore on, the optimism faded. In part, this was because of the lack of specificity that emerged as neuroendocrine tests were studied in increasingly broad patient groups.

Additionally, as the multitude of environmental, clinical, and demographic variables that could affect the results of these tests became known, the complexity of controlling for so many variables increased, requiring either prohibitively large samples or patient samples that had been so highly selected that the findings were of questionable generalizability.

Rather than attempt an exhaustive review of this literature, this chapter will highlight the strengths and weaknesses of this approach by focusing on one of the most rigorously studied neuroendocrine challenges in psychiatry: the growth hormone response to clonidine. Clonidine is an α_2-adrenergic receptor agonist that causes an increase in the secretion of growth hormone by the anterior pituitary as well as a decrease in the plasma MHPG, blood pressure, and sedation [89]. The release of growth hormone by α_2-adrenergic receptor agonists is mediated by the activation of postsynaptic α_2-adrenergic receptors within the hypothalamus exerting a stimulatory effect on the secretion of growth hormone releasing hormone (GHRH) [90]. However, regulation of growth hormone secretion includes many other neurotransmitters, including 5HT and DA. For example, depletion of both 5HT and NA in rats leads to a weaker growth hormone response to clonidine than does depletion of NA alone [91].

One of the most consistent findings from research investigating the growth hormone response to clonidine in humans is that this response is blunted in drug-free depressed patients [92–94]. Acute administration of desipramine also causes an increase in the release of growth hormone. Depressed patients also show a blunted growth hormone response to desipramine, supporting the hypothesis that aspects of NA function may be different in depressed individuals [95–97].

Importantly, a blunted growth hormone response to clonidine is not specific to depression, as it is also reported in patients with panic disorder [98], generalized anxiety disorder [99], social phobia [100], obsessive-compulsive disorder [101], alcohol dependence [102], Gilles de la Tourette syndrome [103], and schizoaffective disorder [104]. In alcoholic subjects with concurrent major depression, the blunted growth hormone response to clonidine was present during retesting, even though depression and anxiety symptoms had remitted [105]. By contrast, one study in 24 children (aged 7–14) with anxiety disorders and 15 age-matched control children did not find evidence of blunting, rather clonidine-stimulated growth hormone secretion was increased in the anxious children relative to the controls [106].

In a study of patients with OCD, the growth hormone response to GHRH was normal, while the response to clonidine was blunted compared with healthy controls [101]. This suggests that the blunted response is not simply because of a deficit at the pituitary level. There is a high correlation between the release of growth hormone by GHRH and that caused by clonidine [107].

A variety of factors have been shown to affect the growth hormone response to clonidine. Some antidepressants appear to cause a diminution of the response [108,109]. These effects have been observed to last as long as 6 months after the exposure to desipramine in healthy subjects [110]. Weight loss increases [111] the GH response to clonidine and menstruation and recent alcohol use reduce the response [112,109].

2. Neurotransmitter Depletion Challenges

As the synthetic pathways for monoamines were discovered, it became possible to specifically enhance or block a particular step. Experimentation with various substrates for the newly discovered synthetic enzymes revealed that chemical modification of some precur-

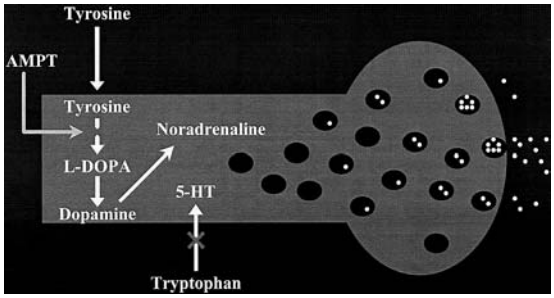

Figure 1 Neurotransmitter depletion.

sors caused them to act as reversible inhibitors of key rate-limiting enzymes. One example of this was alpha methyl para tyrosine (AMPT). AMPT reversibly inhibits tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of NA and DA synthesis (Fig. 1).

AMPT treatment in humans (600–4000 mg/day) decreases urinary excretion of catecholamine metabolites by up to 75% [113]. A 3g/day dose of AMPT reliably reduces urinary MHPG by 70% and reduces CSF levels of the DA metabolite, homovanillic acid (HVA), by 61% with no change in the 5HT metabolite, 5HIAA (114). Maximum reduction of catecholamine metabolites during AMPT treatment occurs within 2 to 3 days of initiation of treatment and returns to normal within 3 to 4 days after withdrawal of the drug [113,114].

Initial studies with AMPT were poorly controlled and difficult to interpret. Even after administration over several months to patients with general medical illnesses, AMPT did not seem to cause significant changes in mood [113]. Most nonpsychiatric patients and healthy subjects demonstrate little behavioral change other than sedation during AMPT treatment, with rebound insomnia frequently seen for 1 to 2 days after AMPT discontinuation [113–115]. AMPT was reported to decrease craving for opiates and amphetamines in a small case series [116], decrease tic movements in Tourette's syndrome [117], reduce oral tardive dyskinesia [118], and potentiate antipsychotic efficacy in schizophrenia [119]. In an open-treatment trial of AMPT in patients with essential hypertension, 6 of 20 hypertensive patients had a history of a previous depressive episode. Three of these six became agitated on AMPT, requiring drug discontinuation [113]. In a double-blind trial, AMPT reduced manic symptoms in five of seven bipolar patients in the manic phase, but two had an increase in manic symptoms [114,120]. In the same study, three of four psychotic depressed patients became more depressed during AMPT treatment [114,120]. In three depressed patients having had a therapeutic response to imipramine, AMPT had no significant effect on the antidepressant response [121]. In one of these patients, AMPT was started prior to initiation of antidepressant treatment and that patient went on to have a therapeutic response to imipramine [121].

The first carefully controlled behavioral studies with AMPT suggest that the most prominent effects are in NRI-treated depressed patients [49–51]. Patients who achieved and maintained a treatment response to desipramine were more likely to have a temporary return of depressive symptoms during exposure to AMPT 3 g/day than those successfully treated with fluoxetine or sertraline [49,50]. These findings are the mirror image of the results with the use of rapid plasma tryptophan (TRP) depletion, a method for reducing

brain 5HT synthesis [24]. In those studies, TRP depletion was more likely to cause a temporary return of depressive symptoms in depressed patients successfully treated with and taking fluoxetine than those treated with and taking desipramine [53].

Unexpectedly, unmedicated, currently depressed patients did not worsen during exposure to either AMPT 3 g/day [51] or TRP depletion [122]. Severity of depression did not alter the behavioral response to either type of depletion: patients did not become more depressed, whether mildly ill or severely ill. These results suggest that the pathophysiology of depression is unlikely to be understood by focusing on monoamine neurotransmission alone [123].

A recent meta-analysis of previously published data with TRP and catecholamine depletion revealed differences in the symptoms that emerge during each type of depletion [124]. Data from 123 depressed patients on and off antidepressants (44 catecholamine depletion and 79 TRP depletion) who participated in one of several prior studies [50–53,125] including a subset of 11 patients who had both TPR and catecholamine depletion [126], were pooled for analysis. Change from baseline during depletion was calculated for Hamilton Depression (Ham-D) scale scores. Both TRP and catecholamine depletion led to statistically significant increases in total HAM-D score, significantly increasing scores on the following items: depressed mood, loss of interest, retardation, agitation, psychic and somatic anxiety, loss of energy, loss of sexual interest, decreased appetite, decreased concentration, and helplessness, hopelessness, and worthlessness. The magnitude of increase in item score for "impaired concentration," "loss of interest," and "retardation" was significantly greater for catecholamine than TRP depletion. The magnitude of increase in somatic anxiety was greater for TRP than catecholamine depletion. Figure 2 graphically shows these changes. One can infer that the symptoms evoked by NA depletion were more similar than different to those evoked by 5HT depletion. As might have been expected, NA depletion impaired cognitive symptoms more than 5HT depletion, while 5HT depletion led to more somatic anxiety. The similarities and differences in symptoms suggest that NA and 5HT share considerable overlap in modulating brain areas mediating the symptoms of depression but have less of an overlap with some symptoms of anxiety

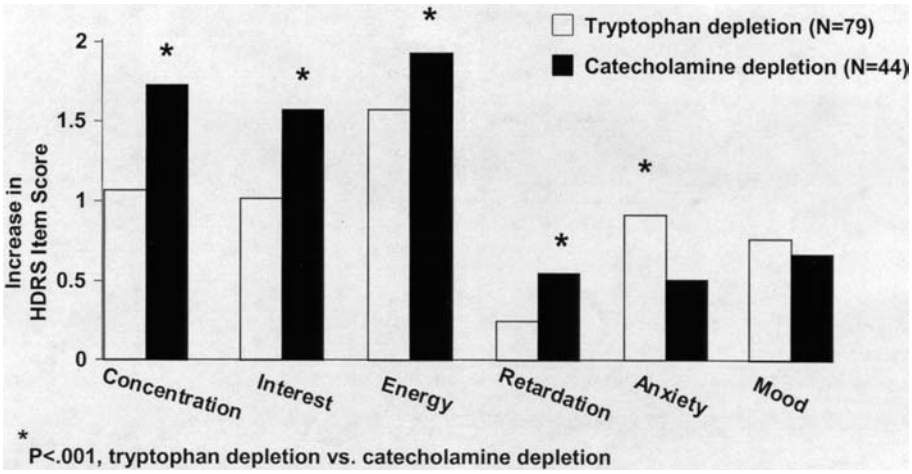

Figure 2 AMPT versus tryptophan depletion.

and cognition. This may have relevance to putative differences between 5HT-selective, NA-selective, and dual-action antidepressants.

Studies in healthy volunteers with no personal or family history of depression showed that depletion alone could not bring about depressive syndrome [127–129], although healthy women report more negative mood states and irritability during NA depletion [130]. However, in patients who had recovered from recurrent episodes of major depression and who were medication-free, it was observed that the majority of the patients experienced a transient return of depressive symptoms in response to TRP depletion [131,127]. Similar findings have recently been reported with catecholamine depletion [52].

E. Treatment Studies

Theories about the neurobiology of mood and anxiety disorders have been important to the field, but unless the predictions from these theories are borne out in treatment research, the theories must be questioned. Therefore, clinical efficacy trials serve both to advance treatment and to test biological theories. However, it is important also to keep in mind that evidence for efficacy does not necessarily inform about pathophysiology. For example, the fact that topical corticosteroids alleviate rashes does not show that rashes are due to a corticosteroid deficiency.

Since the early 1950s, when MAOIs and imipramine were coincidently discovered to have therapeutic effects in the treatment of depression [132], and imipramine was found to be effective for panic attacks in patients with agoraphobia [133], a steady stream of new medications for depression and anxiety became available for clinical use. The first of these drugs were simple modifications of the original tricyclic or MAOI compounds. However, as our understanding of the pharmacology of these compounds evolved, newer drugs were tailored to have specific neurochemical effects. The vast majority of newer compounds have been designed to potently enhance NA and/or 5HT neurotransmission without anticholinergic, antihistaminergic, or antiadrenergic properties. It was hoped that this would lead to drugs with fewer side effects and/or greater efficacy and faster onset of action.

The drugs we call antidepressants appear to have a broad efficacy profile, treating most mood and anxiety disorders. However, as NA and 5HT selective reuptake inhibitors have emerged, there is a growing body of literature suggesting that differences in the therapeutic profile exist. NA- and 5HT-selective drugs appear to have comparable efficacy for treatment of major depression, but 5HT-selective drugs and dual-action drugs may be more effective in a broader range of anxiety disorders than selective NA reuptake inhibitors. The full implications of this for the underlying biological differences between mood and anxiety disorders remain to be elucidated.

The following sections provide a selected review of data from clinical studies investigating the efficacy of several drugs with potent effects of NA and/or 5HT for treatment of major depression and several anxiety disorders. Figure 3 shows the relative potency at inhibition of NA and 5HT reuptake for common antidepressants.

1. NA Reuptake Inhibitors (NRIs)

Desipramine is one of the classical NRIs and there are extensive data available regarding its clinical efficacy profile. It is well established as effective in the acute treatment of major depressive episodes in 45 to 63% of outpatients and 48 to 63% in inpatients [134].

	NE (nM)	5HT (nM)	NE/5HT
Reboxetine	8	1070	0.007
Desipramine	9	76	0.118
Amitriptyline	14	9	1.6
Nefazodone	586	339	1.7
Duloxetine	7.5	0.8	9.4
Imipramine	97	5	19.4
Venlafaxine	1668	13	128
Fluoxetine	625	2	417
Paroxetine	72	0.1	1200
Citalopram	5453	1	4957
Sertraline	1207	0.22	5486

Figure 3 Comparative inhibition of reuptake.

Like other tricyclic antidepressants, it has a host of pharmacological properties that contribute to its side-effect burden. These include anticholinergic, antihistaminergic, and antiadrenergic effects [134]. Desipramine has been reported to be effective in the treatment of panic disorder [l35–138], although clomipramine was even more effective [138]. Desipramine is not efficacious in treatment of OCD [139–141], or post-traumatic stress disorder (PTSD) [142,143]. Bupropion is a very weak DA reuptake blockade inhibitor; its behavioral profile in laboratory animals and humans is that of a central nervous system (CNS) stimulant and indirect DA agonist [144]. It increases locomotor activity and causes stereotyped behaviors in laboratory animals. In humans, it can cause restlessness, insomnia, anorexia, and psychosis. Bupropion is structurally related to phenylethylamines and unrelated to the TCAs, SSRIs, or MAOIs. It has no significant potency at binding to any known neurotransmitter receptors. It has no significant effects at blocking reuptake of 5HT or NA, although its primary metabolite (hydroxybupropion) is a potent NA reuptake inhibitor [145]. Hydroxybupropion is produced rapidly in humans, with peak plasma levels of up to 3 times those of bupropion and a half-life of 24. Clinical studies have demonstrated that bupropion is effective in the treatment of major depressive episodes [134]. While early studies suggested that bupropion might be less likely to cause hypomania or mania in bipolar patients, subsequent studies suggested that it can cause mania and psychosis in bipolar patients, especially those with high pretreatment levels of the DA metabolite HVA [146,147]. In a recent open-label study, bupropion was not effective for treatment of PTSD [148]. However, contrary to commonly held clinical impressions, bupropion was reported to have therapeutic effects in a patient with social phobia [149]. Additionally, a recent review contrasting the relative efficacy of bupropion and sertraline in treatment of anxiety symptoms in patients with major depression showed that baseline Hamilton Anxiety Scale (Ham-A) scores did not predict response to either drug [150] and both drugs equally reduced Ham-A total score [151].

Reboxetine was approved for use as an antidepressant in much of western Europe, South America, and Mexico in 1998. Reboxetine is the only truly selective NRI. It potently inhibits the reuptake of NA without having significant effects on the reuptake of 5HT or DA. It does not inhibit MAO, nor does it bind to $5HT_{1A}$ or $5HT_{2A}$, DA_1 or DA_2, α- or β-adrenergic, muscarinic cholinergic, gamma aminobutyric acid (GABA), benzodiazepine, or histamine H_1-receptors. Reboxetine has primarily been studied in European studies

involving about 665 nonelderly and 56 elderly (age > 65 years) depressed patients [152]. It is administered twice per day at doses ranging from 4 to 12 mg/day. The European data show that reboxetine is more effective than placebo and comparable to imipramine, desipramine, and fluoxetine for the treatment of major depression. Reboxetine has also been found to be equally effective as imipramine in severely ill depressed patients and melancholic depressed patients (see Ref. 48 for review). One study found that while reboxetine restored normal function as measured on a social adjustment scale, fluoxetine did not [153]. The finding of improved social adjustment was interpreted to support the specific involvement of the NA system in "sustaining drive" [153]. A recent study showed reboxetine to be highly effective in the treatment of panic disorder [154].

2. SSRIs

The five drugs in this class are fluoxetine, sertraline, paroxetine, fluvoxamine, and citalopram. All are relatively new and were developed because they are potent and selective 5HT reuptake inhibitors [155]. They share similar side effects and therapeutic spectrum of action, being effective in the treatment of MDE, OCD, and panic disorder. Early clinical experience suggests that while these drugs are more similar than different, there are differences in side-effect profiles between the five that are unexplained by current knowledge. These include sedation/asthenia versus activation and propensity to cause nausea. In general, fluvoxamine and paroxetine are more frequently associated with a sedation-like "blah" feeling referred to as "asthenia" and nausea, whereas fluoxetine and sertraline are more "activating." Citalopram is intermediate in most side effects compared to other SSRIs, probably accounting for its popularity in Europe and the U.S. Another interesting difference is the potency of sertraline in blocking DA reuptake [144] and in binding to sigma receptors [156] and of paroxetine for inhibition of NA reuptake [157]. These data suggest that sertraline may be problematic in psychotic depression and that in high doses paroxetine may be a dual NA/5HT reuptake inhibitor. Paroxetine is also an inhibitor of the enzyme nitric oxide synthetase [158], possibly contributing to a slightly higher rate of sexual dysfunction compared with other SSRIs [159].

3. Clinical Differences Between SSRIs and NRIs in Depression Studies

Nelson [160] recently published a comprehensive review of prior studies comparing an NRI to an SSRI in patients with major depression. Sixteen studies were reviewed [160]. The NA selective agents included desipramine, nortriptyline, reboxetine, lofepramine, and maprotiline and SSRIs included fluoxetine, paroxetine, sertraline, fluvoxamine, zimelidine, and citalopram. A total of 1563 patients were included in these studies. Response rates were similar, 65% and 60%, for the SSRIs and NRIs. When baseline symptoms that predict response were examined, there were no consistent findings across studies, although some studies have found baseline anxiety to predict preferential response to SSRIs versus NRIs [161,162]. The topic of whether NRIs are as effective for anxiety symptoms/disorders as SSRIs continues to be an important focus of debate. In part, this debate is fueled by the lack of efficacy of NRIs for OCD [139–141] and PTSD [142,143,148], as well as a generally held perception that SSRIs are more effective in depressed patients with moderate-to-severe anxiety symptoms [163]. Arguing against a significant class difference in efficacy in patients with comorbid major depression and anxiety symptoms are the studies showing NRIs to be effective in panic disorder [135–137,154] as well as a recent study showing that baseline Ham-A score did not predict response to either sertraline or bupropion during treatment of depression [150].

4. Dual NA/5HT Reuptake Inhibitors

Approved by the FDA for the treatment of major depression in 1994, venlafaxine is the oldest of the newer antidepressants and stands out from most of the others in having minimal effects on blocking neurotransmitter receptors. It is as potent at blocking 5HT reuptake as imipramine, but is weaker at blocking NA reuptake, making it slightly more selective for 5HT than NA, especially at lower doses. It does not inhibit MAO and does not significantly bind to 5HT, NA, DA, muscarinic cholinergic, α_1-, α_2-adrenergic, or histamine H_1-receptors [164].

Venlafaxine has fared well in placebo-controlled studies and has shown efficacy in inpatients and outpatients with major depression and in patients with major depression with melancholia [165,166]. In contrast to SSRIs, there is evidence for a dose-response relationship, with higher doses being more likely to lead to successful antidepressant responses than lower doses. This has been hypothesized to be due to the likelihood that at lower doses (less than 150 mg/day) venlafaxine is predominantly an SSRI and at higher doses the NA reuptake inhibition begins to contribute to its action [167,168].

Several studies suggest that venlafaxine may lead to higher rates of response and a more "robust" profile of response (when more stringent response criteria are used) when compared to fluoxetine, imipramine, or paroxetine. This is especially true in severely ill or melancholic depressed patients [169–172].

Venlafaxine has been suggested to have a faster onset than imipramine [168] and fluoxetine [169]. Early onset has also been reported in placebo-controlled trials [173]. While early onset is more likely to be seen with aggressive dose escalation, it is thought to result from the dual action of venlafaxine on 5HT and NA systems [174].

Venlafaxine may have as broad an efficacy profile as SSRIs. Small open-label studies have suggested efficacy in OCD [175–178]. Venlafaxine has also been found effective for the treatment of panic disorder [179,180], attention deficit hyperactivity disorder [181,182], and social phobia [183].

5. NA Receptor Agonists and Antagonists

Mirtazapine. FDA-approved for the treatment of depression in the summer of 1998, mirtazapine is unique among antidepressants by virtue of the fact that it does not inhibit the reuptake 5HT, NA, or DA. Its primary mechanism of action relates to its potent antagonism of α_2-adrenergic receptors and $5HT_2$ receptors. It is also a potent antagonist of $5HT_3$ and histamine H_1-receptors, effects that influence its side-effect profile. Mirtazapine has no effects on DA, cholinergic, or α_1-adrenergic receptors [184]. By blocking α_2- but not the α_1-adrenergic receptors, mirtazapine leads to an increase in firing rate and release of both NA and 5HT. This is because α_2-adrenergic receptors are localized on both NA and 5HT neurons. On NA neurons, presynaptic α_2-receptors function as autoreceptors, inhibiting the release of NA. Blocking these receptors leads to an increased firing rate and release of NA in most brain regions. NA released near the cell bodies of 5HT neurons activate α_1-adrenergic receptors located on 5HT cell bodies, and since these receptors act in an excitatory fashion, the firing rate of 5HT neurons is increased. 5HT neurons also have α_2-adrenergic receptors but, in this case, the receptors are localized on 5HT terminals and function to inhibit the release of 5HT. Blocking these α_2-adrenergic receptors enhances the amount of 5HT released each time the neurons fire [184].

Mirtazapine has been shown to be more effective than placebo in both hospitalized patients and outpatients, and patients with "severe" depression (17-item Hamilton Depres-

sion Scale score > 25). It has comparable efficacy with amitriptyline [185], doxepin [186], and clorimipramine [187], and has been shown to be more efficacious than trazodone [188] and fluoxetine [189] in severely ill depressed patients.

Most studies have documented improvement in both depression and anxiety and many studies have shown efficacy in anxious depressed patients [190]. However, there are no published reports of mirtazapine treatment for anxiety disorders, although several studies are currently in progress.

Other Adrenergic Antagonists and Agonists. Comprehensive review of the literature on the use of NA receptor antagonists and agonists is beyond the scope of this chapter, but given the theoretical importance, a brief overview is called for. In general, the studies conducted support the interpretation that drugs that reduce NA activity decrease anxiety and cause fatigue and sedation and drugs that increase NA release (yohimbine) increase anxiety, especially (but not exclusively) in individuals with anxiety disorders. There are many published studies on the use of β-adrenergic receptor antagonists (most often propranolol, atenolol, or metropolol), α_2-adrenergic agonists (mostly clonidine), and α_2-adrenergic antagonists (mostly yohimbine) in a variety of anxiety disorders, mixed depression and anxiety states, and anxiety-inducing situations. Many open-label and controlled trials have been conducted and, in general, the results for all anxiety disorders have been mixed with comparable numbers of positive and negative studies (for reviews of these data, see Refs. 191–194). While sometimes effective, treatment with these drugs is most often limited by cardiovascular side effects, sedation, fatigue, and asthma [191–193]. Recent work with the α_1-adrenergic antagonist prazosin in patients with PTSD found it to have evidence of efficacy, improving sleep and decreasing nightmares [195] (Table 1).

IV. OVERVIEW OF NA IN MOOD AND ANXIETY DISORDERS

There has been substantial progress in elucidating many of the normal functions of NA in the brain. The LC appears to be a critical component in the brain's attention system [43]. NA modulation of the PFC explains one aspect of how we modulate our focus on rapidly changing external events [40–42]. In the periphery, NA serves to ready the body for a potential challenge in the classic flight-or-fight paradigm. CNS NA also serves to modify the storage of emotion-laden memory, perhaps conveying aspects of emotional significance to memory of events [44,45]. Chronic activation of NA causes adaptive changes in NA neurons and postsynaptic brain areas, possibly contributing to the symptoms of stress [30–32]. Increasing NA release in healthy people results in mild anxiety [7] and decreasing it results in mild decreases in attention, fatigue, and irritability (49–52, 115–118, 121, 130, 196). More marked differences have been observed in various patient groups, but the fact that such differences exist suggests that NA dysfunction may not be the central pathophysiological component in most patients with affective and/or anxiety disorders.

The assumptions underlying early models for the neurobiology of depression were greatly influenced by the discovery of the CNS DA deficiency in patients suffering from Parkinson's disease [1] and by the remarkable therapeutic effects of the DA precursor, L-DOPA [197]. It was in this historical context that the therapeutic and pharmacological effects of antidepressant drugs were discovered and the first neurobiological theories of depression and anxiety disorders were proposed. The research of the past 30 years suggests that new, more complex models are in order.

Table 1 Clinical Profile of Action of Selected Drugs with Potent NA and/or 5HT Effects

Drug	Major depression	Social anxiety disorder	GAD	Panic disorder	PTSD	OCD
SSRI[a]	++++	++++	++++	++++	++++	++++
Venlafaxine	++++	++++	+++	++++	+++	++++
Reboxetine	++++	NA	NA	+++	NA	NA
Desipramine	++++	NA	NA	++	+/(−)(−)	(−)(−)(−)
Bupropion	++++	+	NA	++	(−)(−)	NA
Mirtazapine	++++	NA	NA	+	++	+
β-Antagonists[b]	NA	++/ (−)(−)(−)	+++/ (−)(−)(−)	+++/ (−)(−)(−)	+[c]	(−)(−)
Clonidine	NA	NA	+/(−)(−)	+/(−)(−)	++	(−)

++++, Randomized placebo-controlled trials with comparison drug showing greater efficacy than both placebo and comparator; +++, at least one placebo-controlled trial or more than one open-label trial in a total of 50 or more patients showing efficacy; ++, two or more case reports with a total of 10 or more patients reporting efficacy in over half of the patients; +, at least one case suggesting efficacy; NA, no data available; (−), at least one case suggesting lack of efficacy; (−)(−), two or more case reports with a total of 10 or more patients failing to show evidence of efficacy in over half of the patients; (−)(−)(−), at least one placebo-controlled trial or more than one open-label trial in a total of 50 or more patients failing to show efficacy.

[a] SSRI (selective serotonin reuptake inhibitors) includes citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline.

[b] Includes propranolol and atenolol.

[c] Propranolol blocked some of the symptoms of acute stress in persons recently traumatized (Ref. 42).

Many studies have identified differences in measures of NA between patients with major depression or anxiety disorders and healthy controls. Of the prior research, one of the most consistent findings has been the blunted growth hormone response to clonidine in drug-free depressed patients. The major problem with prior interpretations of this finding is that it is highly nonspecific, being present in a wide variety of psychiatric conditions. Few of the other markers have been comparably researched in multiple patient populations, but the ones that have suggest considerable overlap between diagnostic groups exists.

It is likely that many of the differences identified in prior studies of NA function in clinical samples are a consequence of the differences in levels of stress between the groups. One of the most serious limitations of prior work investigating NA function in patients with affective and anxiety disorders is the fact that healthy controls were usually used as a comparison. It is well established that the NA system is highly stress responsive and that adaptive changes to chronic stress include many of the same abnormalities identified in patient populations [14,23,30–32]. Therefore, rather than using healthy controls, studies investigating the NA system in clinical samples should ideally be using "stressed" healthy controls. This would help to disentangle whether differences in NA measures reflect a nonspecific effect of stress or the pathophysiological underpinnings of a psychiatric disorder.

Some of the most important studies addressing the role of NA in clinical syndromes are those that have utilized NA depletion. The fact that NA depletion does not lead to depressive symptoms in healthy controls, nonantidepressant-treated clinical samples, or in depressed patients taking SSRIs (49–52, 115–118, 121, 130, 197) suggests that NA dysfunction may contribute to the symptoms of affective and anxiety disorders, but is highly unlikely to be causal. On the other hand, the results of NA depletion studies confirm the importance of NA to the antidepressant response of NRIs relative to SSRIs [49–52]. Similar studies in patients suffering from anxiety disorders have not been conducted to date.

It is likely that biological vulnerability to affective and anxiety disorders is heterogeneous, with many factors converging to cause a common brain dysregulation. While the etiology of these conditions may be heterogeneous, the brain systems involved in determining specific emotions, cognitions, and somatic processes are likely to be similar across patients. In spite of the sometimes dramatic differences in individual symptoms between patients with major depression, most antidepressant drugs are comparably effective across patients. This suggests that the drugs are causing widespread effects in many brain regions rather than simply reversing a common neurochemical abnormality. Considerable evidence supports the idea that specific brain networks and the neuroanatomical circuits that join them underlie emotional experience, cognition, and somatic function [198]. While our current understanding of the role and function of these circuits and networks remains primitive, a rough outline is beginning to emerge [199].

If this view is correct, then patients suffering with similar symptoms most likely have similar dysfunction of the same networks and circuits. Recent data support this and suggest that depression may be associated with localized reductions in brain morphology in the frontal cortex [200]. The etiology of the dysfunction may differ between patients, but the net functional impact may be similar. An analogous situation would be a cardiac arrhythmia leading to an increase in heart rate. In some situations, this could be caused by an electrolyte imbalance, but in many others it will arise from dysfunction of specific conduction pathways in the heart. The same symptom arises from different pathological processes, but the symptom arises from dysfunction of a specific circuit. These types of

integrative models begin to account for the diversity of clinical presentations and offer explanations of how neurotransmitter systems, brain circuits, and life experiences that affect the development of these systems could interact to produce vulnerabilities to depression and anxiety disorders.

None of the data reviewed provide insights into the how the biology of affective and anxiety disorders might differ. It is unlikely that any differences that exist reside in differences in NA function. Until we fully understand the brain networks involved in modulating discrete emotions, it is unlikely that we will have sufficient understanding of the brain to distinguish between these conditions or to even know if anxiety and affective disorders are part of a related spectrum. The treatment data (Table 1) suggest that there will be significant overlap between most anxiety and affective disorders. OCD and PTSD may turn out to be important exceptions to this.

In particular, with the clinical conditions in which most antidepressants seem to work equally, currently available drugs may be acting to cause an alteration in the function of postsynaptic neurons residing in critical brain circuits, an effect that would account for the delay [31,201]. Only after this had occurred would behavioral improvement follow. Abnormalities in the functioning of the postsynaptic receptors may be subcellular, for example, in the G-protein coupling, second- or third-messenger systems, or at the level of gene transcription. Some evidence already exists to suggest that the monoamines share common pathways at this level (31,202,203).

While it is well established that there are considerable interactions between NA and 5HT neurons in the brain, it is also clear that there can be independent effects of changing neurotransmission in either system [31,48–53]. This would explain why the pharmacological profile of the antidepressant drug that the patient is taking is so important to the effects of monoamine depletion. While both neurotransmitter systems generally innervate most brain regions, the pattern of innervation sometimes involves different layers of the cortex; there can be selective innervation of some brain regions; and both common and dissimilar effects can be observed in intracellular responses in postsynaptic neurons (31,204,205).

In considering the possible role of NA in mood and anxiety disorders, it is clear that much more work is needed to further elucidate the role of NA in normal brain function and mental disorders. Future work will benefit from a broader perspective, incorporating research from the cognitive neurosciences, systems neuroscience, molecular and cellular research, as well as neuropsychopharmacology.

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17

Benzodiazepines, Benzodiazepine Receptors, and Endogenous Ligands

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I. PHARMACOLOGICAL AND CLINICAL ACTIONS OF BENZODIAZEPINES

The term "benzodiazepines," as used in this chapter, includes two properties, namely a chemical structure and a certain mechanism of action leading to a characteristic pharmacological profile. Thus it also includes structurally unrelated compounds that have a mechanism of action similar to that of the classic benzodiazepines.

Structurally, benzodiazepines are best considered as derivatives of the prototype diazepam (Fig. 1A, B). Over the years, more than 3000 benzodiazepine derivatives have been synthesized and pharmacologically investigated [1,2]. Approximately 50 benzodiazepines and compounds with a similar mechanism of action have been, or still are, available as therapeutics worldwide.

The classic benzodiazepines have a characteristic profile of activity by which they can easily be distinguished from other neuropsychotropic drugs, such as antipsychotics or antidepressants. They differ in their relative potencies and by the relative prominence of single components of the profile. Typically, benzodiazepines generally have a low toxicity and there is a wide separation between the lowest pharmacologically active doses and those producing marked undesired or even fatal effects [3]; they are virtually devoid of direct actions on cells outside the central nervous system and produce a wide variety of centrally mediated effects that can be grouped conveniently into anticonvulsant activity, behavioral effects related to their clinical antianxiety effect, sedative effects (reduction of arousal state and attention level, facilitation of sleep, potentiation of effects of other centrally depressant agents, depression of consciousness and production of anterograde amne-

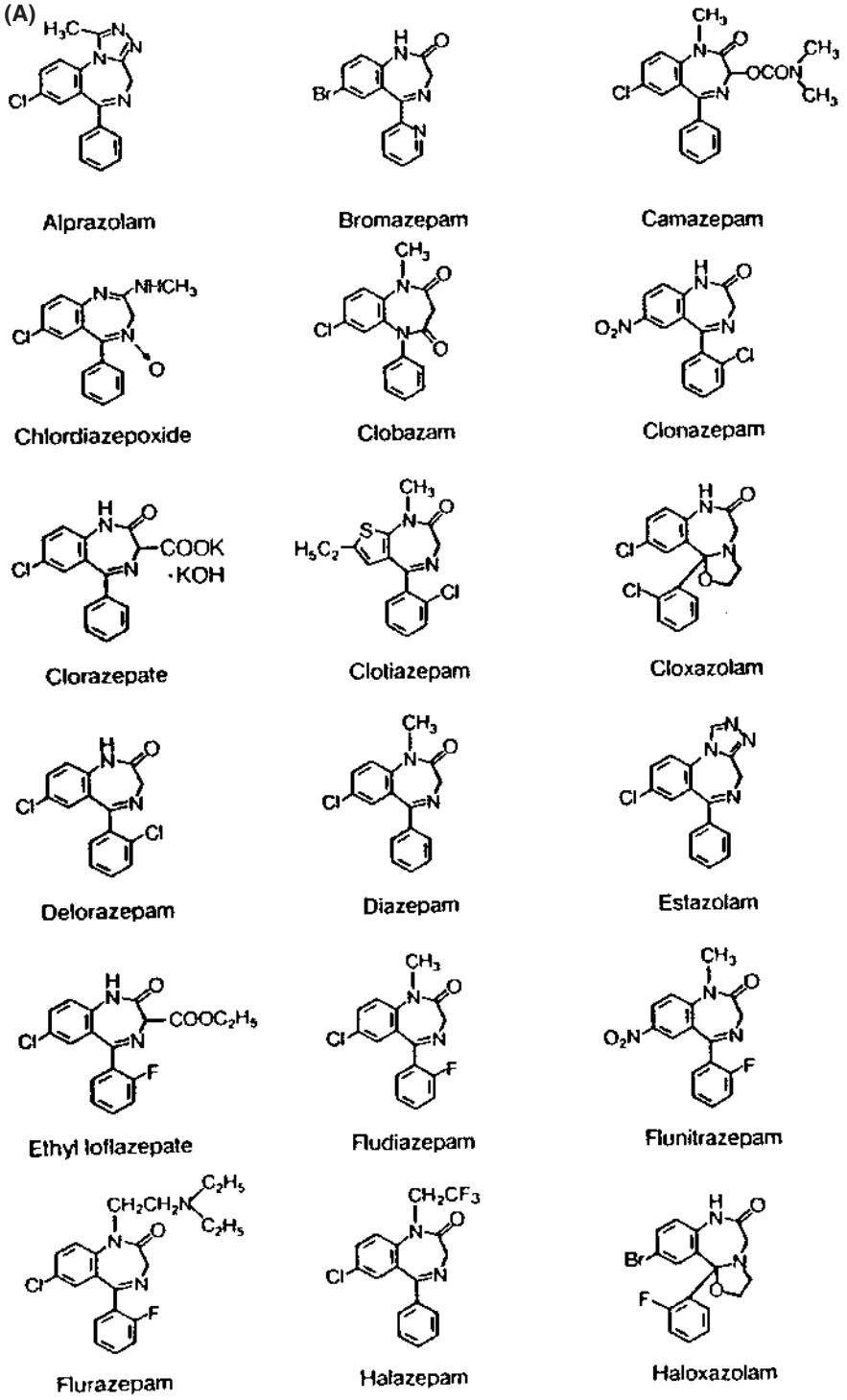


Figure 1A Commercially available benzodiazepines. (From Ref. 1.)

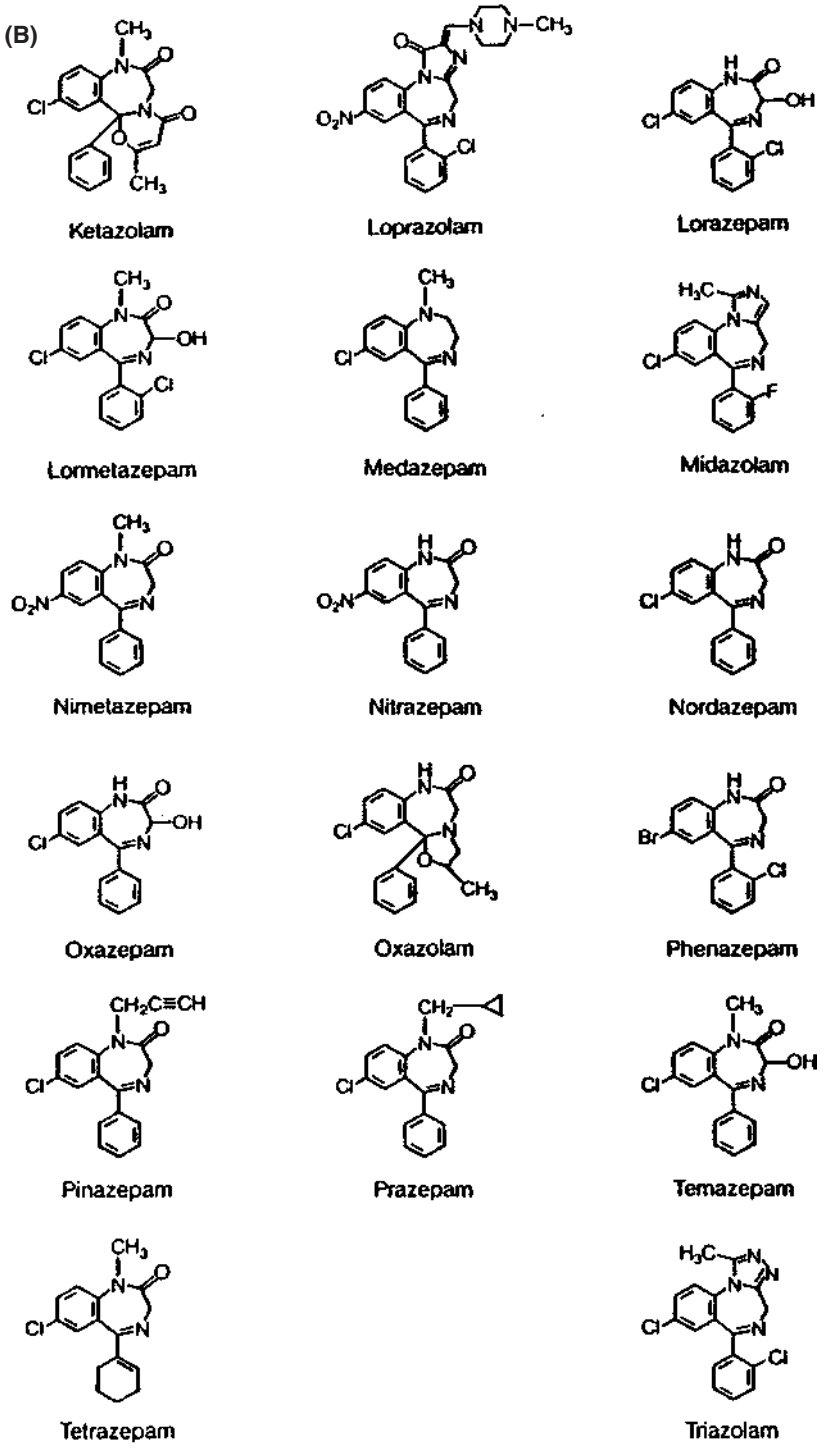


Figure 1B Commercially available benzodiazepines (continued).

sia), modulation of the central control of vegetative functions (autonomic nervous and endocrine systems), reduction of skeletal muscle tone, and impairment of motor coordination [3].

Because of these pharmacological actions, beneficial as well as adverse effects are associated with the use of benzodiazepines, especially at higher doses. Adverse effects include amnesia, ataxia, sedation, increase of the effects of ethanol, development of tolerance and physiological dependence, and occurrence of withdrawal symptoms on discontinuation of the drugs. Furthermore, it is precisely because of their positive attributes—safety, tolerability, and rapid onset of action—that the benzodiazepines may be misused or abused. While the vast majority of patients are treated correctly, benzodiazepines may be prescribed inappropriately, for the wrong patients, and/or for the wrong time period. There is also a small, but definite, problem of the illegal abuse of benzodiazepines by those for whom they were *not* prescribed. Such abuse, however, is usually by those who already abuse other drugs [4,5].

The reactions to these problems by regulatory authorities in different countries has led to major variations in the use of benzodiazepines. According to their particular concerns, countries will impose strict or less strict controls on who should receive benzodiazepines and when. The actions they take are related to their culture, their attitudes to psychological problems, their methods of drug prescription and distribution, and so on [5].

Furthermore, in the absence of hard evidence on the benefits and the risks of benzodiazepines in the clinical setting, decisions are often based on the opinions of experts, the media, and politicians who are either strongly in favor or strongly against their use. What is required is firm evidence of the real scenario regarding benzodiazepines in society, examining both the data on the benefits to the millions of anxiety sufferers and the true extent of misuse and abuse—and the costs of that to society [5].

A. Anticonvulsive Actions

Benzodiazepines are the most potent known anticonvulsants. They are effective in virtually all forms of spontaneous (genetic), chemically, or electrically induced forms of epileptiform activity [6]. It is assumed that benzodiazepines predominantly act by inhibiting the spread of paroxysmal activities, but stabilization of overexcited neurons in the epileptic focus might also be of importance. Benzodiazepines are especially potent in model systems where seizures, are elicited by a suppression of GABAergic inhibition, such as the pentylenetetrazole-induced seizures or seizures elicited by inhibitors of GABA synthesis (thiosemicarbazide). In these models, the activity of benzodiazepines correlates very well with their affinity for the benzodiazepine receptor (see below) and their potency in human patients.

They are much less potent in inhibiting strychnine-induced seizures or seizures elicited by supramaximal electroshock. The potency of benzodiazepines in the latter model does not correlate at all with their affinity for the benzodiazepine receptors and the anticonvulsive effects in this model might thus not be produced by these receptors [7]. Benzodiazepines are also potent anticonvulsants in a variety of human epilepsies. They are the drugs of first choice for status epilepticus and convulsions due to drug poisoning, and are effective in 80% of cases [8]. Unfortunately, however, the anticonvulsant action of the benzodiazepines in humans as well as in animal experiments is reduced over time because of tolerance development, and thus their long-term therapeutic application against epilepsy is limited.

B. Anxiolytic Actions

The anxiolytic action of benzodiazepines usually is investigated in anticonflict tests in animal experiments. There are a variety of different anticonflict tests and these tests are usually based on measuring reactions to punished spontaneous or learned behavior. Benzodiazepines over a wide dose range enhance the number of punished responses of the animals without influencing unpunished behavior [3,7]. This specific action of benzodiazepines can also be observed with barbiturates in a small-dose range and with the tranquilizers meprobamate and metaqualone, but not with neuroleptics and antidepressants. Benzodiazepines are also active in other test procedures such as the "social interaction test" and positively enhance exploratory behavior in an unfamiliar surrounding (test for neophobia, see Ref. 3).

The relatively selective effect on anxiety is probably related to the fact that benzodiazepines suppress activity in many limbic and other brain areas involved in anxiogenesis, including the septal area, amygdala, hippocampus, hypothalamus, locus coeruleus, and raphé nuclei. They also decrease the turnover of acetylcholine, noradrenaline, serotonin, and dopamine in these areas. Suppression of noradrenergic and/or serotonergic pathways appears to be of particular importance in relation to anxiolytic effects [8].

In addition to the anxiolytic action of benzodiazepines these drugs might also be involved in the reduction of the hyperactivity of the vegetative nervous system [9]. Benzodiazepines reduce the secretion of peripheral stress parameters such as catecholamines or cortison and reduce stress-induced stomach ulcera. Thus, these drugs might also be beneficial in the treatment of cardiovascular and gastrointestinal disturbances caused by psychosomatic disorders. It is not clear, however, whether these drugs modulate stress and anxiety only, or in addition have beneficial effects on the accompanying somatic diseases.

The anxiolytic and stress-reducing action of the benzodiazepines is the pharmacological basis for the widespread therapeutic use of these drugs in treatment of anxiety, stress, tension, and excitation. Anxiolytic effects are exerted in doses that cause minimal sedation, although the hypnotic, muscle relaxant, and perhaps amnesic actions may all contribute to relief of associated tension and insomnia [8]. The major clinical advantage of benzodiazepines as anxiolytics is the rapid onset of action, usually apparent after a single dose. This immediate effect contrasts with the delayed anxiolytic effects of antidepressants, buspirone, and psychological treatments. In addition, benzodiazepines are relatively nontoxic and safer than most of the alternative drugs [8]. Unpleasant side effects such as indifference, distance from reality, or reduced effects, which are sometimes observed, might be explained by an overreduction of anxiety, but the sedative component of the benzodiazepine action might also be involved [7].

The anxiolytic action of benzodiazepines might also be responsible for the antiaggressive activity of these drugs in various animal models. It is assumed that benzodiazepines do not reduce aggression per se but are only active when aggression is caused by anxiety [7]. Under certain conditions, however, when aggression is suppressed by anxiety or social subordination, benzodiazepines can also induce aggression. Such mechanisms might explain the relatively rarely observed paradoxical induction of aggressive behavior in humans by benzodiazepines [7,8].

C. Sedative-Hypnotic Actions

The sedative-hypnotic activities of the benzodiazepines can be studied in animal experiments by investigating the exploratory behavior or the prolongation of sleep time caused

by these drugs. In addition, benzodiazepines improve the quality of disturbed sleep in animals and humans. Benzodiazepines, in general, hasten sleep onset, decrease nocturnal awakenings, increase total sleeping time, and often impart a sense of deep, refreshing sleep [8]. They do, however, alter the normal sleep pattern: stage 2 (light sleep) is prolonged and mainly accounts for the increased sleeping time, while the duration of slow-wave sleep and rapid eye movement (REM) sleep may be considerably reduced. The onset of the first REM sleep episode is delayed and dreaming is diminished [8]. The suppression of the REM sleep may initially be helpful in decreasing nightmares, but may also be an important factor in determining rebound insomnia in drug withdrawal.

The typical changes in sleep stages occur with most benzodiazepines in most patients, but individual variations in response are considerable and are influenced by dosage, duration of treatment, type of benzodiazepine, age and clinical state. The increase in total sleeping time appears to be greatest in patients who complain of insomnia and in those with short baseline sleep duration [8].

The sedative-hypnotic activities of the benzodiazepines are also responsible for the unwanted side effects of these drugs, such as sedation during the day, somnolence, and reduced attention and reactivity. In contrast, even high doses of benzodiazepines do not produce narcosis. Thus, benzodiazepines alone in contrast to barbiturates, cannot be used as narcotics.

D. Amnesic Actions

Although benzodiazepines cannot directly cause narcosis, they are frequently used as a premedication for anesthesia to sedate the patients. Especially with intravenous application anterograde amnesia often can be observed [7,8]. This effect might be beneficial, for instance, when applied as premedication for endoscopy, where the patients, although having been awake, cannot remember the unpleasant procedure. Amnestic effects can also be observed in animal experiments. However, in these animal experiments as well as in patients, a reduction in cognitive abilities rather than true amnesia is probably produced. These effects can be used to test for compounds that might possibly improve cognitive abilities.

E. Central Muscle Relaxation

It is not easy to verify the central muscle relaxant activities of the benzodiazepines because the simultaneous reduction in vigilance caused by these drugs also can elicit changes in muscle tonus. Nevertheless, a variety of experimental tests have confirmed the central muscle relaxant activities of benzodiazepines [6]. These effects seem to be produced by dampening of supraspinal activatory influences on motor neurons and by inhibition of polysynaptic reflexes or enhancement of presynaptic inhibition at the spinal level. Ataxia caused by high doses of benzodiazepines might also be caused by a direct influence of these drugs on cerebellar circuits.

The muscle relaxant activity of the benzodiazepines can sometimes be used in a variety of motor disorders. These include a range of dystonias and involuntary movements, myoclonus, akithesia, restless legs syndrome, and muscle spasm associated with pain. However, tolerance develops with long-term use and the drugs are not always effective and may give rise to withdrawal problems [8].

But the muscle relaxant activity of the benzodiazepines is also the cause of severe side effects, such as muscle weakness and disturbed walking, and therefore should not be

used in patients with myasthenia or ataxia. Disturbance of walking and subsequent falls with risk of bone fractures can be caused by overdosage of benzodiazepines and are especially dangerous in old patients [7]. Muscle relaxation by benzodiazepines might also cause a disturbance of breathing [8]. A direct influence of benzodiazepines on breathing is usually only observed with very high, nontherapeutic doses of these drugs. Like other drugs that depress respiration, they should be avoided in patients with severe chronic obstructive airways disease [8].

F. Toxic Actions

Benzodiazepines exhibit an extremely low toxicity. Lethal doses of benzodiazepines for laboratory animals are extremely high with ED_{50} values in the range of many 100 mg/kg [6]. Clinical experiences over the last 30 years also indicate that lethal benzodiazepine intoxication is extremely rare and in most cases occurs because of secondary factors, such as undercooling, or because of a combined treatment with other centrally active drugs or ethanol [4]. Some toxicological effects of benzodiazepines in laboratory animals, such as their lethal actions, are not correlated with their affinity for the benzodiazepine receptors and might thus be due to toxic effects at very high doses caused by mechanisms presently not known [6].

G. Plasma Levels and the Pharmacokinetic–Pharmacodynamic Relationship

The relationship between plasma levels of benzodiazepines, free benzodiazepine concentration, and receptor occupation in the brain has been extensively investigated [10–12]. These investigations clearly demonstrated that the free plasma concentration, which is in equilibrium with the benzodiazepine concentration in the brain, determines the extent of benzodiazepine receptor occupation. And the extent of receptor occupation determines the pharmacodynamic action of the benzodiazepines. Although different doses are needed for different benzodiazepines to elicit specific actions, these doses are in a clear relationship to the doses necessary for half-maximal occupation of the receptors. In case of the pentylenetetrazole test, for instance, it was demonstrated that for all compounds investigated in the range of their pharmacological ED_{50} values, receptor occupation is about 25%. In addition, it was also demonstrated that a different extent of occupation of the benzodiazepine receptors is required for different pharmacological actions. These data indicate that there is a clear correlation between the receptor occupation and the pharmacological effect and that the classic benzodiazepines exhibit quite similar pharmacological interactions with their receptors [7].

Although in certain tests there is a good correlation between the pharmacokinetic elimination velocity and the length of the pharmacodynamic action of the benzodiazepines, this does not hold true for all benzodiazepine effects observed. In many cases, distribution of the drug between brain and peripheral tissues is an important factor that determines the length of action of benzodiazepines. Thus, it has been demonstrated that the length of the hypnotic effect of benzodiazepines does not depend on the pharmacokinetic elimination velocity but is strongly determined by the distribution of the drugs from the brain into peripheral tissues [11]. These considerations are valid, however, only for the hypnotic action of benzodiazepines that requires a high concentration of these drugs in the brain. Hangover effects of benzodiazepines, such as difficulties in concentration and fatigue in

the morning after the use of the drugs, for instance, are influenced by the elimination half-life of the drug.

H. Tolerance Development

A variety of studies has demonstrated the development of functional tolerance in animals [13] and humans [4,8] after chronic treatment with benzodiazepines. Development of tolerance is more prominent for the sedative-hypnotic and anticonvulsant properties of the benzodiazepines than for their anxiolytic actions. Tolerance to the hypnotic effects develops rapidly, sometimes after only a few days of regular use [8]. Although development of tolerance against the sedative-hypnotic properties is desirable when benzodiazepines are used as anxiolytics, the development of tolerance against the anticonvulsive properties significantly limits the use of benzodiazepines in the treatment of epilepsy. The mechanism of tolerance development is not yet well understood, partially because the effects observed in animals not only depend on the drugs used but also on the intensity and duration of chronic treatment, the time of analysis post-treatment, and the brain area evaluated [14]. But it is generally assumed that tolerance is caused by a downregulation of receptor density or function.

I. Addiction, Dependence, Withdrawal

Animal experiments have indicated a weak tendency for self-application of benzodiazepines when these drugs were added to the drinking water. Although self-administration of these drugs was increased on parenteral injections of the benzodiazepines, it was still much less frequent than self-administration of cocaine, codeine, or barbiturates [15]. Similar results were obtained in double-blind placebo-controlled experiments with probands for a variety of classic benzodiazepines. Although there was a slight preference for benzodiazepines over placebo, this preference was much less than that of barbiturates [4,15]. Since there were only small differences in preference between different benzodiazepines, it can be concluded that all benzodiazepines have a low potential for inducing addiction. Clinical experiences indicate that patients taking benzodiazepines stay with the same dose over years and there is no tendency to increase the dosage.

But, of course, a certain form of physical dependence develops on long-term use of benzodiazepines that is indicated by withdrawal symptoms on abrupt discontinuation of the drug. In animals, this can lead to increased motor activity, tremor, increased muscle tonus, and seizure susceptibility [4,15]. In humans, rebound anxiety and insomnia, some perceptual and motor disturbances, and sometimes even seizures can occur [4,8]. Chronic benzodiazepine doses needed to elicit such effects, however, are higher than the relevant pharmacological ED_{50} values in the animal species investigated. Potent benzodiazepines with relatively short elimination half-lives (triazolam, alprazolam, lorazepam) appear to carry the highest risk of causing problems with dependence [8]. In contrast to classical benzodiazepines that presumably exhibit similar potential to cause addiction and dependence, partial benzodiazepine receptor agonists (see below) seem to be less prone to eliciting these effects. It is not yet clear, however, whether this also holds true for patients.

II. BENZODIAZEPINE RECEPTORS

After the discovery of specific high affinity binding sites for benzodiazepines on brain membranes [6,17], a wealth of experimental data have accumulated indicating that these binding sites are heterogeneous. Most of the benzodiazepine binding sites have been identified to represent allosteric modulatory sites on γ-aminobutyric $acid_A$ ($GABA_A$) receptors.

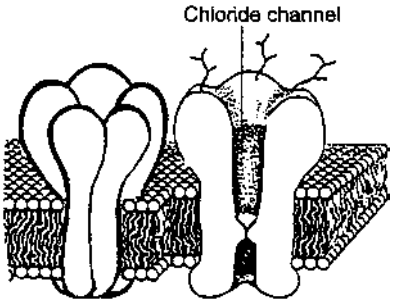


Figure 2 Model of the $GABA_A$ receptor.

Since there is an excellent correlation between the clinical potency of benzodiazepines and related compounds and their ability to displace radiolabeled diazepam or flunitrazepam from these $GABA_A$-receptor-associated binding sites, it is now clear that most, if not all, of the clinically relevant effects of benzodiazepines are mediated by modulating the function of $GABA_A$ receptors [1,3,7,14].

In addition to these "central" benzodiazepine receptors (that actually are no receptors, but modulatory binding sites, see Ref. 18), other benzodiazepine binding sites have been identified in several peripheral tissues as well as in the central nervous system [17,19]. These "peripheral" benzodiazepine receptors, although exhibiting high affinity for some benzodiazepines, are not associated with $GABA_A$ receptors. They appear to represent a heteromeric complex of at least three different proteins [20] and there is no significant correlation between the clinical potency of benzodiazepines and their affinity for the "peripheral" binding sites. The physiological functions of these sites are not known, but they might be involved in steroidogenesis, in the regulation of cell proliferation, and in the adaptation of the organism to stress and brain damage (for review, see Ref. 20).

Finally, "micromolar" benzodiazepine binding sites have been identified [21] that differ from the central and peripheral benzodiazepine binding sites not only in their much lower affinity for benzodiazepines but also in their binding properties and kinetic and pharmacological characteristics. Evidence has accumulated that these binding sites are able to modulate Ca^{2+} channel processes [22,23], but probably are not involved in most of the clinical actions of benzodiazepines.

III. BENZODIAZEPINE MODULATION OF $GABA_A$ RECEPTORS

GABA is the major inhibitory transmitter in the central nervous system. It is estimated that about 30% of all synapses of the brain use GABA as a transmitter [14]. Most of the physiological actions of GABA are mediated by $GABA_A$ receptors. These receptors are composed of five protein subunits forming a central chloride ion channel that can be opened by GABA (Fig. 2). So far, at least 6α, 3β, 3γ, 1δ, 1ε, 1π, 1θ, and 3ρ subunits have been identified in the mammalian nervous system [18] and, depending on their subunit composition, receptors exhibit distinct pharmacological and electrophysiological properties [14]. Recent immunocytochemical studies have indicated that individual subunits exhibit a distinct and often widespread distribution throughout the nervous system [24,25]. The resulting expression of multiple subunits in the same neurons suggest the existence of a large variety of $GABA_A$-receptor subtypes in the brain.

Evidence has accumulated that the majority of the $GABA_A$ receptors are composed

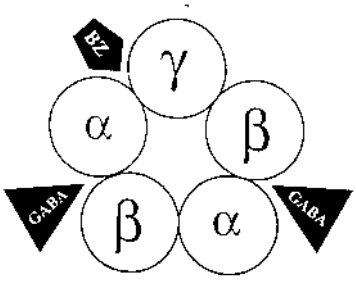


Figure 3 Stoichiometry and subunit arrangement of $GABA_A$ receptors. A mirror arrangement is equally possible. BZ or GABA = site of interaction of benzodiazepines or GABA with $GABA_A$ receptors, respectively.

of 2α, 2β, and 1γ subunit [26]. Minor receptors appear to be pentamers composed of αβδ, αβε, αβπ, αβ, or homo- and hetero-oligomeric ρ subunits. Because two different α and/or two different β subunits can be present in the same receptor, probably more than 500 distinct $GABA_A$-receptor subtypes do exist in the brain. Their subunit composition and regional and cellular distribution, however, presently are not known. Only recently, methods have been developed that allow the identification of the composition and quantitative importance of receptor subtypes in different brain regions [27]. Because of the widespread distribution and quantitative importance of the GABA system, even minor $GABA_A$-receptor subtypes probably exhibit an abundance that is comparable to that of some monoamine, serotonin, or peptide receptors.

A. Action of GABA on $GABA_A$ Receptors

Evidence has accumulated that the GABA binding site of $GABA_A$ receptors is located at the interface between α and β subunits (Fig. 3) [28]. Since there are two α/β interfaces on $GABA_A$ receptors, these receptors contain two more or less equivalent GABA binding sites. Binding of GABA to both of these sites seems to be necessary to induce the conformational change that leads to the opening of the chloride ion channel integrated into the $GABA_A$ receptor [29]. The direction and strength of the chloride flux depends on the membrane potential and the chloride concentrations on both sides of the membrane. In most cases, GABA induces an influx of chloride ions and thus a hyperpolarization and an inhibition of the electrical activity of the cell. So far, no GABA binding site ligands are available that could significantly differentiate between different $GABA_A$-receptor subtypes.

B. Action of Benzodiazepines and Related Ligands on $GABA_A$ Receptors

Benzodiazepines do not bind to the GABA binding site. They bind to a separate site on $GABA_A$ receptors that is present only on receptors containing a γ subunit (Fig. 3). This benzodiazepine binding site is located at the interface between the α and γ subunit of $GABA_A$ receptors [30] and the exact type of the α and γ subunit forming this site strongly influences its pharmacological properties [14]. Since there are six different α and three different γ subunits, up to 18 different $GABA_A$-receptor-associated benzodiazepine binding sites do exist in the brain. By binding to these sites, benzodiazepines cause a conforma-

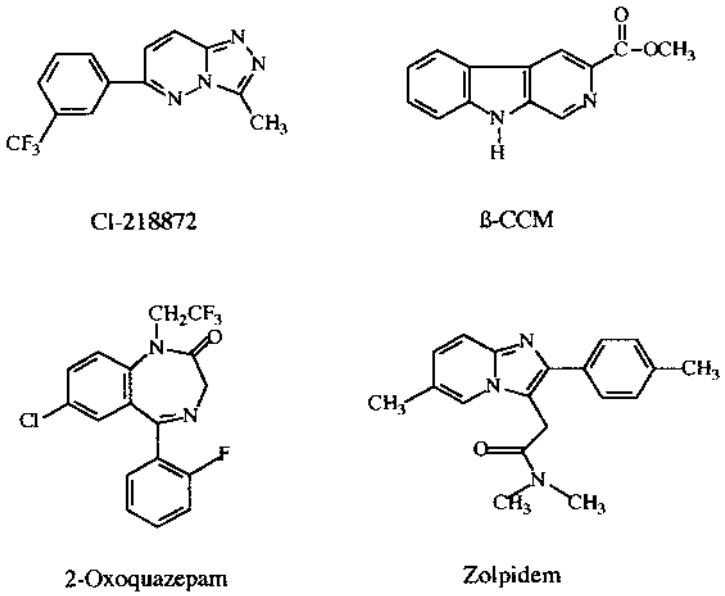


Figure 4 Partially selective benzodiazepine site ligands. β-CCM: β-carboline-3-carboxylate methylester.

tional change in the receptor that usually enhances GABA-induced chloride flux. These drugs, however, cannot directly activate the chloride ion flux of $GABA_A$ receptors in the absence of GABA. They are just only modulating ongoing GABAergic activity. This explains the extremely low toxicity [1,4] of benzodiazepines.

The classic benzodiazepines all have a similar action. This is not surprising because all these compounds exhibit comparable affinities for the benzodiazepine binding sites of most of the benzodiazepine-sensitive $GABA_A$-receptor subtypes in the brain [14]. They enhance GABA-induced chloride flux and at relatively low concentrations exhibit anticonvulsant and anxiolytic actions. At higher concentrations they exhibit sedative-hypnotic and muscle-relaxing effects.

In a search for compounds with a more selective action, many ligands with a benzodiazepine or nonbenzodiazepine structure were identified that exhibited a high affinity for the $GABA_A$-receptor-associated benzodiazepine binding site [1,2,18]. Some of these compounds exhibited a certain selectivity for $GABA_A$-receptor subtypes (Fig. 4). Thus, the benzodiazepines quazepam and oxoquazepam, several β-carboline-3-carboxylates, imidazopyridines such as zolpidem or alpidem, or the triazolopyridazine Cl-218872 exhibited a high affinity for the benzodiazepine binding site of $GABA_A$ receptors composed of $\alpha 1$, β, and $\gamma 2$ subunits (old nomenclature: type I receptors, BZ1 receptors, ω_1 receptors), and a 10 to 15 times lower affinity for the benzodiazepine binding sites of other $GABA_A$ receptors (old nomenclature: type II receptors, BZ2 receptors, ω_2 receptors) (see Ref. 18). It is now clear that the latter type of receptors represents a mixture of many different receptors. This small selectivity in the binding properties of these compounds, of course, is not sufficient to avoid coactivation of all the other receptors, and it thus was no surprise that most of these compounds exhibited properties that were only marginally different from those of classic benzodiazepines [31,32].

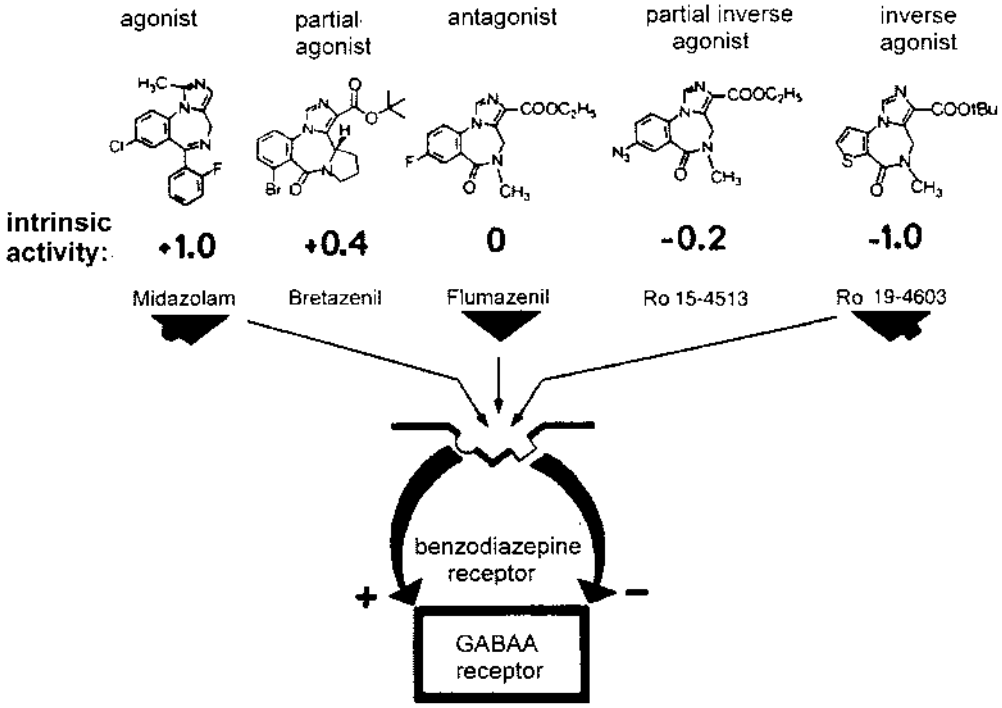
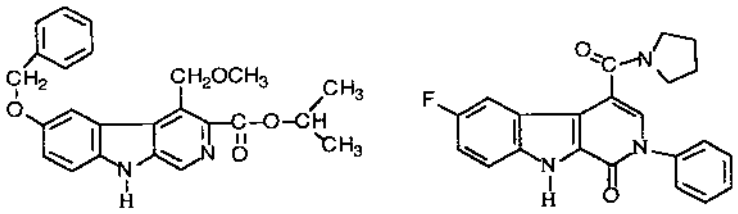
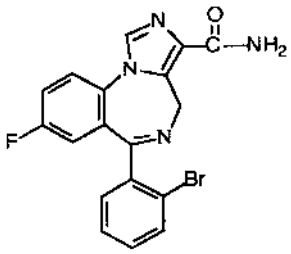


Figure 5 Schematic presentation of the various classes of ligands for the benzodiazepine binding site of $GABA_A$ receptors. (Modified from Ref. 128.)

Interestingly, the β-carboline-3-carboxylates exhibited actions opposite to that of benzodiazepines although interacting with the benzodiazepine binding site of $GABA_A$ receptors. This discrepancy was solved when the efficacy of various benzodiazepine binding site ligands for modulating $GABA_A$ receptors was systematically investigated. It was demonstrated that some of these ligands, the "benzodiazepine binding site agonists" like the classic benzodiazepines, cause a conformational change in $GABA_A$ receptors that enhances GABA-induced chloride ion flux (positive intrinsic efficacy). These compounds exhibit anxiolytic, anticonvulsant, muscle relaxant, and sedative hypnotic properties. Other ligands, the "benzodiazepine binding site inverse agonists" cause a conformational change that reduces GABA-induced chloride ion flux (negative intrinsic efficacy). These compounds have an action opposite to that of agonists: they produce convulsant, stimulant, and anxiogenic effects [33,34]. The agonist as well as the inverse agonist actions are produced via the same (benzodiazepine) binding site, because there is also a third group of ligands, the benzodiazepine binding site antagonists, that bind with high affinity to the benzodiazepine binding site of $GABA_A$ receptors but have either no or only weak intrinsic efficacy for changing GABAergic transmission. These compounds, therefore, have no or only weak intrinsic effects when given to animals or humans, but are able to inhibit the effects of both benzodiazepine site agonists or benzodiazepine site inverse agonists. Between these extreme actions, compounds were identified (partial agonists or partial inverse agonists) with intermediate actions (Fig. 5). Such compounds have less positive or negative intrinsic efficacy than full agonists or inverse agonists [1,34].





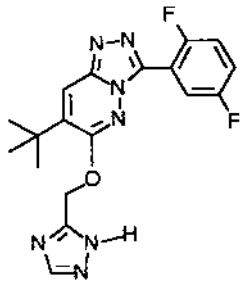


Figure 6 Partial agonists at the benzodiazepine site.

Partial agonists recently have become quite interesting clinically, because it was demonstrated that a different extent of receptor occupation is necessary to elicit the anxiolytic, anticonvulsant, muscle relaxant, and sedative-hypnotic effects of benzodiazepines [35,36]. Thus, full agonists are able to elicit their anxiolytic and anticonvulsant effects at a relatively low receptor occupancy. Since partial agonists only weakly enhance GABAergic transmission, they need a higher receptor occupancy to elicit comparable effects. To elicit muscle relaxant and sedative effects, however, full agonists require a higher receptor occupancy. Because of their weaker enhancement of GABAergic transmission, partial agonists no longer can elicit muscle relaxant and sedative effects with comparable strength and thus exhibit less side effects than full agonists [35,36].

Some partial agonists, such as bretazenil (Fig. 5) or abecarnil (Fig. 6), raised high hopes because in animal experiments they acted as potent anxiolytics and anticonvulsants without tolerance development and sedation and little potentiation of alcohol intoxication. In clinical experiments, however, these compounds were disappointing because of side effects, particularly sedation [32]. Imidazenil (Fig. 6), a compound related to bretazenil but not yet clinically tested, produced a preclinical profile similar to bretazenil but unlike diazepam, did not produce amnesia at dosage levels with maximal anticonvulsant efficacy [31]. It is unclear at present why the pharmacological profile of these compounds should be unfavorable in humans.

Some newer benzodiazepine site ligands, L-838.417 [37] or SL-651.498 (Fig. 6) [38], exhibit an even more restricted action on various $GABA_A$-receptor subtypes and in animal experiments again exhibit predominantly anxiolytic, but no sedative actions. The investigation of their clinical effects is eagerly awaited.

Benzodiazepine binding-site agonists, inverse agonist, or antagonists were identified

in each class of compounds interacting with the benzodiazepine binding site of $GABA_A$ receptors. In addition, it was demonstrated that the efficacy of compounds for activating or inhibiting the function of $GABA_A$ receptors differs for different $GABA_A$-receptor subtypes. Thus, a compound can be a full agonist for a certain receptor and a partial agonist or even antagonist for other receptor subtypes. The degree of partial agonism also can vary between different receptor subtypes [31,39], although the compound might exhibit a similar affinity for all of them! The often observed slightly different spectrum of action of different benzodiazepines can thus be explained by a difference in their ability to activate various $GABA_A$-receptor subtypes.

C. Action of Other Compounds on $GABA_A$ Receptors

In addition to benzodiazepines, a variety of pharmacologically and clinically important drugs such as barbiturates (phenobarbital, pentobarbital), neuroactive steroids (progesterone and deoxycorticosterone metabolites), anesthetics (propofol, halothane, isoflurane, chloralhydrate), and convulsants have been demonstrated to produce at least part of their clinically relevant effects by interacting with allosteric binding sites on $GABA_A$ receptors [14]. In addition, other compounds such as avermectin, γ-butyrolactones, etazolate, etomidate, chlormethiazole, Zn^{2+}, La^{3+}, antidepressant drugs such as amoxapine and mianserine [40,41], or antipsychotic drugs such as clozapine and olanzapine [42] are acting on these receptors [14,43]. The exact number of different allosteric modulatory sites present on $GABA_A$ receptors and their location currently is not known. Binding of any of these drugs again causes a conformational change in the structure of $GABA_A$ receptors and, by this, enhances or reduces GABA-induced chloride ion flux. This comparable mode of action explains the synergistic effects of all these centrally depressant drugs with benzodiazepines as observed in clinical studies.

In addition to their $GABA_A$-receptor modulatory activity and in contrast to benzodiazepines, barbiturates, neuroactive steroids, anesthetics, etazolate, etomidate, or chlormethiazole at higher concentrations are also able to directly activate the chloride ion channel of $GABA_A$ receptors in the absence of GABA. These drugs, therefore, exhibit a much higher toxicity than benzodiazepines [14].

Ethanol shares some properties with barbiturates and benzodiazepines, because it exhibits anxiolytic, anticonvulsant, and sedative-hypnotic activity. A development of cross-tolerance among these compounds has also been observed [44]. Other evidence indicates that ethanol is able to potentiate GABAergic transmission in at least some neuronal systems (for review, see Refs. 14, 45) and might thus mediate part of its pharmacological and toxicological effects via $GABA_A$ receptors. The exact mode of action of ethanol on $GABA_A$ receptors, however, so far could not be determined.

IV. ENDOGENOUS LIGANDS

A. Endogenous Ligands Acting via the Benzodiazepine Binding Site

A variety of evidence indicates that benzodiazepine binding site antagonists, previously described as intrinsically inactive, are able to induce effects in animals and humans under particular circumstances [46]. Although these effects, for instance, also could be explained by a possible dependence of the antagonist efficacy on the previous activity of $GABA_A$ receptors or its state of phosphorylation [47], alternatively, endogenous agonists or inverse

agonists might exist and modulate the function of these receptors. A variety of brain disorders, such as anxiety, insomnia, epilepsy, spasticity, alcoholism, coma, or dementia may then be associated with a disequilibrium of opposing endogenous benzodiazepine site ligands changing the excitability of neurons implicated in these diseases [46].

Over the years, several endogenous ligands [48] acting at the benzodiazepine binding site of $GABA_A$ receptors (endozepines) have been discovered, although a possible physiological function of none of these compounds so far has been definitely established. Thus, the purines inosine and hypoxanthine inhibit diazepam binding with IC_{50} values 400 to 1300 μM and 700 to 3700 μM, respectively [49]. Similarly, adenosine [50] and S-adenosylhomocysteine [51] can inhibit benzodiazepine binding. Concentrations needed for these effects, however, seem to be high compared to the endogenous concentrations of these compounds [52].

The following three groups of substances are currently thought to be the most likely endozepine candiates: diazepam binding inhibitor (DBI) and/or its processing products being the only peptides among them [53]; n-butyl-β-carboline-carboxylic acid ester [54]; and benzodiazepine-like compounds [55,56]. Experimental evidence suggests that DBI and n-butyl-β-carboline esters are inverse agonists, whereas benzodiazepine-like substances are agonists at the benzodiazepine binding site of $GABA_A$ receptors. All these agents were isolated from the brain of various mammals including humans, but they have been shown to fulfill the criteria for modulators of GABAergic inhibition to various degrees [46].

To date, only DBI and its smaller fragment, octadecaneuropeptide, were found colocalized and coreleased with GABA from neurons [57]. Both DBI and n-butyl-β-carboline-carboxylic acid ester induced anxiogenic and proconvulsant effects that were antagonized by the benzodiazepine binding-site antagonist flumazenil (Fig. 5) [53,54]. Whereas n-butyl-β-carboline-carboxylic acid ester binds to the benzodiazepine binding site of $GABA_A$ receptors with high affinity, DBI is a ligand with intermediate affinity. DBI is, however, sufficiently concentrated in the brain to activate $GABA_A$ receptors. Interestingly, DBI and some of its processing products bind also to the "peripheral" benzodiazepine binding sites in the brain and in endocrine tissues, where they promote the synthesis of neurosteroids and thereby indirectly affect $GABA_A$ receptors [46].

In the case of benzodiazepine-like substances, some of which are indeed benzodiazepines (diazepam, desmethyldiazepam), their endogenous source has still not been proven since both plants and gut microorganisms synthesize them [56], perhaps contributing to an environmental-based relaxed behavior. Some experimental data nevertheless suggest that mammalian cells may synthesize similar, not yet identified substances, which are released from neurons, block convulsions elicited by the GABA antagonist bicuculline, and enhance GABA-mediated currents in neurons [46].

B. Increase of Endogenous Ligands in Neuropsychiatric Diseases

Several lines of evidence indicate that endogenous benzodiazepine binding site ligands can be found enriched in neuropsychiatric diseases. For instance, hepatic encephalopathy is clearly associated with increased levels of endogenous benzodiazepines such as diazepam, desmethyldiazepam, and other benzodiazepine-like substances in plasma, urine, CSF, and brain of patients [58], and these compounds play a role in the pathophysiology of this syndrome by potentiating the GABA-induced CNS depression. Flumazenil, the only

benzodiazepine site antagonist studied in detail, rapidly alleviates the symptomatology of hepatic encephalopathy, induces arousal from coma, responding to stimuli, and awareness of the environment without leading to noticeable adverse effects [58].

Coma from other origin was also reported to respond favorably to flumazenil. For example, flumazenil transiently reversed comatose states due to alcohol, carbamazepine, baclofen, halothane, chloralhydrate, and cannabis intoxication [59–62]. Since flumazenil is a selective benzodiazepine site antagonist, the most parsimonious explanation of these unexpected therapeutic effects would be antagonism of a coma-related activation of endogenous benzodiazepine agonists.

In addition, it has been demonstrated that another brain disorder, the idiopathic recurrent stupor, is associated with a large increase of endogenous benzodiazepine binding-site agonists [63]. In a case of idiopathic recurrent stupor, both the prompt abolition by flumazenil of the stupor episodes and a dramatic increase of endogenous benzodiazepine agonists during these attacks in CSF and plasma of the patient indicated an involvement of endogenous benzodiazepine agonists in the pathogenesis of this rare disorder [64].

In some other neuropsychiatric diseases apparently inverse agonists predominate. For instance, DBI was elevated in the CSF of depressed patients with pronounced anxiety [65]. This anxiety might have been directly induced by DBI because it is known that inverse agonists can induce anxiety [66]. Similarly, late-onset alcoholic patients with anxious personality had increased CSF levels of DBI when compared to early-onset alcoholics demonstrating antisocial personality and a genetic predisposition [67]. Release of an endogenous benzodiazepine inverse agonist during withdrawal from ethanol was also suggested by the finding that flumazenil was able to diminish alcohol withdrawal symptoms, such as tremor, sweating, nausea, anxiety, depression, and restlessness [68].

A recent demonstration of increased levels of a less well-characterized endogenous benzodiazepine site ligand in the plasma of anxious and depressed patients, as well as in stressed healthy volunteers, suggests its involvement in processes related to stress and/or anxiety [59,69,70]. If elevated, DBI in CSF produces a reduced GABAergic function, then depression as well as alcoholism coupled to anxiety might possibly be associated with a downregulated $GABA_A$-receptor function, in certain brain areas.

All these results would be consistent with the proposal [46] that endogenous benzodiazepine binding-site ligands are at least partially colocalized with GABA in neurons and coreleased with it in response to defined stimuli according to the spatiotemporal organization of particular neuronal circuits. Endogenous benzodiazepine agonists would then increase and inverse agonists decrease the effect of GABA upon $GABA_A$ receptors, leading to enhanced or reduced inhibition of postsynaptic neurons, respectively. Depending on arrangements of GABAergic neurons in various CNS regions and their roles in integrating behavioral patterns, a disequilibrium of endogenous benzodiazepine ligands with a preponderance of either agonists or inverse agonists would ultimately contribute to different levels of excitability of GABA-receptive neurons that may be critically implicated in the expression of CNS disorders [46].

Since it was shown that the release of a peptidergic cotransmitter is increased upon higher frequency stimulation of presynaptic neurons while that of the principal transmitter remains the same through a range of stimulation frequencies [71], at least in the case of DBI and its metabolites it may be assumed that enhanced neuronal firing of GABAergic neurons would release higher amounts of the peptides, thus reducing the inhibitory effect of coreleased GABA and further increasing neuronal excitability. Such a mechanism might also contribute to the genesis of epilepsy. Flumazenil exhibits a moderate antiepileptic

activity [72–74], which may have been underestimated because of short half-life and low bioavailability of this drug.

C. Endogenous Ligands Acting via Other Sites of $GABA_A$ Receptors

As mentioned above, a variety of endogenous neuroactive steroids (progesterone and desoxycorticosterone metabolites) can allosterically modulate the function of GABA on $GABA_A$ receptors [14,75]. There are regional differences in the sensitivity of $GABA_A$ receptors to modulation by neuroactive steroids [76], and the effects of neuroactive steroids are dependent on the subunit composition of the $GABA_A$ receptor [77]. The location of these sites on $GABA_A$ receptors currently is not known. Since steroids at higher concentrations can directly activate the $GABA_A$-receptor-associated chloride channel, presumably more than one specific steroid binding site is present on these receptors. In addition to steroids that can enhance the actions of GABA on $GABA_A$ receptors, other steroids, such as pregnenolone sulfate, allosterically reduce the actions of GABA on these receptors [14,75].

Some steroids, such as the progesterone metabolites pregnanolone (3α-hydroxy-5β-pregnan-20-one) and allopregnanolone (3α-hydroxy-5α-pregnan-20-one), are synthesized in the brain and their concentration varies depending on the hormonal situation (estrous cycle) of the individual [78]. From animal studies, it is known that these metabolites exhibit anxiolytic, hypnotic, and anticonvulsant effects. Their withdrawal during the estrous cycle might contribute to the symptoms of premenstrual syndrome, such as anxiety and seizure susceptibility [79]. Reduced concentrations of these steroids might also contribute to postpartum or postmenopausal dysphoria [80].

In addition to these direct effects of steroids on $GABA_A$ receptors, indirect effects via genomic receptors also seem to occur. Thus, estrogens regulate $GABA_A$-receptor subunit mRNA expression in regions of the female rat brain known to contain estrogen receptors [81].

Since allopregnanolone has a poor oral availability, a new synthetic neuroactive steroid ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) has been developed and is currently under clinical trials. It is a potent and efficacious anticonvulsant agent for the management of generalized absence and partial seizures [82] as well as for convulsions due to cocaine poisoning [83]. Recently, it was reported that ganaxolone is superior to valproate, ethosuximide, clonazepam, diazepam, and phenobarbital in preventing the pentylenetetrazole-induced convulsions and the behavioral effects of pentylenetetrazole including its depressant effects on locomotor activity and rearing in mice, thereby suggesting that ganaxolone may provide additional benefits in the treatment of epilepsy by controlling anxiety, mood changes, and other behavioral alterations associated with preseizure activity [84].

Other steroids such as allotetrahydrodeoxycorticosterone (5α-THDOC) are synthesized in the adrenal gland, although this compound is found in the brain where its concentration is increased during stress [85]. 5α-THDOC is one of the most potent known steroid modulators of $GABA_A$ receptors and, thus, can directly modulate the function of these receptors via the steroid binding site. In addition, steroids produced in the adrenals directly or indirectly influence the expression of $GABA_A$-receptor subunits in the brain, as shown by adrenalectomy [86]. This indicates that $GABA_A$-receptor function is under constant regulation by such steroids.

Other hormones, such as the thyroid hormones, also directly [87–89] or indirectly

[90,91] modulate the function of $GABA_A$ receptors. Although somatostatin-14, a biologically active tetradecapeptide, is known to mediate its biological actions through the G-protein-coupled membrane receptors [92]; its direct interaction with the $GABA_A$-receptor complex has also been reported [93,94]. Similarly, melatonin has been reported to interact directly with $GABA_A$ receptors [95,96]. Because this effect was observed already at nanomolar concentrations of melatonin, an interaction of melatonin with $GABA_A$ receptors might have some physiological significance.

In addition, neuronal growth factors such as neurotrophin-3 [97] or platelet-derived growth factor [98] seem to be able to directly or indirectly inhibit the function of $GABA_A$ receptors. A low-molecular-weigth factor released from astrocytes is also able to negatively modulate the function of $GABA_A$ receptors [99]. This factor seems not to act via the benzodiazepine binding site because its effect could not be blocked by flumazenil.

In addition, polyamines, such as spermine, spermidine, and putrescine, or arachidonic acid and fatty acids are able to modulate the function of $GABA_A$ receptors [14]. Cis-9,10-octadecenoamide, a fatty acid primary amide that accumulates in the CSF of sleep-deprived cats and may represent a novel signaling molecule, has also been demonstrated to modulate the function of $GABA_A$ receptors. This compound might be involved in the regulation of arousal and has been implicated in the circadian control of physiological sleep [100].

Imidazoleacetic acid is normally present in the brains of rats and humans as well as in human CSF. This compound is a full agonist at the GABA binding site of $GABA_A$ receptors and can be synthesized from minute quantities of histamine in the brain. Its central administration induces a host of centrally mediated effects, including sedation and hypnosis (for review, see Ref. 101). This compound might be involved in a novel mechanism for interactions between histamine and the GABAergic system.

Normal human urine contains inhibitory activity for the enzyme monoamine oxidase (MAO). It has been tentatively termed tribulin and it has been speculated that tribulin might be related to an endogenous anxiety factor acting via the benzodiazepine receptor and bearing structural similarities to β-carbolines [102]. Subsequently, it has been established that tribulin consists of several low-molecular-mass fractions that exhibit four known bioactivities: monoamine oxidase A and B inhibitory activities and peripheral and central benzodiazepine receptor-binding-inhibitory activities [103]. The compounds involved in these activities seem to be differentially distributed in the brain of rats and their level is increased in both human urine and rat tissues by stress or anxiety, and by anxiogenic drugs [104]. Further studies have to be performed to clarify the function of the compounds contributing to tribulin.

The intracellular loops of several subunits of the $GABA_A$ receptor contain consensus sequences for phosphorylation by various protein kinases [14]. Such phosphorylation directly modulates the function of $GABA_A$ receptors and the detailed effects probably depend on the subunit composition of the individual receptor subtypes. Several different kinases have been identified to modulate the function of $GABA_A$ receptors and the effects of phosphorylation are the subject of intensive investigation [14,105–107]. The identity of the corresponding phosphatases so far has been much less investigated. Because the various enzymes involved in phosphorylation and dephosphorylation of $GABA_A$ receptors could be activated by many different transmitter and second-messenger systems, this offers the possibility of short- and long-term modulation of $GABA_A$-receptor function by other transmitter systems, hormones, or growth factors. Furthermore, it should be stressed that

Table 1 Endogenous Ligands Acting on $GABA_A$ Receptors

	Refs.
Ligands acting at the benzodiazepine site:	
DBI	53
β-carbolines	54
benzodiazepines	55, 56
Ligands acting at other sites:	
neuroactive steroids	14, 75
thyroid hormones	87–89
somatostatin	93, 94
melatonin	95, 96
neurotrophin-3	97
platelet-derived growth factor	98
polyamines, arachidonic acid, fatty acids	14, 100
imidazoleacetic acid	101
tribulin	102–104
Ligands indirectly modulating $GABA_A$ receptors via protein phosphorylation	
various transmitters and modulators	105–107

drugs interfering with these regulatory mechanisms could influence the GABAergic system and could lead to specific GABAergic effects.

Endogenous ligands modulating $GABA_A$ receptors are summarized in Table 1. The accumulated data indicate that the function of $GABA_A$ receptors is highly regulated by a variety of ligands and signals and can thus be immediately adjusted to the physiological requirements of the individual.

V. FUNCTION OF $GABA_A$-RECEPTOR SUBTYPES

Although a large variety of allosteric binding sites have been identified on $GABA_A$ receptors, only a few compounds have been discovered so far that exhibit a certain receptor subtype selectivity (see Sec. III). Their selectivity, however, is not sufficient to allow a selective modulation of a single receptor subtype and, thus, to determine its function in the brain.

Several attempts have been made to identify the function of receptor subtypes by generating mouse lines in which the gene encoding for a certain $GABA_A$-receptor subunit was inactivated. A knockout of γ_2-subunits was lethal [108], possibly at least in part because the γ_2-subunit is required for synaptic clustering of $GABA_A$ receptors [109]. By contrast, mice heterozygous for the γ_2-subunit knockout developed and behaved normally. The synaptic clustering of $GABA_A$ receptors was only partly reduced (about 15–30%, depending on the brain region); the unclustered receptors consisted of α- and β-subunits. When exposed to certain fear-inducing stimuli, these animals showed a striking disease phenotype with a high anxiety response to natural and learned aversive stimuli, as well as cognitive bias for threat cues [110]. This indicates that a reduction in the number of $GABA_A$ receptors containing γ_2-subunits might cause a predisposition for anxiety and is

consistent with the fact that benzodiazepines produce their anxiolytic action by interacting with $GABA_A$ receptors containing a γ-subunit [14].

A knockout of the α_6-subunit, which is expressed more or less exclusively in the cerebellar granule cells, did not result in an overt phenotype [111]. However, the α_6 knockout mice were more sensitive to the motor-impairing action of diazepam in an accelerating rotarod test than their wild-type counterparts [112]. Although disruption of the gene encoding the β_3 or δ-subunit produced mice with an epileptic phenotype [113] or an attenuated sensitivity to neuroactive steroids [114], respectively, a possible change in the development and function of the brain, as well as changes in the expression of other receptors or ion channels caused by the lack of these receptors could not be excluded.

In a more subtle attempt to identify the function of receptors that contain specific α-subunits, a new genetic approach was recently developed [115]. This approach was based on introducing a point mutation (His101Arg) into the α_1-subunit of $GABA_A$ receptors, rendering α_1-containing receptors insensitive to allosteric modulation by diazepam without altering their GABA sensitivity. In the absence of any change in signal intensity produced by the mutated receptors, animals possessing this mutation developed normally and the cellular and subcellular location of receptors was unchanged. In these animals, therefore, diazepam mediated its effects through only the α_2-, α_3-, and α_5-subunit-containing receptors, because receptors containing α_4- or α_6-subunits are insensitive to diazepam [14]. A comparison of drug-induced behavioral responses in α_1(His101Arg) mutant and wild-type mice then allowed the identification of diazepam effects that are missing in mutant mice and, thus, to determine the contribution of α_1-containing receptors to the effects of diazepam. It was demonstrated that α_1(His101Arg) mutant mice failed to show the sedative, amnesic, and, in part, anticonvulsant actions of diazepam. By contrast, the anxiolytic-like, myorelaxant, and ethanol-potentiating effects were fully retained, which indicates that these effects are produced via the nonmutated $GABA_A$ receptors that contain α_2-, α_3-, or α_5-subunits [115]. Using a similar approach, most of these findings were confirmed by a different group [37]. The small discrepancies between these studies could be explained by differences in the test procedures applied [38].

In subsequent studies, the function of α_2- or α_3-subunit containing receptors in the brain was investigated. For this, point mutations were introduced into α_2- or α_3-subunits at positions homologous to that of α1His101 [116]. However, in α_3(His126Arg) mice the anxiolytic activity of diazepam, as tested by the light-dark choice test and the elevated plusmaze test, was not impaired compared with wild-type mice. By contrast, in α_2(His101Arg) mice, the anxiolytic activity of diazepam was absent [116]. Thus, the anxiolytic activity of diazepam seems to be mediated by neurons expressing α_2-containing receptors. This finding is consistent with the expression of α_2-subunits in brain areas that are associated with emotional stimulus processing [24,25]. Thus, α_2-containing receptors are abundant in the central nucleus of the amygdala. In addition, these receptors are densely packed on the axon initial segment of principal cells of the cerebral cortex and the hippocampus bringing their output activity under GABA-mediated control [117,118]. Given that α_2-containing receptors constitute only about 15% of diazepam-sensitive $GABA_A$ receptors, ligands selective for α_2-containing $GABA_A$ receptors would be expected to show a much reduced side-effect profile. Such agents would be highly selective drugs compared with the nonselective benzodiazepines in clinical use.

To identify the $GABA_A$-receptor subtypes mediating the action of diazepam on muscle tone, in a subsequent study, the myorelaxant properties of diazepam were investigated in these α_2 or α_3-point mutated mice [119]. Whereas in α_2(His101Arg) mice the myorelax-

ant action of diazepam was almost completely abolished at doses up to 10 mg/kg, the same dose induced myorelaxation in both wild-type and α_3(His126Arg) mice. It was only at a very high dose (30 mg/kg diazepam) that α_2(His101Arg) mice showed partial myorelaxation and α_3(His 126Arg) mice were partially protected from myorelaxation compared with wild-type mice. From this it was concluded that the myorelaxant activity of diazepam seems to be mediated primarily by α_2-subunit-containing $GABA_A$ receptors and at high concentrations also by α_3-containing receptors [119].

Thus, α_2-containing receptors apparently mediate the anxiolytic as well as muscle relaxant action of diazepam. But these actions presumably are elicited by receptors in different brain regions and there seems to be a difference in the dose necessary to exhibit these effects. From this it can be predicted that a partial agonist selective at α_2-containing $GABA_A$ receptors will exhibit anxiolytic but no sedative and muscle relaxant properties. A compound coming close to these properties might be L-838.417 (Fig. 6). This compound, although binding with high affinity to α_1-, α_2-, α_3-, and α_5-subunit-containing receptors, does not enhance GABA-response on α_1-receptors but acts as a partial agonist on α_2, α_3-, and α_5-containing receptors [37]. Studies on the clinical effects of this compound are eagerly awaited.

Interestingly, a recent report has indicated that α_5-subunit deficient mice seem to exhibit increased abilities in learning and memory tasks [120], possibly indicating that α_5-subunit-containing $GABA_A$ receptors mediate the memory-impairing effect of diazepam. This conclusion is supported by the abundant location of α_5-subunits in the hippocampus [24,25], a brain region in which $GABA_A$ receptors are involved in memory encoding and retrieval [121]. Selective partial inverse agonists of $GABA_A$ receptors containing α_5-subunits might thus represent excellent candidates for the generation of memory-enhancing drugs.

In conclusion, recent studies have indicated that the different effects of benzodiazepines not only are produced in different brain regions but are also mediated by partially different $GABA_A$-receptor subtypes. These results should cause a revival of $GABA_A$-receptor research and strongly stimulate the development of drugs with a higher α_2-, α_3-, or α_5-receptor subtype selectivity. Since $GABA_A$ receptors not only are involved in controlling the excitability of the brain [122] and the modulation of anxiety [123,124], but also in the modulation of feeding and drinking behavior [125], circadian rhythms [126], cognition, vigilance, memory, and learning [121,127], a selective modulation of individual receptor subtypes will generate quite selective clinical effects.

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18

Antidepressants for the Treatment of Depression and Anxiety Disorders: Same Mechanism of Action?

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I. INTRODUCTION

Comorbidity of depression and anxiety is common. Approximately 45% of patients with a current anxiety disorder also have a depressive disorder, and about 40% of patients with depression have been found to have an anxiety disorder [1]. Antidepressants are effective for the treatment of depressive illnesses in the majority of patients, regardless of the degree of anxiety symptoms present. They are also an effective treatment for several primary anxiety disorders, which has led to the hypothesis of "affective spectrum disorders," defined by their response to antidepressants and speculation that similar pathophysiological processes underlie these conditions [2]. However, this is predicated on the assumption that antidepressants have the same or similar mechanisms of action in anxiety disorders as in depression. This chapter will briefly review the evidence for the effectiveness of antidepressants in depression and a number of anxiety disorders. Several issues will be explored including: (1) how far are the effects of antidepressants in anxiety disorders due to a nonspecific effect on mood and (2) to what extent do these agents have specific effects on the core psychopathology of anxiety disorders?

If antidepressants work through the same mechanism to alleviate depression and anxiety, then it would be expected that the clinical response to antidepressants would be similar in time course, that similar classes of antidepressants would have similar efficacy, and that similar doses of drug would be required. The clinical response to antidepressants

will be examined in depression and three anxiety disorders—obsessive-compulsive disorder (OCD), panic disorder, and post-traumatic stress disorder (PTSD). Clinical observations suggest that antidepressants work by different mechanisms in at least some anxiety disorders when compared with depression; various possible explanations of these observations will be explored.

II. CLINICAL RESPONSE TO ANTIDEPRESSANTS

A. Depression

The discovery of tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) in the 1950s and 1960s led to the monoamine hypothesis of the pathophysiology of depressive illnesses, implicating both norepinephrine (NE) and serotonin (5HT). The first randomized controlled trial of the effectiveness of these drugs in depression was conducted by the Medical Research Council of the United Kingdom in 1965 [3]. This demonstrated the superiority of imipramine over placebo and, incidentally, the ineffectiveness of phenelzine in the severely depressed group of patients in this study. The advent of the selective serotonin reuptake inhibitors (SSRIs) in the 1980s led to much debate as to whether drugs that acted solely on the 5HT system could be as effective as those that acted on NE system as well. Accordingly, large numbers of comparative studies comparing TCAs and SSRIs have been conducted in patients with varying degrees of severity of depression. Several meta-analyses of these studies have demonstrated that both classes of drugs are equally effective in general [4–6]. Of interest is the conclusion that drugs that are relatively selective at blocking NE reuptake, such as desipramine, produce similar response rates as SSRIs. However, analysis of those studies investigating the treatment of hospitalized patients with severe depressive illnesses suggests that the older TCAs may be superior to SSRIs in this population, with this superiority residing almost entirely with amitriptyline [6,7]. More recently, meta-analysis of studies comparing the NE and 5HT reuptake inhibitor venlafaxine with SSRIs has also demonstrated a superiority of venlafaxine at doses of 150 mg/day and above in moderate to severely ill patients with major depression [8,9]. The reason for the superior clinical potency of amitriptyline and venlafaxine in major depression is unknown, although both drugs are potent in the blockade of both the NE and 5HT uptake mechanism. Further support for the notion that dual action leads to improved clinical outcome comes from studies examining the effect of combining an SSRI with a relatively NE-selective TCA [10], which show increased efficacy in this combination over an SSRI alone. Although the interpretation of these studies is complicated by the potential pharmacokinetic interaction that can occur between SSRIs and TCAs due to the metabolism of the latter by the cytochrome P450 isoenzyme 2D6 being inhibited by the former [11], these results support the notion that blockade of the reuptake of both these amines is more effective than that of 5HT alone. Thus, it can be concluded that in the management of major depression, SSRIs are as effective as selective NE reuptake inhibitors and that drugs that potently block both uptake mechanisms are probably superior in the treatment of the severely ill.

Clinical observations of depressed patients treated with antidepressants demonstrate that response is not immediate. Improvements in mood, cognitive functioning, and somatic symptoms are first observable in patients who eventually respond to tricyclic antidepressants after 1 to 2 weeks [12], with a similar temporal profile seen also in the elderly treated with fluoxetine [13]. However, there is little information in the literature regarding the

total length of time patients take to recover when treated with antidepressants. The Food and Drug Administration (FDA) in the United States recommends that acute treatment studies in depressed patients should be conducted for a period of 6 weeks (*http://www.fda.gov/cder/guidance/old050fn.pdf*), although there appears to be little basis for this opinion. Analysis of a large number of patients treated in placebo-controlled studies suggests that only a few patients who have not shown any signs of improvement after 4 weeks of treatment show some response at 6 weeks [14]. This finding is independent of the nature of the depressive illness or antidepressant used [14], although the elderly may take longer to respond [15]. This has led to clinical guidelines for the management of depression in adults, recommending changes in antidepressant treatment in patients who have either failed to show any response at 4 weeks, or only minimal response at 6 weeks, once the diagnosis has been reviewed and compliance checked [16].

B. Obsessive-Compulsive Disorder

The Epidemiological Catchment Area survey in the United States reported a lifetime prevalence of OCD of 2.5% [17]. Two-thirds of OCD patients have a lifetime history of major depression and one-third satisfy criteria for major depression at the time of first evaluation [18]. Conversely, over 20% of bipolar and 10% of unipolar affective disorder patients, respectively, have comorbid OCD [19]. Despite the high rates of co-occurrence of both depressive and obsessive-compulsive symptomatology in patients, it is possible to selectively investigate effects of treatment on core OCD symptoms using well-validated scales such as the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) [20,21]. This is an important issue because it allows the possibility of examining the specific effects of antidepressants in OCD.

A meta-analysis of randomized controlled trials demonstrates a superiority of clomipramine and SSRIs over placebo in OCD patients as assessed using specific scales such as the Y-BOCS [22]. However, neither nortriptyline [23] nor imipramine [24] show evidence of superiority over placebo. The difference between clomipramine and other TCAs that are less 5HT selective is supported by a meta-analysis of eight direct comparison studies, which reveal clomipramine to demonstrate superior efficacy in alleviating the core symptoms of OCD [22]. Similarly, fluvoxamine has been shown to be superior to desipramine [25]. While it appears that the effect size seen with clomipramine compared to placebo is bigger than that with SSRIs [22], in direct head-to-head comparisons, SSRIs appear to be equally effective as clomipramine in OCD [26]. In addition, the hypothesis that the efficacy of clomipramine in OCD resides entirely in its ability to block 5HT uptake is supported by the finding that the addition of an NE uptake inhibitor (desipramine) to an SSRI in treatment-resistant OCD patients has no effect [27]. Although clomipramine is metabolized to desmethylclomipramine, which is largely an NE uptake blocker, the assumption is that clomipramine acts independently in this condition.

Given the high rates of comorbid depressive symptomatology, an important question is whether antidepressants are effective in OCD simply by ameliorating these symptoms, rather than the core psychopathology of the disorder. As described above, both clomipramine and SSRIs appear to have significant effects compared to placebo on OCD symptoms as assessed using specific rating scales. However, this question has been further investigated by comparing the relative efficacy of clomipramine versus placebo according to the levels of depression of patients at the outset of the trial. Piccinelli and colleagues [22] compared trials in which patients were excluded if they had a primary affective disorder

and/or patients had a baseline Hamilton Depression Rating Scale score lower than 17 with trials in which OCD patients had concomitant depression. Interestingly, clomipramine was superior to placebo in both sets of trials, but the effect was greatest in those trials where patients had little or no concomitant depression.

Not only is there a difference in the range of antidepressants that is effective in the treatment of OCD compared to depression, but the doses needed and the time to response also appear to be different. Many of the randomized controlled studies conducted to date have compared treatments over periods of 8 to 12 weeks. These have often revealed a delayed response to treatment, leading expert consensus to suggest therapeutic trials for a minimum of 8 weeks [28,29], twice the duration recommended for depression [16]. In addition, the dose of antidepressant needed to treat OCD tends to be higher than that needed to treat depression. In a fixed-dose comparison of placebo, 20 mg, 40 mg, and 60 mg of fluoxetine in OCD, a dose-response relationship is apparent with 20 mg being no better than placebo [30]. This is somewhat different from the situation in depression, where 20 mg of fluoxetine is clearly effective and there is little evidence of a dose-response relationship for higher doses [31,32]. These differences may simply be due to OCD being a more severe form of illness than depression. However, such a situation cannot explain the different range of effective treatments in the two conditions.

C. Panic Disorder

Around two-thirds of patients with panic disorder have experienced a major depressive episode at some time [33]. Such comorbidity is associated with a more severe illness and is reflected by a suicide rate twice that seen in depressed patients without comorbid panic disorder [1]. While some studies have suggested that such comorbidity has no effect on response to treatment [34,35], the majority of studies show that comorbid depression leads to a poorer treatment response in panic disorder patients [36]. This suggests a complex interaction between the expression of depressive and panic symptoms in patients and is to a certain extent consistent with the notion that there may be a shared pathophysiology between these conditions. This matter is further confounded as there are differences in the response to treatment between the conditions.

The assessment of the effect of treatments in panic disorder is complicated by the high frequency of occurrence of comorbid conditions. Many studies comparing different treatments have assessed global anxiety, depression ratings, or overall clinical improvement [37]. This makes it difficult to be certain that the treatments are having an effect on core panic disorder symptoms rather than associated or comorbid symptomatology. The clearest, but far from perfect, way of assessing the effect of treatments in panic disorder is simply to measure panic attack frequency. Using such measures, it can be seen that in some patients panic disorder responds to treatment with TCAs such as imipramine [34]. The response to imipramine appears to correlate with the plasma concentration of imipramine itself rather than its active metabolite desmethyl-imipramine [38]. Since imipramine is predominantly a 5HT uptake inhibitor, while its metabolite is an NE uptake inhibitor, this finding suggests that blockade of 5HT uptake is the mechanism by which imipramine acts in panic disorder. This supposition is supported by findings that the tetracyclic antidepressant maprotiline [39], which selectively inhibits NE uptake, and the TCA lofepramine [40], which is relatively NE selective, are ineffective in panic disorder. Conversely, SSRIs appear to be highly effective in the treatment of panic disorder [37,41]. Despite the 5HT uptake blockade potency of the parent compound, imipramine does not appear to be as

effective as clomipramine [42], which is more potent at blocking 5HT uptake. In addition, in a meta-analysis SSRIs have been shown to be more clinically effective in panic disorder than imipramine [41]. These data suggest that 5HT uptake blockade is more important in treating panic disorder than NE uptake blockade, and are reflected in expert consensus statements on the management of panic disorder [43]. However, the picture is not as clear as in OCD [28], because TCAs (i.e., imipramine) other than clomipramine appear to be effective. This may relate to the complex presentation of panic disorder and the problems of assessment of response to treatment.

D. Post-Traumatic Stress Disorder

Post-traumatic stress disorder is characterized by a triad of psychopathology, including reexperiencing a traumatic event, such as recurrent and intrusive flashbacks; avoidance of any situation or activity that directly or indirectly reminds the patient of the trauma; and increased arousal. However, in addition, a high proportion (up to 80%) of patients also suffer from comorbid anxiety disorders and depression [44]. Therefore, it is not surprising that there has been interest for some years in the use of antidepressants for the treatment of PTSD. A recent systematic review of methodologically sound, randomized controlled trials suggests that antidepressants are effective in the treatment of PTSD [45]. Of particular interest is that improvement in the core symptoms of PTSD is seen, rather than just comorbid depression. This would tend to suggest that antidepressants are effective in treating, or at least ameliorating the effect of, the underlying pathophysiology of PTSD.

Most recent interest in the treatment of PTSD has centered on the use of SSRIs. These have been shown clearly to be effective [46]. It has been suggested that the effect size seen with antidepressants in PTSD correlates with their serotonergic specificity [47]. This is supported by a small and short duration study that found desipramine, a relatively NE-selective TCA, to be ineffective [48]. The evidence for the specificity of the SSRIs in this condition is not clear-cut and it is not surprising that expert consensus guidelines recommend the use of SSRIs, nefazodone, or venlafaxine as first-line treatments of PTSD [49], although the inclusion of the latter two drugs does not have great evidence-based support [50].

In addition to possible differences in the range of antidepressants that have efficacy in PTSD compared to depression, there is also a suggestion of a difference in the time course of response. No FDA recommendations exist regarding the duration of controlled studies of medication in PTSD patients, although generally studies range in duration from 4 to 12 weeks. Expert consensus suggests that the time to response is longer than in depressive illness and a duration of 8 weeks is recommended in therapeutic trials [49,50]. However, even this length of treatment may not be sufficient as inspection of several of the longer controlled trials suggests that a significant proportion of nonresponders at 8 weeks may well become responders by week 12 [46,51,52]. This is clearly well beyond the normal response time seen in depressive illness.

III. MECHANISM OF ACTION OF ANTIDEPRESSANTS

A. Depression

Little is known about the mechanism of action of antidepressants in ameliorating the symptoms of depressive illness in humans. Not surprisingly, most hypotheses regarding poten-

tial mechanisms center on effects on monoaminergic systems. Because the clinical response to antidepressants is delayed, any hypothesis needs to explain why this occurs despite inhibition of monoamine uptake or metabolism, or receptor blockade occurring as soon as a single oral dose of an antidepressant is absorbed. Such explanations usually postulate neuroadaptive changes occurring in response to raised monoamine concentrations, based on experimental work in animals.

Postmortem binding studies in suicide victims have led to hypotheses that depression is associated with a supersensitivity of beta-adrenoceptors [53]. Data from animal studies demonstrate that repeated administration of TCAs, MAOIs, and electroconvulsive shocks decrease beta-adrenoceptor numbers in rats [54,55]. This has led to the suggestion that this is the potential mechanism of action of antidepressants [56]. Of note is that changes in the number of beta-adrenoceptors are seen after 7 to 21 days of treatment, but not after a single dose of an antidepressant [55], in line with the time course of response of depressed patients to pharmacological treatment. However, the effect of SSRIs on beta-adrenoceptors appears to be inconsistent, with sertraline [57] reported to cause a similar reduction as TCAs, but no effect of the most selective of the SSRIs, citalopram [58,59].

Other monoamine hypotheses of the mechanism of action of antidepressants have included those connected with 5HT receptors. Rapidly lowering brain tryptophan levels (and hence 5HT) can lead to significant lowering of mood in subjects with a vulnerability to depression, such as patients with a strong family history of depression [60,61] and euthymic subjects with a history of recurrent depression [62,63]. The consequences of rapid lowering of 5HT on the functional activity of the serotonergic system remain to be determined. Deakin and Graeff [64] have suggested that 5HT neurons in the median raphé that project onto postsynaptic $5HT_{1A}$ receptors in the hippocampus maintain adaptive behaviors in the face of aversive stimuli. They further hyphothesize that a failure of this system leads to helplessness in animals and depression in humans [64]. This model would predict a mood-lowering effect of tryptophan depletion in humans as a result of reduced transmission through postsynaptic $5HT_{1A}$ receptors. Several studies have reported a blunted growth hormone response to L-tryptophan in depressed patients compared to controls [65], a neuroendocrine test believed to be an indicator of $5HT_{1A}$ function [66].

There is further evidence that $5HT_{1A}$ receptors are involved in the mechanism of antidepressant action. Antidepressant treatments may lead to a neuroadaptive change that counteracts an impairment of postsynaptic $5HT_{1A}$ receptors. In vivo studies in rodents have demonstrated that a range of antidepressants and electroconvulsive shocks, when given chronically over 14 days, but not single doses, attenuate the function of somatodendritic $5HT_{1A}$ receptors located on raphé neurons [67,68]. Single doses have no effect. Attenuation of these autoreceptors enhances serotonergic transmission generally, including transmission mediated through postsynaptic $5HT_{1A}$ receptors. An overall effect of antidepressants enhancing 5HT transmission through postsynaptic hippocampal $5HT_{1A}$ receptors has also been argued by Blier and de Montigny [69] using in vivo electrophysiological techniques in rats, although they argue for differing mechanisms between antidepressants. For example, repeated TCAs are claimed to lead to a supersensitivity of postsynaptic $5HT_{1A}$ receptors, while SSRIs are said to cause a downregulation of somatodendritic $5HT_{1A}$ receptors [69]. In either case, these effects are seen after 14 days of treatment with antidepressants, but not after single doses.

More recently, there have been hypotheses explaining that antidepressants are effective in depression through mechanisms independent of monoaminergic systems, such as alterations in the expression of glucocorticoid receptors [70] and pathways involved in

neuronal plasticity and cell survival [71]. These hypotheses, particularly the former, relate to the hypercortisolemia seen in depression [72,73] that may be of pathophysiological importance [74]. In common with proposed monoaminergic mechanisms of action, these effects of antidepressants are likely only to become apparent after at least several days of treatment. As such, they are also able to explain the clinical delay in response seen in depressed patients.

B. Obsessive-Compulsive Disorder

The pathophysiology of OCD is far from clear. However, PET imaging studies have suggested that the disorder is associated with increased activity in orbitofrontal-subcortical circuitry [75,76], which appears to normalize upon successful treatment of the disorder [77]. How this might relate to possible 5HT abnormalities is unclear, but given the weight of clinical data regarding the efficacy of serotonergic antidepressants described above, 5HT systems seem to be closely involved at least in the successful pharmacological treatment of the condition.

One of the cornerstones of the proposed mechanism of action of antidepressants in depression is that animal investigations demonstrate adaptive changes to occur over 1 or 2 weeks; this is similar to the time course seen in the clinical response in depression. However, as discussed above, the clinical response in OCD tends to take a much longer period of time, and this is at odds with the earlier animal data published in investigations concerned with depression. However, recent investigations have shown that administration of an SSRI desensitizes 5HT autoreceptors and increases 5HT release in the orbitofrontal cortex after 8 weeks of treatment, but this effect is not seen after 3 weeks [2]. This is of particular interest given the findings suggesting the importance of this region in OCD [75]. In addition, this effect also appears to require larger doses of SSRIs that are effective in the treatment of depression [78]. Therefore, it may well be the case that some antidepressants are clinically effective in OCD at high doses because of their ability to desensitize 5HT autoreceptors in the orbitofrontal cortex, as opposed to effects seen at lower doses on other 5HT pathways that are important in the relief of depression.

C. Panic Disorder

Many systems have been hypothesized as involved in the pathophysiology of panic disorder [79]. However, given the evidence illustrating a preferential effect of serotonergic versus norepinephrinergic antidepressants, the 5HT system is likely to play a major role. Two possibly conflicting hypotheses have been proposed.

The first posits that panic disorder results from an excess of 5HT and/or a supersensitivity of postsynaptic 5HT receptors. This is supported by findings that fenfluramine, which induces neuronal release of 5HT, increases the level of anxiety in panic disorder patients [80]. In addition, the nonselective $5HT_2$ agonist mCPP increases anxiety in healthy subjects as well as in patients with panic disorder [81]. The second 5HT hypothesis of panic disorder is the complete reverse, postulating that the disorder arises because of a 5HT deficit. Deakin and Graeff [64] have proposed that the serotonergic input to the periaqueductal gray matter (PAG) is particularly involved in the mediation of panic. The PAG receives its 5HT input from the dorsal raphé and it is hypothesized that increased 5HT activity to the PAG reduces panic-like response. It is important to note that this proposed pathway (dorsal raphé to PAG) is different from that hypothesized by the same authors as being involved in depression (median raphé to hippocampus) [64]. This 5HT

deficit hypothesis is supported by findings that an infusion of tryptophan appears to decrease anxiety in panic disorder patients [82]. Further, while tryptophan depletion does not lead to panic attacks directly, it does make patients more sensitive to panicogenic challenges [83].

On theoretical grounds, the action of SSRIs in alleviating panic disorder is explicable on the basis of either a 5HT excess or a deficit hypothesis. In the former case, SSRIs may initially increase anxiety [84] because of increased postsynaptic availability. However, the delayed therapeutic response may result from a downregulation of postsynaptic 5HT receptors, compensating for their pathological supersensitivity and/or an excess of 5HT. Conversely, if panic disorder results from a 5HT deficit, then SSRIs may act by repeated administration, leading to a downregulation of somatodendritic $5HT_{1A}$ autoreceptors in the raphé, reducing feedback inhibition of the 5HT system and so increased 5HT availability in PAG. This is a similar explanation as that for the action of antidepressants on the 5HT system in depression, except that it is an action on somatodendritic $5HT_{1A}$ autoreceptors in dorsal, rather than median, raphé nuclei that is important for the treatment in panic disorder.

D. Post-Traumatic Stress Disorder

One of the most prominent areas of research into the pathophysiology of PTSD is in relation to abnormalities in the hypothalamic–pituitary–adrenal (HPA) axis. These studies have revealed striking differences compared with depression. Severe depressive illness is associated with hypercortisolemia [72,73] and cortisol nonsuppression in the dexamethasone suppression test (DST) [85]. The hypercortisolemia may arise because of a hypothalamic overdrive because raised corticotrophin-releasing hormone (CRH) is also found in the CSF of depressed patients [86]. Alternatively (or additionally), raised cortisol and an abnormal DST may arise because of abnormal or reduced glucocorticoid receptors, which are involved in the feedback control of the HPA axis, because these are found to be reduced in lymphocytes [87] and in the brains of suicide victims [88]. The situation in PTSD is rather different. Patients with this condition have been found to have increased CRH in CSF, as in depression, but this is associated with normal or even low cortisol levels [89]. In addition, the DST in PTSD patients is either normal [90] or even enhanced [91], regardless of comorbid depression. These findings have been suggested to result from an increase in glucocorticoid receptors, which has been demonstrated in lymphocytes [92].

Given these differences in pathophysiology between depression and PTSD, it seems unlikely that antidepressants act in identical ways in the two conditions. Specifically, the hypothesis that antidepressants act in depression by increasing the expression of glucocorticoid receptors [70] seems to be an unlikely mechanism of action in PTSD, where these receptors may already be overexpressed.

With regard to 5HT systems in PTSD, it has been demonstrated that there is a reduction in 5HT uptake sites on platelets in patients, regardless of comorbid depression [93]. Investigations in depressed patients show a similar change [94,95]. In both cases, there appears to be a normalization of 5HT uptake sites upon successful treatment with an SSRI. This argues for a possible similar mechanism of action of antidepressants in PTSD and depression. However, there is also evidence of an abnormal sensitivity of postsynaptic $5HT_2$ receptors in PTSD similar to that seen in panic disorder, in that mCPP provokes symptoms of PTSD in patients [96]. Therefore, it may be the case that the therapeutic effect of antidepressants in PTSD may involve similar 5HT pathways as are relevant

in panic disorder—that is, an effect on dorsal raphé projections to PAG rather than median raphé projections to the temporal lobe.

IV. SUMMARY

Because antidepressants are effective in the treatment of depression and a number of anxiety disorders, it has been postulated that similar pathophysiological mechanisms may underlie both conditions [97]. This seems unlikely to be the case. As described here, there are differences in the spectrum of drugs that are effective in treating depression and a number of anxiety disorders. Furthermore, the clinical response varies according to the diagnosis of the patient, with differing time courses and core symptoms of the anxiety disorders being differentially affected relative to comorbid depressive symptoms. In addition, there is evidence for several anxiety disorders of a distinct psychopathology that is separate from that found in depression. Most of the systems postulated to be involved in the pathophysiology of these conditions, for example, the 5HT system, are extremely complex with multiple pathways. It seems likely that the differences in clinical response may reflect differential effects of antidepressants on different systems and/or pathways. Given the frequent comorbidity of depression and many of the anxiety disorders, together with the nosological difficulty in defining such disorders as generalized anxiety, panic, and social phobia, it is sometimes hard to draw definitive conclusions. Nonetheless, there is no reason to suppose that, because a particular patient responds to an antidepressant, this agent is acting in one specific way in every case. Benefits may result from the action of the drug on several distinct neurochemical pathways.

It is unfortunate that the group of drugs that we know as antidepressants was termed in this way. Their efficacy in several conditions other than depression, probably by acting in a way that is distinct from the way they act in this disorder, clamor for a change in name. It is difficult to characterize these agents simply from the description of a simple pharmacological action (e.g., specific serotonin reuptake inhibition) because their efficacy in some/all conditions may or may not reside in this action. All involved in this rapidly developing field should be aware of the many-faceted actions of the antidepressant drugs and beware of attributing their actions to alleviation of depression alone.

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Studies on the Neurobiology of Depression

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I. INTRODUCTION

Major depressive disorders are common, severe, chronic and often life-threatening illnesses. It is estimated that more than 15% of all adults will experience a major depressive episode at some point in their lives [155,280]. Suicide is estimated to be the cause of death in up to ~15% of individuals with major depressive disorders and, in addition to suicide, many other deleterious health-related effects are increasingly being recognized [231,309]. Indeed, there is a growing appreciation that, far from being diseases with purely psychological manifestations, major depressive disorders are systemic diseases with deleterious effects on multiple organ systems [231,309]. For example, major depressive disorders represent a major risk factor for both the development of cardiovascular disease, as well as for death after an index myocardial infarction [231,309]. Furthermore, a recent study, which controlled for physical illness, smoking, and alcohol consumption found that the magnitude of the increased mortality risk conferred by the presence of high depressive symptoms was similar to that of stroke and congestive heart failure [309]. The cumulative effects of recurring bouts of affective episodes lead to an increased rate of marital and family breakdown, unemployment, impaired career progress, and consequent financial difficulties. The costs associated with disability and premature death from major mood disorders represent an economic burden of tens of billions of dollars annually in the United States alone [126,378].

Despite the availability of a wide range of antidepressant drugs, clinical trials indicate that 30 to 40% of depressed patients fail to respond to first-line antidepressant treat-

ment despite adequate dosage, duration, and compliance [344]. There is mounting evidence that recurrent mood disorders—once considered "good prognosis diseases"—are, in fact, often very severe and life-threatening illnesses. For many patients, the long-term outcome is often much less favorable than previously thought, with incomplete interepisode recovery, and a progressive decline in overall functioning observed [347]. While there is often a complete remission of symptoms, and general functioning returns to premorbid level in some patients, in a large proportion of cases, some symptoms of the episode persist for long periods of time and functioning may be severely impaired. Indeed, according to the Global Burden of Disease Study, mood disorders are among the leading causes of disability worldwide and are likely to represent an increasingly greater health, societal, and economic problem in the coming years [230]. Despite the devastating impact that these diseases have on the lives of millions worldwide, there is still a dearth of knowledge concerning their underlying etiology and pathophysiology. This dearth of knowledge regarding the cellular underpinnings of depression has undoubtedly contributed to the lack of development of new treatments for depression. Studies on pathophysiology of mood disorders involve genetics, neurotransmitter systems, intracellular signal transduction pathways, neurotrophic factors, neuroimaging, endocrine abnormalities, neurodegeneration, vascular disease and increased platelet aggregability, pineal function and circadian rhythm, and immunological function. This chapter will review studies on the pathophysiology of major depression.

II. GENETICS OF MOOD DISORDERS

Genetics undoubtedly plays a major role in the etiology of mood disorders. By determining the rates of illness in different types of relatives, genetic epidemiological studies can provide information about the familial and genetic nature of a disorder (e.g., depression). The probability that a person will develop a mood disorder is influenced by a number of factors, including premature death of a parent, a history of traumatic event, inadequate rearing by parents, poverty, malnutrition, medical illness (e.g., diabetes), personal or family history of affective episodes, extent of psychosocial support, and recent stressful life events [152]. In terms of the genetic component of major depression, such studies have been conducted and provide much information about the genetic transmission of mood disorders. Three types of studies have been used to determine the role of genetic factors in mood disorders: (1) family studies; (2) twin studies; (3) adoption studies.

A. Family Studies

Family studies address the question of whether a disorder is familial. These types of studies determine whether the rate of depression in the family members of the probands with the disorder is greater than that of the general population. The studies conducted on the families of probands with unipolar depression reveal morbid risks for depressive disorders among first-degree relatives, which are two to three times those of the general population. These data argue strongly for a genetic component of mood disorders. Depressive disorders have also been reported to occur at a higher rate in the families of probands with bipolar disorders, and the rate of bipolar disorder is also elevated in the families of probands with depressive disorders. The morbid risk of bipolar disorder in first-degree relatives of bipolar disorder probands is reported to range between 3 and 8%. This rate contrasts markedly with the 1% rate in the general population. Weissman and colleagues

[364] reported that the first-degree relatives of patients with bipolar disorder were at least 24 times more likely to develop bipolar disorder than relatives of control subjects. A recent meta-analysis found that, across five family studies, there was strong evidence for an association between major depression in the probands and major depression in first-degree relatives [336]. However, family studies cannot establish that a disorder is hereditary, as familial aggregation of a disorder (e.g., major depression) may reflect a shared environment [255]. Other types of genetic studies address some of the limitations of family studies.

B. Twin Studies

The family study data clearly indicate that affective disorders are familial. However, such studies cannot distinguish whether genetic or environmental factors are responsible for familial transmission. Families might share a variety of different environmental factors that could transmit the illness. Such factors might be common exposure to a particular culture, diet, behavior, virus, toxin, or other brain insult. Twin studies provide a powerful approach to teasing out genetic from environmental factors. Twin studies compare the concordance rate for illness in pairs of monozygotic (identical) twins with the rate of dizygotic (nonidentical) twins. The rationale is that monozygotic twins share identical genes, whereas dizygotic twins share only half their genes. Typically, twin pairs are selected who have been raised together, so that environmental factors are shared equally. Thus, if monozygotic twins show a greater concordance rate for a particular psychiatric disorder than do dizygotic twins, this is believed to be evidence for a genetic contribution to development of the disorder. The marked difference in concordance rates between monozygotic and dizygotic twins strongly supports the hypothesis of a major genetic contribution to the development of unipolar depression [151,152,213,214]. These studies find that the concordance rate for mood disorder in monozygotic twins is two to four times that in dizygotic twins. Since the concordance rate for monozygotic twins is not 100%, other nonheritable environmental factors are at work and also play a significant role in mood disorders.

C. Adoption Studies

Adoption studies provide an attempt to tease out the genetic and environmental factors in familial transmission. Mendlewicz and Rainer [220] found a threefold increase in the rate of bipolar disorders in biological relatives of probands with bipolar disorders. They also found a twofold increase in the rate of depressive disorders in biological relatives [220]. Similarly, Wender and colleagues [362] found evidence for a genetic effect on the transmission of major depression. The authors reported a threefold increase in the rate of unipolar disorder and a sixfold increase in the rate of completed suicide in the biological relatives of mood disorder probands. Using offspring of psychiatrically disturbed and normal biological parents, adopted at birth, Cadoret found that the incidence of depression was significantly higher in the affective-parented adoptees (3 depressed of 8 adoptees) than in the remaining adoptees whose biological parents had other psychiatric conditions or were apparently psychiatrically well (8 depressed of 118). The results suggest a genetic factor in affective disorders. In contrast, the study by von Knorring et al. [358] did not find an increase in mood disorders in the biological parents of adoptees with mood disorders. Unfortunately, this method of study has not been pursued much in the last two decades, thus limiting the amount of data with which to make any firm conclusions.

III. MODE OF TRANSMISSION

Although the genetic basis for affective disorders has been recognized, the exact mode of transmission remains unknown. An initial report in the Amish community found that the gene responsible for manic-depressive illness was found to be positioned on chromosome 11 and was inherited by autosomal dominant transmission [107]. Unfortunately, subsequent analysis on an expanded data set of the same population was unable to confirm the investigators' original findings [149]. Other investigators have reported linkage between bipolar disorder and the X chromosome [45], chromosome 4 [44], chromosome 5 [71], chromosome 6 [348], chromosome 11 [107], chromosome 12 [80], chromosome 16 [112], chromosome 18 [36], chromosome 18q for bipolar II [217], and chromosome 21 [335]. Recently, Souery and colleagues [328] failed to detect an association between the A2186C polymorphism of the trytophan hydroxylase gene and bipolar and unipolar affective disorders.

While the study of the mode of transmission in mood disorders has certainly advanced in the past decade, the exact mode of transmission still remains unknown.

A. Neurotransmitter Hypothesis

1. Norepinephrine and Adrenergic Receptors

The noradrenergic system was one of the first neurotransmitter systems studied in the pathophysiology of depression. Early theories of depression postulated that an imbalance in the metabolism of norepinephrine was responsible for affective disorders [56,305]. The hypothesis that the noradrenergic system was responsible for the pathophysiology of affective disorders was referred to as the "catecholamine hypothesis" of affective disorders. The noradrenergic dysfunction in the pathophysiology of depression has been reported to occur at many different levels, including problems with precursor availability, neurotransmitter synthesis, storage, release, presynaptic autoreceptor function, neurotransmitter reuptake, metabolism, and postsynaptic neurotransmitter receptors.

Norepinephrine, epinephrine, and dopamine are all catecholamines, and the principal metabolite of norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), is generally considered to reflect presynaptic activity of noradrenergic neurons. This metabolite can be measured in cerebrospinal fluid (CSF), plasma, and urine. In these earlier studies, MHPG was found to be elevated in CSF in patients with depression, mania, and schizoaffective disorder [305]. The predictive value of CSF levels of the norepinephrine metabolites and response to antidepressant treatment has received much attention. The CSF level of norepinephrine metabolites has been studied in patients receiving treatment with tricyclic antidepressants (desipramine, nortriptyline, amitriptyline), maprotiline, bupropion, flouxetine, and fluvoxamine. Asberg and colleagues [13] reported that treatment with clomipramine in 14 depressed patients resulted in significant decreases in the levels of 4-hydroxy-3-methoxyphenyl glycol MHPG. Aberg-Wistedt and colleagues [1] reported that high levels of MHPG in CSF in depressed patients at baseline predicted a better response to desipramine than those with low levels of this norepinephrine metabolite. Dahl and colleagues [81] reported that in spite of a significant improvement in depressive symptoms in depressed outpatients treated with desipramine 150 mg/day for 6 weeks, there were no consistent changes in MHPG in CSF. In addition, the pretreatment level of CSF MHPG had no predictive value for the outcome of desipramine treatment. Mendlewicz and col-

leagues [221] reported that treatment with 150 mg/day amitriptyline for 14 days in depressed inpatients with moderate-to-severe illness resulted in a significant decrease in CSF levels of MHPG. However, they did not find a relationship between therapeutic response, plasma antidepressant levels, or pretreatment CSF MHPG levels in this small group of patients. Nordin et al. [246] reported that treatment with nortriptyline in depressed patients for 3 weeks was associated with a 31% mean decrease in the MHPG in CSF. The same group [247] later reported an 18% mean decrease in MHPG in CSF in five depressed patients treated with E-10-hydroxynortriptyline (the major metabolite of nortriptyline). Timmerman and colleagues [346] found no correlation in clinical response and CSF MHPG in depressed patients treated with maprotiline for 3 weeks. Golden and colleagues [123] reported that there was a trend toward a reduction in CSF MHPG following successful bupropion treatment in depressed patients, although this change did not achieve statistical significance. Recent studies have documented that fluvoxamine or fluoxetine treatment of depression is associated with significant reductions of CSF MHPG concentrations, confirming the notion that even selective serotonergic antidepressants may ultimately affect the noradrenergic system [317]. Martensson et al. [200] reported that CSF MHPG concentrations decreased significantly in depressed patients treated with the serotonergic antidepressant fluoxetine. Similarly, DeBellis et al. [86] reported that CSF MHPG levels for nine depressed patients, although no different from those of control subjects, decreased significantly after treatment with fluoxetine.

Some authors [315] have suggested that caution should be exercised when interpreting CSF MHPG levels in depressed patients without correction for the diffusion of MHPG across the blood–brain barrier. Kopin and colleagues [162] reported that concentrations of free MHPG in the plasma and CSF are highly correlated, but that concentrations in the CSF are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from a tumor of the adrenal medulla. MHPG that is synthesized, but not catabolized, in the CNS maintains CSF fluid levels at an increment over those in plasma.

Elevated plasma norepinephrine and norepinephrine metabolite concentrations [180,289,354], and elevated urinary concentrations of norepinephrine and its metabolites, have been reported in patients with unipolar depression. Rudorfer and colleagues [292] reported that supine plasma norepinephrine levels were significantly lower in bipolar patients than in either unipolar depressives or normal volunteers. Following the orthostatic challenge, the fractional norepinephrine increase in both patient groups exceeded that in the controls. Siever and colleagues [321] reported that plasma MHPG responses to clonidine were reduced in depressed patients compared to controls, suggesting a reduced responsiveness of α_2-adrenergic receptors in depression. While depressed patients have high basal plasma concentrations of norepinephrine, those with melancholia are reported to have even greater elevations in plasma norepinephrine levels when subjected to orthostatic challenge than do either nondepressed control subjects or depressed patients without melancholia [288]. Furthermore, depressed patients who are dexamethasone (DST) nonsuppressors have significantly higher basal and cold-stimulated levels of norepinephrine than do depressed patients who are DST suppressors [288]. Following treatment with tricyclic antidepressants (TCA), urinary excretion of norepinephrine and its metabolites diminishes with plasma norepinephrine concentrations [65,123].

The association between CSF and plasma norepinephrine metabolites and suicide has not been thoroughly examined. Secunda and colleagues [302] reported that in de-

pressed inpatients who participated in the NIMH Collaborative Study on the Psychobiology of Depression that the CSF and plasma MHPG did differentiate between suicide attempters and nonattempters.

Initial research examining urinary MHPG levels attempted to stratify patients with unipolar depression based on MHPG values. Patients with unipolar depression have been classified as having low, intermediate, or high urinary MHPG values [307]. Those patients in the low category are considered to have diminished activity of the noradrenergic system and are reported to respond to treatment with tricyclic and tetracyclic antidepressants and fluoxetine [137,185]. Patients with unipolar depression and high MHPG values are believed to have increased activity of presynaptic noradrenergic neurons. These groups of patients are also reported to have high plasma cortisol levels, to be nonsuppressors on the DST tests, and to be resistant to treatment with conventional antidepressants. The utility of intermediate MHPG values in unipolar patients remains unknown.

Aberg-Wistedt et al. [1] reported that in depressed patients urinary excretion of MHPG decreased significantly during desipramine treatment. Bipolar patients are reported to have significantly elevated plasma norepinephrine and epinephrine concentrations during manic episodes than during depression or euthymia [191]. Manic patients also exhibit significantly increased urinary concentrations of norepinephrine in comparison to depressed patients or control subjects [338]. In a later study, Swann and colleagues [339] noted that an individual's "environmental sensitivity" had a significant effect on urinary norepinephrine excretion. Manic patients whose episodes were environmentally sensitive demonstrated elevated norepinephrine excretion, compared with patients with manic episodes that were unrelated to external stressors.

Much research has emphasized the quantitation of catecholamine metabolites to obtain more homogeneous subtypes of depressed patients. With regard to urinary excretion of MHPG, unipolar depressed patients are more heterogeneous than are bipolar I patients [184]. Plasma and urinary concentrations of MHPG were significantly elevated in bipolar patients when they were manic compared with MHPG concentrations when these patients were depressed [130]. Furthermore, depressed bipolar patients were found to have significantly diminished urinary MHPG concentrations than unipolar depressed patients or healthy control subjects [306].

More recent studies suggest that reduced urinary MHPG concentrations are characteristic of bipolar I depressed patients but not bipolar II depressed patients, with MHPG levels similar to those of patients with unipolar depression [303]. Patients with unipolar depression generally have greater urinary MHPG concentrations than bipolar I patients. Kopin and colleagues [163] argue that the measurement of urinary MHPG may have little value in assessing the noradrenergic system because less than 20% of MHPG is derived from brain norepinephrine metabolism. Furthermore, because unconjugated MHPG is readily diffusible, there is a free exchange of this metabolite among plasma, CSF, and nerve tissues (including brain and spinal cord). The CSF MHPG levels, after correction for the plasma contribution of the metabolite, more accurately reflect its rate of formation in the CNS. Hollister and colleagues [137] have also emphasized early on that collecting urine for MHPG determination in depressed patients is not easy, that the variability of excretion within patients is considerable, and the range of MHPG excretion closely parallels that in normal persons. Tsuji et al. [349] found correlations among the CSF concentrations of MHPG and 3,4-dihydroxyphenylglycol (DHPG) in CSF and plasma, but no significant correlations were found between the urinary levels and either plasma or CSF levels of these metabolites. Furthermore, neither the MHPG nor DHPG levels in the urine of

depressed patients revealed any significant correlation with their clinical assessments using the Hamilton rating scale score.

The relationship between sympathoadrenal and hypothalamic–pituitary–adrenal (HPA) axis activity has also been an active area of investigation in patients with major depression. Several [303], but not all [186], investigators have reported significant positive correlations between urinary cortisol and urinary MHPG concentrations in depressed patients. A similar positive correlation has been reported for plasma cortisol and epinephrine concentrations [334]. Previous studies regarding the levels of CSF norepinephrine and CSF corticotropin-releasing hormone (CRH) receptor have reported normal, reduced, and increased levels [118,234,270,288]. Recently, Wong and colleagues [376] reported that, around the clock, patients with melancholic depression have elevated levels of CSF norepinephrine and plasma cortisol, but not CSF CRH or plasma adrenocorticotropic hormone (ACTH).

In summary, the data suggest that plasma or CSF noradrenergic metabolites (MHPG and DHPG) provide more important information than those in the urine. However, these types of studies have fallen into disuse over recent years and have not been replicated. Over the last several years, there has been a steady shift in utilizing less invasive, in vivo, high-resolution methods (functional magnetic resonance imaging, positron emission tomography, and single-photon-emission computed tomography) to study the catecholamine function in the CNS of depressed patients instead of peripheral measures of catecholamine function.

Additional evidence of noradrenergic dysfunction in the pathophysiology of depression comes from studies that use α-methylparatyrosine (AMPT) to deplete central norepinephrine stores [225]. In these studies, administration of AMPT to depressed patients who had been successfully treated with desipramine or mazindol (both are NE uptake inhibitors) resulted in a rapid return of depressive symptoms. Interestingly, administration of AMPT to depressed patients did not worsen the core symptoms of depression but did cause worsening of some neurovegetative symptoms, in particular, anergy and tiredness [225].

Neurotransmission in the noradrenergic system is mediated by a number of different neurotransmitter receptors whose function has been implicated in either the pathophysiology of depression or in the mechanism of action of antidepressants. The α_2-adrenergic receptors and β-adrenergic receptors have been the focus of considerable research on their role in the pathophysiology of depression. Increased α_2-receptor density is seen in the brains of suicide victims [218]. Treatment with antidepressants has been associated with decreases in the density and sensitivity of these receptors on platelets. Several methods have been used to investigate the role of the α_2-receptor in depression. An indirect method for studying α_2-receptors is to challenge subjects with the α_2-receptor agonist clonidine. Administration of clonidine induces growth hormone secretion, primarily through postsynaptic α_2-receptors. Several abnormalities in growth hormone release in patients with major depression have been reported. Growth hormone response to clonidine is blunted in unipolar depressed patients [202,321,322], supporting the concept of decreased responsiveness of postsynaptic α_2-adrenergic receptors in depressed patients.

Charney and colleagues [66] reported that long-term desipramine treatment significantly attenuated the effects of clonidine on plasma MHPG level and blood pressure, indicating that during desipramine treatment α_2-adrenergic receptors had become subsensitive. In addition, they reported that plasma MHPG levels were significantly reduced during long-term desipramine treatment. Neuroendocrine tests have also been used in an attempt

to better subtype depression. Price et al. [273] reported that the cortisol response to yohimbine (an α_2-adrenergic antagonist) was significantly greater in depressed patients than in controls, despite similar MHPG responses between groups.

Maes and colleagues [189] reported that major depression is accompanied by decreased platelet α_2-adrenoreceptor density, and that subchronic treatment with TCAs, but not fluoxetine, resulted in a decreased affinity of rauwolscine (a selective α_2-adrenergic antagonist) for platelet α_2-adrenoceptor. Another method of examining the function of α_2-receptors is to measure intracellular response following α_2-receptor activation. Garcia-Sevilla and colleagues [116] measured the functional status of α_2-receptors in depressed patients by assessing both inhibition of adenylate cyclase activity and induction of platelet aggregation. They concluded that the latter measure was a better marker to assess changes in the α_2-receptor in depressed patients.

The β_1- and β_2-adrenergic receptor subtypes are found postsynaptically and also stimulate the intracellular adenylate cyclase system. Preclinical studies have shown that chronic antidepressant administration produces desensitization of the β-adrenergic receptor. The β-adrenergic receptor downregulation hypothesis posits that the mode of action of clinically effective antidepressants is by decreasing the number of β-adrenergic receptor sites. Initial measurements of these β-adrenergic receptors in depressed patients have yielded mixed results. Biegon et al. [37] reported a significant, 50% increase in β-adrenergic receptor density found in prefrontal cortical homogenates in the postmortem brain tissue of suicide victims. They reported that the increased binding is selective, appearing in some cortical regions but not in basal ganglia or white matter areas. Mann and colleagues [197] also reported increased β-adrenergic receptor density in post-mortem brain tissue of suicide victims. However, Crow and colleagues [79] demonstrated decreased density of hippocampal β-adrenergic receptors in the postmortem brain tissue of depressed patients who had been hospitalized. In this latter group, previous antidepressant treatment may have induced β-adrenergic receptor downregulation. Similarly, contradictory results have been reported from studies determining the B_{max} (number of binding sites) for β-receptors on leukocytes. Some investigators have reported reduced B_{max} for peripheral β-receptors in depressed patients, but others have documented no difference between depressed patients and healthy control subjects [132]. Monoamine oxidase (MAO)-A is an important target of a number of antidepressant drugs that inhibit its activity. Based on this pharmacological action and other evidence, a number of researchers reasoned that depression may have its biological basis in disruption of central monoamine systems. A number of investigators have measured MAO activity in brains from suicide victims and nonsuicide control subjects. Mann and Stanley [197], Sherif et al. [319], and Galva et al. [115] demonstrated no change in MAO-A activity in the frontal cortex of suicide victims compared to that of control subjects. Ordway and colleagues [253] also failed to find any differences in MAO-A distribution at any level of the locus ceruleus or raphe nuclei between subjects with major depression or psychiatrically normal controls.

Lecrubier et al. [172] supported the noradrenergic hypothesis of depressive states, especially the involvement of β-adrenergic receptors in depression. They reported that the use of salbutamol, a β-adrenergic stimulant, depressed patients had in some cases led to a clear and rapid antidepressant effect.

2. Serotonin

The serotonergic system has also received much study in the pathophysiology of depression. The hypothesis that the serotonin is responsible for the pathophysiology for affective

disorders has been referred to as the "serotonin hypothesis" of affective disorders [219]. There is considerable evidence of abnormalities in the serotonergic neurotransmitter system in patients suffering from depression [219]. The serotonergic dysfunction in the pathophysiology of depression has been reported to occur at many different levels, including problems with precursor availability, neurotransmitter synthesis, storage, release, presynaptic autoreceptor function, neurotransmitter reuptake, metabolism, and postsynaptic neurotransmitters receptors.

Serotonin is synthesized from the amino acid tryptophan, which is derived from the diet. The rating-limiting step in serotonin synthesis is the hydroxylation of tryptophan by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan. Under normal circumstances, this rating-limiting enzyme is not saturated by substrate, so tryptophan concentration can impact the rate of synthesis. Tryptophan is then taken up into brain via a saturable carrier mechanism. Tryptophan actively competes with other large neutral amino acids for transport; brain uptake of tryptophan is determined both by the amount of circulating tryptophan and by the ratio of tryptophan to the other large neutral amino acids. This ratio may be affected (elevated) by carbohydrate intake, which induces the release of insulin and the uptake of many large neutral amino acids into peripheral tissues. High-protein foods tend to be relatively low in tryptophan, thus lowering this ratio. The administration of specialized low-tryptophan diets has been found to produce significant declines in the brain serotonin levels.

Pretreatment plasma tryptophan has been reported to be lower in depressed patients than in healthy controls and to differentiate certain subgroups of depression [187,219]. Depressed patients exhibit reduced plasma concentrations of 5-hydroxytryptophan after ingestion of test doses of oral L-tryptophan [82]. Other studies report that, in normal control subjects, lowering of plasma tryptophan via dietary manipulation produces a depressed mood [89]. The occurrence of depression following acute tryptophan depletion has been reported to occur more commonly in healthy subjects with first-degree relatives with affective disorders than in healthy subjects with no family history of a mood disorder [33,109]. Lower pretreatment plasma tryptophan has been reported to predict antidepressant treatment [181,227]. The depletion of dietary L-tryptophan has also been reported to induce relapse in recently remitted depressed patients [89,239]. In contrast to these findings, several studies have recently found that the effect may be less consistent than previously reported. Moore and colleagues [228] observed no effect on mood in fully remitted patients medicated with selective serotonin reuptake inhibitors (SSRIs). Leyton and colleagues [177] also reported that acute tryptophan depletion did not induce relapse or change in mood in fully remitted, medication-free, former patients with major depression. In another study [1] of patients who had responded to treatment with citalopram, only 5 of 12 patients relapsed, and the effect seemed to be clinically significant in only 1 patient. While depletion of dietary L-tryptophan has been reported to induce relapse in recently remitted depressed patients, Cassidy and colleagues [63] did not find that depletion of L-tryptophan results in relapse in patients who had successful treatment with ECT. These previous studies suggest that acute tryptophan depletion reflects a reversal of mechanisms involved in the therapeutic effects of antidepressants but not of ECT. Depletion of plasma tryptophan has been reported to cause dysphoria in the first-degree relatives of patients with depression.

A series of neuroendocrine challenge paradigms have been conducted to more closely examine the presynaptic serotonergic neurons in patients with depression. In normal subjects, the intravenous infusion of tryptophan increases prolactin plasma levels

[274]. However, in depressed patients, this release of prolactin to intravenous tryptophan is blunted compared to healthy controls [62,274]. The neuroendocrine challenge with the appetite suppressant fenfluramine also produces similar results to the intravenous infusion of tryptophan. The administration of fenfluramine causes a rapid increase in the plasma levels of prolactin in normal healthy subjects. However, when fenfluramine is administered in depressed patients, the prolactin release is blunted [226,314]. The blunted prolactin response has been reported to normalize with successful treatment and has been proposed as a test for predicting response to antidepressant treatment [195,314].

Recent neuroendocrine challenge studies have incorporated the use of PET technology. Instead of measuring prolactin, PET scans measure the blood flows in response to fenfluramine challenge [198]. In this study, similar to the results obtained with serum prolactin levels, depressed subjects who were challenged with fenfluramine had a significant decrease in cerebral glucose utilization compared to normal controls. Another neuroendocrine challenge test that has been used is m-chlorophenylpiperazine (m-CPP) [8]. m-CPP has mixed pharmacological actions at the postsynaptic serotonin receptors. Two studies did not find differences in neuroendocrine measures between depressed and healthy controls following a challenge with m-CPP [8,274].

The utility of directly measuring 5-hydroxyindolacetic acid (5-HIAA), the principal metabolite of serotonin, in CSF in patients with depression remains unclear. Several studies have detected reduced CSF concentrations of 5HIAA in some depressed patients, in patients who committed suicide, particularly by violent means, independent of their psychiatric diagnosis, and in patients who had poor impulse control [353,356].

Another method for evaluating presynaptic serotoninergic function is by the measurement of serotonin transporter (SERT) binding. SERT's role is to pump serotonin from the synaptic space back to the presynaptic neuron. Since SERT is transcribed from a single copy gene, both the platelet and CNS SERT are identical. Thus, abnormalities in platelet SERT most likely reflect problems with CNS SERT. The SERT has been studied both in platelets and in post-mortem tissue of patients who were depressed at the time of death.

A number of studies on the concentration of the SERT on platelets have been measured with [^{3}H]-imipramine binding and [^{3}H]-paroxetine binding. The results of these studies have been mixed, with some studies reporting no differences in the B_{max} for the platelet SERT between depressed and healthy control subjects [50,51]. However, a large meta-analysis reported that the B_{max} value for platelets was significantly lower in depressed compared to healthy control subjects [110].

A number of studies investigating SERT in projection regions of serotonergic cell bodies in suicide victims with depressive disorder have been conducted on post-mortem brain tissue. The results of these studies are mixed. Early studies primarily focused on suicide victims and used [^{3}H] imipramine, a less than optimum radioligand, for measuring serotonin transporter. The earlier studies reported increases, decreases, or no change in [^{3}H] imipramine binding to frontal cortex in suicide victims [12,79,330]. More recently, other radioligands including [^{3}H] paroxetine, [^{3}H] citalopram, and [^{125}I] cyanoimipramine have been identified as superior ligands for measuring serotonin transporters [129]. Unfortunately, the results with these newer ligands in subjects with depression are also mixed. Some studies reveal either significant decreases [10,145] or no changes [40,197].

Several different serotonin receptor subtypes have been identified in recent years. Subtyping is based in part on the characteristics of binding to serotonin, other agonists, or antagonists. Three main classes—$5HT_1$, $5HT_2$, and $5HT_3$ receptors—are further subdivided into subtypes $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_{1F}$. The $5HT_2$ receptor appears to

be virtually identical to the $5HT_{1C}$ receptor; this class may be divided into $5HT_{2A}$ and $5HT_{2B}$ subtypes. The $5HT_{1A}$ receptor, in particular, has been implicated in the pathophysiology of mood disorders through evidence that major depressives have blunted physiological responses to $5HT_{1A}$-receptor agonists in vivo and abnormal $5HT_{1A}$-receptor binding postmortem [46,178,333]. During $5HT_{1A}$-receptor agonists challenge, the physiological increases of plasma concentrations of ACTH and cortisol are attenuated in unmedicated subjects with major depressive disorder [77]. Postmortem studies of cerebral $5HT_{1A}$-receptor binding and mRNA expression in major depressive disorders and bipolar disorder suggest $5HT_{1A}$-receptor dysfunction in mood disorders, but these data are limited to two studies involving small sample sizes [46,178]. Lopez and colleagues [178] showed $5HT_{1A}$-receptor mRNA levels were abnormally reduced in the hippocampus in six subjects with major depressive disorder who died by suicide, and Bowden et al. [46] found reduced $5HT_{1A}$-receptor binding to [^{3}H]8-OH-DPAT in the temporal polar and posterior ventrolateral prefrontal cortex in seven medication depressives with major depressive disorder or bipolar disorder dying of natural causes. Supporting the postmortem findings, Drevets and colleagues [98] reported that the regional $5HT_{1A}$-receptor binding of depressed subjects with primary, recurrent, familial mood disorders determined by using positron emission tomography was significantly reduced compared to healthy controls. The investigators found that the mean $5HT_{1A}$-receptor binding potential was reduced by 42% in the midbrain raphe and 25 to 33% in limbic and neocortical areas in the mesiotemporal, occipital, and parietal cortex.

Cowen and colleagues [76] have suggested that major depression may be associated with impaired sensitivity of $5HT_{1A}$ autoreceptors and that the decrement in serotonin neurotransmission at postsynaptic $5HT_{1A}$ receptors in depression is due to decreased serotonin release rather than impaired responsivity of postsynaptic $5HT_{1A}$ receptors.

Mann and colleagues [197] reported an increased number of postsynaptic $5HT_2$ receptors in the brains of depressed patients. Mattsubara and colleagues [203] documented an increase in the number of $5HT_1$ and $5HT_2$ receptors in the prefrontal cortex of suicide victims, although other investigators have not replicated this finding [332].

ECT has been reported to enhance central serotonergic responsivity [313]. Chronic administration of effective antidepressant therapy, including ECT, induces an upregulation in the number of $5HT_{1A}$ postsynaptic receptors [131]. Even antidepressants such as desipramine, which act primarily on norepinephrine neurons, appear to exert potent effects on $5HT_{1A}$ receptors [182]. Conversely, most antidepressants—including MAOIs, TCAs, SSRIs, nefazodone, and trazodone—induce downregulation of postsynaptic $5HT_2$ receptors [75,124]. One notable exception is ECT, which results in upregulation of $5HT_2$ receptors [174].

$5HT_2$-receptor binding to platelet membranes and serotonin-induced shape change and aggregation have been used to study $5HT_2$ receptors in patients with mood disorders. Biegon and colleagues [38] suggested that the increase in number of $5HT_2$ receptors on platelets may be viewed as a state-dependent marker in major depression. In their study of 15 depressed patients, 3 weeks of treatment with maprotiline were associated with significant decreases in $5HT_2$-receptor binding in responders; in contrast, receptor binding increased or remained the same in the nonresponders.

The human platelet concentrates serotonin from plasma via the serotonin transporter in a fashion similar to that seen in the brain, and therefore it has been posited as a model of central serotonin neurons. [^{3}H] Imipramine binds to the serotonin transporter on the presynaptic nerve terminal and on the platelet. A reduction in the number of the platelet

[^{3}H] imipramine binding sites in depressed patients has been reported by several groups [50,235]. Perry et al. [260] reported decreased density of [^{3}H] imipramine binding sites in the hippocampus and occipital cortex of depressed patients, confirming the original studies by Stanley and colleagues [330].

Recently, Malison and colleagues [194], observed decreased serotonin transporter binding in the midbrain of depressed patients compared to control subjects using [^{123}I] β-carbomethoxy-3 β-(4-iodo-phenyl) tropane [^{123}I] β-CIT) and SPECT imaging.

In recent years, [^{3}H] imipramine has been found to have less specificity than [^{3}H] paroxetine [236]. Nemeroff and colleagues [237] reported that [^{3}H] paroxetine-binding sites are reduced in the platelets of drug-free depressed patients. This may ultimately prove a more useful tool than [^{3}H] imipramine.

Brain $5HT_2$ receptors have also been examined in living depressed patients that used either single-photon-emission computed tomography or PET. Agren and colleagues [2] reported that a significantly lower uptake of [^{11}C] 5HTP across the blood–brain barrier in depressed patients, regardless of phase of illness compared to healthy volunteers. The authors suggest that the transport of 5HTP across the blood–brain barrier is compromised in major depression. In the only study that used single-photon-emission computed tomography, D'Haenen and colleagues [90] reported an increase in uptake of 2-ketanserin labeled with iodine 123 [^{123}I] in parietal cortex bilaterally and right greater than left asymmetry in the inferofrontal region of depressed patients compared with control subjects. A PET study [43] reported a decrease in uptake of altanserin labeled with fluorine [^{18}F] in the right anterior part of insular cortex and right posterolateral orbitofrontal cortex of depressed patients compared with controls. PET studies suggest mixed results, two [16,379] reported a decrease in [^{18}F] setoperone binding in the frontal cortex of depressed patients compared with control subjects, while the other [224] found no difference between the two groups.

The cortisol response to 5-hydroxytryptophan administration has been found to be enhanced in unmedicated depressed patients and to decrease following treatment with antidepressants [164]. Noting a significant negative correlation between baseline plasma cortisol levels and the cortisol response to 5-hydroxytryptophan in nondepressed control subjects, Koyama and Meltzer [164] postulated that serotonin may play an important role in the simulation of basal plasma cortisol secretion.

Because serotonin activates the HPA axis, increased serotonin activity could play a role in the increased HPA axis activity and pathogenesis of psychotic depression. In a review of the literature, Schatzberg and Rothschild [304] noted that increased serotonin uptake into platelets and increased CSF levels of 5HIAA have both been reported in patients with psychotic depression.

3. Dopamine

A relevant preclinical model derives from the crucial role of mesoaccumbens dopamine (DA) in the neural circuitry of reward and/or incentive motivational behavior [371]. Loss of motivation is one of the central features of depression, and indeed anhedonia is one of the defining characteristics of melancholia. Thus, a deficiency of DA systems stands out as a prime candidate for involvement in the pathophysiology of depression [368]. The strongest finding from clinical studies implicating DA in depression is reduced homovanillic acid (HVA), the major DA metabolite in the CSF of depressed patients. Indeed, this is one of the most consistent biochemical findings in depression [269]. There is also evidence of a decreased rate of CSF HVA accumulation in subgroups of depressed patients,

including those with marked psychomotor retardation versus agitation [366]. Furthermore, low levels of HVA may be associated with cognitive impairment, both in depressed patients and patients suffering from Parkinson's disease [372].

Preclinical studies show that chronic administration of antidepressants and electroconvulsive shock (ECS) enhances mesolimbic DA functioning [367]. Thus, in some animal models of depression, the efficacy of antidepressants involves increased transmission through DA synapses, particularly in the mesolimbic system [367]. Similarly, d-amphetamine-induced locomotor activity, a behavior dependent on the integrity of the mesoaccumbens DA neurons, is enhanced following chronic use of antidepressants. Since the responses to either DA or apomorphine (direct acting agonists) are also enhanced following chronic antidepressants use, they appear to be due, at least in part, to increased postsynaptic DA receptor sensitivity [192].

The mechanisms by which antidepressants enhance postsynaptic DA function in the accumbens remain unknown, and binding studies have failed to reveal consistent alterations in the density of either D_1 or D_2 receptors. However, recent studies on chronically stressed animals suggest that the effect may involve both D_1 and D_2 receptors or an interaction between them. Despite the opposite effects of D_1 and D_2 receptors on adenylyl cyclase activity, these receptors also couple to multiple G proteins and effectors such that D_1 stimulation can facilitate a variety of D_2 responses [196]. Furthermore, D_1- and D_2-receptor stimulation may be required for maximal production of vigilance, alertness, and more sensitive control of reactivity [251].

The preclinical study of ECS on dopaminergic function is especially relevant because ECT is especially effective in those psychiatric syndromes which likely involve impaired dopaminergic transmission: depression with psychotic features or marked psychomotor retardation, mania, and catatonia. Indeed, ECT is remarkably efficacious in the prototypic "dopamine deficiency" condition, Parkinson's disease [15]. A dramatic improvement in motor symptoms occurs with maintenance of a therapeutic regime of direct and indirect DA agonists, suggesting that ECT exerts some effects via postsynaptic mechanisms. Although many behavioral studies suggest enhancement of postsynaptic DA receptor sensitivity after ECS, there are conflicting reports about changes in presynaptic DA receptor sensitivity. Studies of D_2-receptor density have consistently failed to reveal any alterations following chronic ECS. Newman and Lerer [240] showed enhancement of D_1 stimulated adenylyl cyclase activity in the caudate following chronic ECS. In humans, ECT appears to increase DA receptor responsiveness, as indexed by apomorphine's effect on plasma prolactin [23]. Moreover, chronic ECT increases both CSF HVA and 5-hydroxy-indoleacetic acid (5HIAA), suggesting increased DA and serotonin (5HT) turnover [293].

Interestingly, monoamine oxidase inhibitors (MAOIs) constitute the only pharmacological monotherapy, which is reported to be effective in 50% or more of patients who fail to respond to the full range of tricyclic antidepressants. Nolen et al. [245] reported a controlled trial pointing to the superior efficacy of tranylcypromine (average dose of approximately 80 mg/day) in those patients. Tranylcypromine is superior to imipramine in chronic, mild unipolar patients [212] and in "anergic" bipolar depression [135,343], and phenelzine is superior in unipolar patients refractory to imipramine [210]. An open-label study of high-dose tranylcypromine (average 120 mg/day) in 14 unipolar patients who "had a clear history of nonresponse to at least two prior medication treatments" yielded an impressive 50% "complete" (21-item Hamilton depression rating scale score < 10) response rate [6]. It was speculated that "at higher plasma concentrations of tranyl-

cypromine, the inherent sympathomimetic (amphetamine-like) activity of the drug emerges." Studies with dopamine reuptake inhibitors such as nomifensine have shown clear antidepressant effects in major depression. Similarly, bromocriptine, a postsynaptic dopamine receptor agonist, has been reported to have efficacy comparable with standard tricyclic antidepressants [323] and to be useful in antidepressant-resistant depression [142], and in relapses that occur with SSRI treatment [211]. Other dopaminergic treatments, such as stimulants [243] and ECT [93], have also been reported to be useful for the treatment of depression in patients with Parkinson's disease.

Neuroimaging studies have been conducted to directly assess the in vivo availability of dopamine D_2 receptors in patients with major depression. Four studies compared the binding of the SPECT radiotracer [^{123}I]IBZM, a selective D_2/D_3 antagonist [169] in patients with major depression and control subjects. The results of these studies are mixed. Two of the four studies reported higher [^{123}I]IBZM specific binding in the striatum of depressed subjects compared to control subjects [91,311], whereas two studies reported no changes [106,161].

The in vivo binding of [^{123}I]IBZM is affected by competition with endogenous dopamine [170], and it has been proposed that increased [^{123}I]IBZM binding in depression might be due to decreased synaptic dopamine concentration and subsequent upregulation of D_2 receptors [91]. Thus, in vivo neuroreceptor binding techniques can be used to measure acute changes in the concentration of endogenous transmitters in the vicinity of receptors [171]. Psychostimulant-induced dopamine release was recently examined in patients with unipolar depression [256]. The authors did not find alterations in striatal D_2-receptor density in depressed patients and concluded that stimulant-induced dopamine release is not altered in major depression.

4. Acetylcholine

Janowsky and colleagues [146] postulated than an increased ratio of cholinergic to adrenergic activity may underlie the pathophysiology of depression, whereas the reverse occurs in mania. Although the adrenergic–cholinergic balance hypothesis appears incomplete in the face of increasing data on the preeminent role of alterations in serotonergic and noradrenergic neurotransmission in major depression, the importance of cholinergic mechanisms in some forms of depression is supported by both preclinical and clinical data.

Animal models of depression (e.g., the forced swim test) are exacerbated by the administration of cholinesterase inhibitors such as physostigmine [216], whereas muscarinic receptor antagonists reverse the effects of these agents. Additional evidence concerning the importance of acetylcholine in the maintenance of mood is the induction of depressed mood following administration of physostigmine or cholinergic agonists (e.g., arecoline) in euthymic control subjects [111], unipolar depressed patients [147], and bipolar manic patients [147]. Another neuroendocrine test that has been conducted to examine the acetylcholine system in depression is the enhanced growth hormone release in response to pyridostigmine challenge in depressed subjects relative to healthy comparison subjects. The investigators found that the mean delta growth hormone (the difference between basal and maximal growth hormone following pyridostigmine) was significantly greater than comparison subjects in patients with major depression. The responses observed in patients with schizophrenia and alcohol dependence syndrome did not differ from the comparison group. The sensitivity of the pyridostigmine/growth hormone test was 63% for major depression. These authors suggest that the pyridostigmine/growth hormone test may help

distinguish depression from schizophrenia, alcohol-dependence syndrome, or panic disorder [72].

Depressive symptoms, including psychomotor retardation and depressed mood, are often a complication of acetylcholinesterase inhibitor treatment of Alzheimer's disease. Such sensitivity to the mood-lowering effects of cholinergic drugs appears dependent on the presence of an underlying psychiatric disorder. If cholinergic mechanisms contribute in some manner to the pathophysiology of major depression, then anticholinergic drugs should be effective antidepressants. Although physostigmine-induced dysphoria may be reversed with atropine [111], little evidence indicates that anticholinergic medications have significant antidepressant properties [147]. Moreover, the newer antidepressants, including the SSRIs, have little affinity for muscarinic cholinergic receptors, yet remain effective antidepressants. Sitaram and colleagues [324] proposed that primary affective illness (specifically bipolar disorder) may be characterized by a state-independent cholinergic hypersensitivity in conjunction with a state-dependent noradrenergic supersensitivity (during late depression and early mania), which return to normal during remission.

5. γ-Aminobutyric Acid

γ-Aminobutyric acid (GABA) is the principal mediator of inhibitory synaptic transmission in the mammalian brain and, therefore, is likely to participate in wide-ranging aspects of both normal and abnormal CNS function. GABA regulates many CNS functions, such as seizure threshold, and it also has inhibitory input into other neurotransmitter systems such as norepinephrine and dopamine. Clinical and pharmacological data suggest that GABA metabolism may be altered in affective disorders.

Several lines of clinical investigation suggest that the GABA system may contribute to the pathophysiology of depression. Several clinical trials have demonstrated potent antidepressant and mood-stabilizing properties of several compounds with GABA-mimetic activity [46,259,264]. The anticonvulsants valproic acid and carbamazepine were serendipitously noted to have a mood-stabilizing effect, decreasing the intensity and frequency of manic and perhaps depressive phases of bipolar disorder. All antimanic agents (including lithium) have been suggested to stabilize mood in part by increasing GABAergic transmission, leading to the hypothesis that a relative GABA deficiency plays a role in mania.

Several investigators have reported that CSF and plasma GABA concentrations in depressed patients are significantly lower than in nondepressed control subjects; this finding has been replicated in several studies [261,262]. Low levels of GABA are not specific to cases of depression or mania but are also seen in alcoholism [263].

Recently, Sanacora and colleagues [297] measured in vivo brain GABA levels with proton magnetic resonance spectroscopy and found that the depressed patients demonstrated a highly significant (52%) reduction in occipital cortex GABA levels compared with the group of healthy subjects.

6. Glutamate

Excitatory amino acid receptors are the principal mediators of excitatory synaptic transmission in the mammalian brain and, therefore, are likely to participate in wide-ranging aspects of both normal and abnormal CNS function. Glutamate-mediated excitotoxicity has been implicated in certain neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease [67]. Glutamatergic neurotrans-

mission dysfunction has also been implicated in the pathogenesis of schizophrenia and obsessive-compulsive disorder, and is now being explored as a potentially novel mechanism of action for antipsychotic drugs [254,284]. Given the potential role of glutamate in CNS injury and neurodegenerative diseases, several treatment strategies have been implemented to reduce glutamate-mediated excitotoxicity. There is evidence suggesting that there may also be glutamatergic neurotransmission dysfunction in depressed patients. Glutamine plasma levels were found to be high in depressed patients compared with a population control group [156,201]. Altamura et al. [5], studying platelets and plasma in 15 patients with unipolar and bipolar depression and comparison subjects, found lower plasma glutamine levels in the depressed patient group compared with a neurological control group (10 subjects). Mauri et al. [204] found elevated plasma and platelet levels of glutamate in depressed patients compared to controls. Recently, Berk and colleagues [34] reported that the platelet glutamate receptors may be supersensitive in schizophrenia and depression with psychotic features, but not in mania with psychotic features compared to normal controls.

Functional imaging also suggests that the glutamatergic system may be involved in the pathophysiology of depression. Ongur et al. [252] reported a reduction of glial-cell mass in the subgenual frontal cortex of mood-disorder patients. This, and the absence of reports indicating an increase of glial cell mass in brain areas of affective patients, suggests that neuronal metabolic rather than glial processes are responsible for the high glutamine levels. A temporary reduction in a number of neurobiochemicals including myo-inositol (mI) and glutamate was noted in two patients with secondary depression during tamoxifen/Neupogen chemotherapy for breast cancer [74]. Auer et al. [17] found a significant decrease of glutamate in severely depressed patients in the anterior cingulate cortex. Levine et al. [176], using a proton magnetic resonance spectroscopy (MRS) technique found that, compared with the control group, the depressed patient group had significantly higher CSF glutamine concentrations. Disturbances in glutamatergic neurotransmission appear to be present early in life in patients with mood disorder. In one study, 10 children with bipolar affective disorder, aged 6 to 12 years, compared to a control group using proton MRS were found to have elevated levels of glutamate/glutamine in both frontal lobes and basal ganglia [64].

In postmortem studies, Nowak et al. [249] reported that the proportion of high-affinity, glycine-displaceable [^{3}H] CGP-39653 binding to glutamate receptors was reduced in suicide patients compared with control subjects.

In the 1950s, D-cycloserine (a partial agonist at the N-methyl-D-aspartate (NMDA)-receptor glycine site used as a part of multidrug antituberculosis treatment) was reported to have a mood-elevating effect [133]. Since then, there is increasing evidence for an association between alterations of brain glutamatergic neurotransmission and the pathophysiology of mood disorders. A growing body of preclinical research suggests that the NMDA class of glutamate receptors may be involved in the pathophysiology of major depression and the mechanism of action of antidepressants [325]. NMDA-receptor antagonists such as MK-801 and AP-7 have demonstrated antidepressant effects in animal models of depression, including the application of inescapable stressors, forced-swim, and tail-suspension-induced immobility tests in learned helplessness models of depression and in animals exposed to a chronic mild stress procedure [258]. Conversely, antidepressant administration has been shown to affect NMDA-receptor function [248,249] and receptor-binding profiles [257]. Furthermore, the role of glutamatergic dysfunction in depression

is further supported by the fact that repeated antidepressant administration regionally alters expression of mRNA that encodes multiple NMDA-receptor subunits [325] and radioligand binding to these receptors within circumscribed areas of the CNS [325].

Recently, Berman et al. [35] reported the first placebo-controlled, double-blind trial assessing the treatment effects of a single dose of an NMDA-receptor antagonist, ketamine, in seven patients with depression. The authors reported that subjects with depression evidenced significant improvement in depressive symptoms within a short period of time (72) after taking ketamine but not placebo. In this study, treatment with ketamine was associated with transient cognitive deficits, euphoria, and greater scores on the Brief Psychiatric Rating Scale, especially the positive symptoms. Similarly, another antiglutamatergic agent, lamotrigine, was found to be effective in acute bipolar depression [61].

B. Intracellular Signal Transduction Pathways and Depression

1. Stress and Depression

Preclinical and clinical studies demonstrate that neuronal atrophy and cell death occur in response to stress and in the brains of depressed patients. Although major depressive disorders undoubtedly have a strong genetic basis, recent evidence has shown that severe stressors are associated with a substantial increase in risk for the onset of major depressive disorder in susceptible individuals. In rodents, certain stressors are capable of producing dendritic atrophy, death, or endangerment of hippocampal CA3 pyramidal neurons [209]. Activation of the HPA axis appears to play a critical role in mediating these effects, since stress-induced neuronal atrophy is prevented by adrenalectomy, and increased by exposure to high concentrations of glucocorticoids [209]. These observations are noteworthy with respect to the pathophysiology of major depressive disorders, because a significant proportion of patients with major depressive disorder display an excessive activation of the HPA axis, and the subtypes of depression most frequently associated with HPA activation are those most likely to be associated with hippocampal volume reductions. The mechanisms by which glucocorticoids exert these deleterious effects on the hippocampus need to be fully elucidated, but likely involve the facilitation of glutamatergic signaling and inhibition of glucose transport [209]. The reduction in the resilience of hippocampal neurons may also reflect the propensity for various stressors to decrease the expression of brain-derived neurotrophic factor (BDNF) in this region [209].

In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume, cell number, and cell body size. Baumann and associates [27] reported reduced volumes of the left nucleus accumbens, the right putamen, and bilateral pallidum externum in postmortem brain samples obtained from patients with unipolar depression or bipolar disorder.

Several recent postmortem stereological studies of the prefrontal cortex also have demonstrated reduced regional volume, cell numbers, and/or sizes. Morphometric analysis of the density and size of cortical neurons in the dorsolateral prefrontal cortex and orbitofrontal cortices has revealed significant reductions in mood-disorder patients as compared to control subjects [276,277]. Marked reductions in the density of large neurons (corresponding to pyramidal glutamatergic excitatory neurons) were found in layers III and V of the dorsolateral prefrontal cortex in bipolar disorder and major depressive disorder. In other prefrontal regions such as rostral orbitofrontal cortex, the most prominent neuronal reductions in major derpressive disorder are confined to layer II cells. Reductions in the

density of specific populations of layer II nonpyramidal neurons containing the calcium binding protein, calretinin, also have been reported in the anterior cingulate cortex in subjects with a history of mood disorders.

Additional morphometric studies also have reported layer-specific reductions in interneurons in the anterior cingulate cortex, and reductions in nonpyramidal neurons (~40% lower) in CA2 of the hippocampal formation in bipolar disorder subjects compared to controls [32]. Overall, the layer-specific cellular changes observed in several distinct brain regions, including the prefrontal cortex, anterior cingulate cortex, and hippocampus suggest that multiple neuronal circuits underlie the neuropathology of mood disorders. This is not altogether surprising since the behavioral and physiological manifestations of the illnesses are complex and include cognitive, affective, motor, and neurovegetative symptomatology, as well as alterations of circadian rhythms and neuroendocrine systems, and are thus undoubtedly mediated by networks of interconnected neurotransmitter systems and neural circuits [276].

In addition to neuronal pathology, unexpected reductions in glial cell number and density also have recently been found in postmortem brains of both major depressive disorder and bipolar disorder patients. Thus, a histological study of area sg24 located in the subgenual prefrontal cortex found striking reductions in glial cell numbers in patients with familial major depressive disorder (24% reduction) and bipolar disorder (41% reduction) as compared to controls [252]. This observation is consistent with this group's neuroimaging report on reductions in cortical gray matter volume found in the same brain region in a similar diagnostic group. Marked decreases in overall and laminar (layers III-IV) glial cell packing densities also were found in subjects with major depressive disorder compared to nonpsychiatric control subjects [277]. Comparable reductions in glial densities were also detected in the dorsolateral prefrontal cortex from subjects with bipolar disorder across all cortical layers (except layer IV) [276]. In view of the growing appreciation of the critical roles of glia in regulating synaptic glutamate levels, CNS energy homeostasis, liberation of trophic factors, and, indeed, the very existence of synaptic networks of neurons and glia [78,179] all suggest that the prominent glial loss observed in major depressive disorder and bipolar disorder may be integral to the pathophysiology of the disorders and worthy of further study.

Recent morphometric neuroimaging studies have demonstrated that patients with both bipolar disorder and major depressive disorder display morphometric changes that suggest cell loss and/or atrophy [316,318]. The preponderance of evidence from recent volumetric neuroimaging studies suggests an enlargement of third and lateral ventricles, as well as reduced gray matter volumes in the orbital and medial prefrontal cortex, the ventral striatum, and the mesiotemporal cortex in patients with mood disorders [316,318,331]. Reductions in frontal lobe volumes, and striking ~40% reductions in the mean gray matter volume in the region located ventral to the genu of the corpus callosum have recently been demonstrated in bipolar disorder depressives and familial unipolar depressives [95]. Additional studies suggest that these subgenual prefrontal cortex gray matter volume reductions may be particularly evident in "enriched" patient populations, namely those with positive family histories of mood disorders [136]. Reductions in the volume of the hippocampus have also been observed in subjects with a history of major depressive disorder, findings that may persist for decades after the depressive episodes have resolved [316,318]. Interestingly, the loss of hippocampal volume appears to be correlated with the total lifetime duration of major depressive disorder but not with the

age of the patients [318], leading to the suggestion that these changes may represent the sequelae of repeated and/or prolonged episodes of depression [53,300].

2. Neurotrophic Factors and Depression

In recent years, the cellular and molecular model basis for the effects of antidepressants and stress on the survival of hippocampal neurons in the pathophysiology of depression has received much attention [100]. They proposed that a decrease in serotonin and norepinephrine transmission may lead to decreased levels of brain-derived neurotrophic factor (BDNF), which may be decreased even further by glucocorticoids, eventually leading to neuronal cell death. Their model suggests that antidepressants may prevent the downregulation of BDNF in response to stress. In addition, the genetic expression of some factors involved in neuronal atrophy and survival is influenced by antidepressant drug treatments [102]. Further evidence supporting their model is that chronic, but not acute, administration of various classes of antidepressants upregulates the cAMP-CREB cascade and the expression of BDNF in the rodent hippocampus and the number of new cells apparent in the dentate gyrus granule cell layer [66,101]. Recently, Chen and colleagues [66] reported that in the learned helplessness model rats that received bilateral microinjection of herpes simplex virus-CREB into the dentate gyrus showed significantly fewer escape failures in the subsequent conditioned avoidance test than those injected with control vector, suggesting that overexpression of CREB in the hippocampus results in an antidepressant effect. Further research is underway to determine whether hippocampal neurogenesis plays any direct role in the therapeutic actions of antidepressants and whether antidepressants exert major effects on neurotrophic factors and the signaling cascades.

C. Neuroimaging Studies

Neuroimaging studies have also been used to better understand the pathophysiology of mood disorders. In this section, we review the controlled structural (CT and MRI) and functional (PET [positron emission tomography] and SPECT [single photon emission computed tomography]) imaging studies of patients with affective disorder.

1. CT and MRI Studies

Many CT and MRI studies have demonstrated increased ventricle size in patients with unipolar and bipolar disorders. However, these studies are confounded by numerous methodological problems. For example, most of these studies estimate lateral ventricle size by using a ventricular brain ratio. Unfortunately, few conclusions can be drawn from the CT literature on ventricular enlargement in affective disorder. More consistent, however, are the CT and MRI studies of increased ventricular size in depression in geriatric patients (individuals ages 65 and older). Some investigators believe that ventricular enlargement is a nonspecific structural brain alteration [108].

The hippocampus, a structure of paramount importance to learning and memory, and which has high concentrations of glucocorticoid receptors, appears particularly vulnerable to the effects of stress on the brain [299]. Several groups have examined volumetric changes in the hippocampus of those with major depression. Some have found no difference between the hippocampal volume of nonpsychiatric control subjects and that of patients with major depression [22]. In contrast, Sheline and colleagues [316] reported smaller left and right hippocampal volumes in patients with major depression. In addition,

the authors reported that the extent of hippocampal atrophy was significantly correlated to the duration of depression; there was no difference in whole-brain volume between these groups [316]. In one study, the depressed group was divided into two subtypes based on the individual's response to treatment. They analyzed volumetric changes in cerebral and cerebellar regions. Although they did not find significant differences when comparing depressed patients as a whole with control subjects, they did find significant results when depressed subjects were divided into groups of responders and nonresponders. Recently, Vakili and colleagues [350] reported that there were no significant differences observed between the hippocampal volumes of patients with major depression and control subjects; however, the authors found a significant correlation between the left hippocampal volume of men and their depression scores at baseline.

The abnormal presence of hyperintensities of gray and white matter has been reported in multiple MRI studies of geriatric patients with affective disorder, particularly those with late-onset depression (i.e., elderly depressed patients who experience their first depression after age 60). Subcortical hyperintensities in the elderly are age-dependent and may reflect a variety of life-long insults and/or from pathophysiological processes related to cerebrovascular disease, including arteriosclerosis of the small lacunar arterioles that supply the basal ganglia and subcortical white matter [127,175]. Hypertension is associated with white matter hyperintensity in both depressed and nondepressed control individuals [175]. Study of nondepressed control subjects has also revealed that increasing age is associated with reduction of the size of the putamen [141] and caudate nuclei [166], as well as of the size of the midbrain [310] and pituitary gland [167]. A recent study reported that elderly adults (>60 years old) with severe white matter lesions are 3 to 5 times more likely to have depressive symptoms as compared to persons with only mild or no white matter lesions [84]. Elderly adults with severe subcortical but not periventricular white matter lesions were more likely to have a history of depression with an onset after 60 years compared with persons with only mild or no white matter lesions. Severity of white matter hyperintensity predicts poorer response to antidepressant therapy [134]. Ahearn and colleagues [3] in a post hoc analysis reported that unipolar patients with a history of attempted suicide demonstrated significantly more subcortical gray matter hyperintensities compared with patients without such history.

To assess whether abnormal brain structure is associated with abnormal brain function, investigators used CT and MRI studies in association with depression rating scores, neuroendocrine stimulation tests, neuropsychological testing, monitoring of patients' clinical course, and response to treatment. For example, postdexamethasone cortisol levels are significantly correlated with ventricular brain ratio [278] and pituitary volume [21]. Furthermore, in depressed elderly patients, caudate hyperintensities are associated with an increased risk for the development of TCA- and ECT-induced delirium [113,114], as well as neuroleptic-induced Parkinsonism [114].

2. PET and SPECT

Compared with nondepressed individuals, patients with unipolar depression have had a lateralized decrease in activity in the left lateral prefrontal cortex repeatedly documented by PET investigations [28,140]. Similar reduction in activity of the left frontal cortex has been observed in depression secondary to other CNS disorders, including epilepsy, human immunodeficiency virus, Huntington's chorea, and Parkinson's disease [359].

Of particular interest is the decreased neuronal activity of the caudate and putamen, and globus pallidus. The basal ganglia can be visualized as the motor behavioral effector

mechanism that underlies the cognitive expression of emotion. Vulnerability to affective dysfunction might derive from disruption of connections between the basal ganglia or from interruption of pathways connecting the basal ganglia to other parts of the brain, specifically the limbic system and prefrontal cortex [167]. Recent PET neuroimaging studies support a distinction between the neural substrates of normal sad mood states and major depression (e.g., left amygdala); such physiological states do not mimic the influence of major depression on frontal cortical function. Using PET images, Drevets and colleagues [95] localized abnormally decreased activity in the left prefrontal cortex ventral to the corpus callosum genu in both familial bipolar and unipolar depressed patients (patients with a family history of bipolar and unipolar depression, respectively). This diminished activity was explained in part by a corresponding reduction in volume in this same area as demonstrated by MRI. Resting neuroimaging studies of adult depressed patients have shown left-sided or bilateral hypoactivity in the dorsolateral, inferior, and medial frontal regions as well as the temporal limbic regions, particularly the amygdala [94,95,205].

Functional neuroimaging studies during resting and activation have also been conducted. Resting-state hypoactivity in the medial prefrontal cortex has been correlated with cognitive impairment [31]. Blunted left cingulate activation has been observed in younger depressed patients performing a response interference task [117]. de Asis and colleagues [83] reported bilateral activation deficits in the dorsal anterior cingulate gyrus and hippocampus of depressed geriatric patients. The depressed patients also had memory deficits that correlated with lower hippocampal activity during both rest and activation.

Furthermore, PET studies of depressed patients often reveal significant correlation between the severity of depressive symptoms and the reduction in left frontal cortical function. Clinical response of depressed patients to antidepressant treatment may be associated with increased blood flow or metabolism within the basal ganglia, cingulate, or prefrontal cortex [94,140]. However, one group has documented reductions in cerebral blood flow, both global and in anterior cortical regions, in manic patients and depressed individuals who have shown clinical remission of their symptoms following ECT [244] or treatment with oral antidepressants [244]. Mayberg et al. [206] reported that the metabolic response of depressed patients' rostral anterior cingulate metabolism differentiated antidepressant treatment responders from nonresponders.

Functional neuroimaging studies may have future potential in identifying effective treatment strategies tailored to the individual depressed patient or to those with certain symptom complexes [31]. Recent PET and SPECT studies of depressed patients have found differences in regional brain activity evident in pre- and post-treatment scans that distinguish treatment responders from nonresponders. Hyperactivity in the cingulate region of the frontal cortex prior to overnight sleep deprivation was associated with a positive clinical response of depressive symptoms to sleep deprivation. Nonresponders exhibited normal cingulate gyrus activity before and after sleep deprivation [377]. Mayberg and colleagues [207] found that patients with unipolar depression who responded to fluoxetine treatment after 6 weeks showed higher pretreatment glucose metabolism in a rostral region of the anterior cingulate cortex (Brodmann's area 24a/b) than nonresponders and nonpsychiatric comparison subjects. Pizzagalli and colleagues [266] reported that, in a group of individuals treated for major depression, baseline theta activity within a rostral region of the anterior cingulate cortex involving areas 24 and 32 predicted degree of response 4 to 6 months later. Depressed patients showing a better response had higher theta activity than those responding less well, and they also tended to have higher activity than comparison subjects. This study replicates the findings by Mayberg and colleagues [206].

Brody and colleagues [52] reported that at baseline subjects with major depressive disorder had higher normalized metabolism than controls in the prefrontal cortex and lower metabolism in the temporal lobe. With treatment, subjects with major depressive disorder had metabolic changes in the direction of normalization in these regions and that paroxetine-treated patients had a greater mean decrease in Hamilton Depression Rating Scale score than did subjects treated with interpersonal psychotherapy. Kennedy and colleagues [153] reported that successful paroxetine therapy was associated with significant increases in metabolic activity in dorsolateral, ventrolateral, and medial aspects of the prefrontal cortex (left greater than right), parietal cortex, and dorsal anterior cingulate.

Sackeim [296] points out that in contrast to the general agreement about baseline imaging deficits in major depressive episode, changes following treatment are uncertain, with reports of normalization of baseline deficits, increased abnormal activities, and no change [296]. He identifies many factors as possibly responsible for the inconsistency in findings including small sample sizes in the initial and retest studies, technical difficulties in obtaining images across two time points, and studies that are semiquantitative.

3. Anatomical Circuits Implicated in Major Depressive Disorder and Bipolar Disorder

These data implicate two interconnected circuits in familial major depressive disorder and bipolar disorder: a limbic-thalamo-cortical circuit involving the amygdala, the mediodorsal nucleus of the thalamus (in the medial thalamus), and the ventrolateral and medial prefrontal cortex; and a limbic-striatal-pallidal-thalamic circuit involving related parts of the striatum and ventral pallidum as well as the components of the other circuit [94]. The limbic-striatal-pallidal-thalamic circuit constitutes a disinhibitory side loop between the amygdala or prefrontal cortex and mediodorsal nucleus. The amygdala and the prefrontal cortex send excitatory projections to overlapping parts of the ventromedial striatum [94]. Because the major depressive disorder criteria likely encompass a group of conditions that are heterogeneous with respect to etiology, neuroimaging measures may differ across pathophysiologically distinct depressive subtypes. For example, lesions involving the parts of the prefrontal cortex that participate in the limbic-thalamo-cortical circuit and diseases of the basal ganglia (e.g., Parkinson's disease and Huntington's disease) are associated with higher rates of major depression than other similarly debilitating conditions [97], suggesting that dysfunction at multiple points within this system may give rise to the depressive syndrome. Because these conditions affect this system in different ways, imbalances within these circuits rather than overall increased or decreased synaptic activity within a particular structure may be associated with the depressive syndrome [94].

4. Specificity of Elevated Limbic-Thalamo-Cortical (LTC) Activity

Other studies identified clinical distinctions that may also identify elevated limbic thalamic cortical activity in major depressive disorder. Wu et al. [377] and Ebert et al. [105] found metabolism in the amygdala and anterior cingulate abnormally elevated in major depressive disorder subgroups who were responsive to sleep deprivation, and Bremner et al. [48] reported that medicated, remitted major depressive disorder subjects who relapsed during serotonin depletion had higher baseline amygdala and orbital cortex metabolism than those who did not. Other studies of unmedicated depressives with major depressive disorder have also reported increased cerebral blood flow and metabolism in the orbital cortex relative to healthy controls, and longitudinal studies in which depressives are imaged before and during treatment consistently show that orbital cortex cerebral blood flow

and metabolism decrease following effective antidepressant drug therapy, ECT, phototherapy, repeated transcranial magnetic stimulation, and sleep deprivation (reviewed in Ref. 96). In contrast, studies reporting data acquired from subjects receiving psychotropic drugs at the time of scanning or which included subjects with depression secondary to neurological disorders have not found elevated activity in these structures (reviewed in Ref. 96).

D. Endocrine Studies

1. HPA Axis in Affective Disorder

Numerous studies suggest hypercortisolemia in depression [24,26]. The HPA axis has been well established in the physiological response to stress and the hippocampus has been suggested as a regulator involved in the negative-feedback control of cortisol [144]. Hypercortisolemia may induce neuronal damage in the hippocampus [298] because of the high levels of glucocorticoid receptors in the hippocampus [281]. Hippocampal atrophy may then result from a long-term exposure to higher levels of glucocorticoids.

HPA axis hyperactivity occurs in depression [267] and its magnitude can be similar to that seen in mild Cushing's disease, and is linked to suicidal ideation [57] and risk of suicide [73]. It is also associated with severity of depressive illness [58,69], endogeneity [69], psychotic symptoms [286], and inpatient status [188] or symptoms associated with the need for hospitalization [268]. Adrenal gland hypertrophy, an index of centrally mediated hyperstimulation of the adrenal cortex, occurs in up to 70% of depressed subjects and resolves upon treatment [290]. HPA dysfunction precedes the resolution of affective illness, whereas unresolved or recurrence of HPA dysregulation may be prognostically ominous in both unipolar and bipolar disorders [139,282,308]. Even modest chronic exposure to excess glucocorticoids can also have medical consequences such as accelerated bone loss [143].

The rates of DST nonsuppression in depression vary widely according to study. Nelson and Davis performed a meta-analysis of studies comparing DST suppression test results and found an overall nonsuppression rate of 36% among inpatient melancholic patients but only 22% among outpatients and, when inpatient status was controlled for, melancholia did not predict nonsuppression [232]. However, Rush et al. reported nonsuppression rates of 62% among Research Diagnostic Criteria (RDC) endogenous inpatients, with similar rates for unipolar and bipolar patients subgroups [295]. Inpatient status and severity of illness may predict HPA axis abnormality. Patients with high spontaneous cortisol levels or cognitive impairment have a poorer response to treatment with antidepressants [68,70] and some, but not other, studies suggest that DST nonsuppression is associated with poor response to TCAs [7]. Frequent recurrent episodes of mood disorder [340,341] or long duration of episodes may increase treatment resistance [30,327] and limbic neuroendangerment; all of which could be hypothesized as related to elevated HPA activity. It should be noted that given the likely day-to-day oscillations in HPA axis activity in patients with major depression some patients may be hypercortisolemic overall even though they may suppress in response to DST on a given day.

Corticotropin-releasing factor (CRF) is both a direct regulator of secretion of ACTH from the corticotrophs in the anterior pituitary and is a neurotransmitter that is abundantly located in extrahypothalamic brain areas. It has been hypothesized that a major role of CRH is to regulate stress responses throughout the CNS, coordinating the endocrine, immune, autonomic, and behavioral responses to stress. In this capacity and as the principal regulator of ACTH secretion, the CRF system is believed to be involved in the pathophysi-

ology of depression [238]. Hypercortisolinemia, DST nonsuppression, and the stress intolerance displayed by depressed patients all suggest dysregulation of the CRF system in these patients.

The CSF CRF concentration in untreated depressed patients is increased compared to healthy controls [234]. Other research groups have also replicated this finding. An increased CSF CRH concentration is also elevated in suicide victims [11].

Among the biological abnormalities tentatively associated with risk for suicide, those involving the HPA axis have shown particular promise. In postmortem studies, persons who died by suicide, in comparison to matched subjects who died by other violent means, had greater adrenal weights [92,342], fewer binding sites for CRF in the frontal cortex, and higher levels of CRF in CSF. Each of these findings associates suicide with HPA axis hyperactivity. Studies examining whether abnormal DST results are associated with suicide have been mixed, some studies finding an association between abnormal DST results and suicide and other studies not finding this association. More recently, Coryell and Schlesser [73] reported on the predictive validity of abnormal DST and suicide in 78 inpatients with major depressive disorder or schizoaffective disorder, depressed type, followed for up to 15 years. Survival analyses showed that the estimated risk for eventual suicide in patients with abnormal DST was 27% compared to only 3% among patients who had normal DST results.

More than a dozen studies have examined patients' ACTH responses to administration of exogenous CRH in mood disorder and the majority of studies included both unipolar and bipolar subjects [121,122,283,357]. Subjects were generally inpatients with melancholic features. Gold et al. found that bipolar depressed subjects had only half the peak and net integrated ACTH response to CRH, whereas there was no significant difference from placebo in mania or recovered patients [120]. Despite methodological differences, 11 of 13 studies found that, compared to normal controls, the depressed subjects had blunted plasma ACTH and normal cortisol responses that were disproportional to the ACTH responses, occurring in the context of CRH stimulation, indicative of adrenal hyperresponsiveness [42]. A blunted plasma ACTH response in association with nonblunted cortisol responses is indicative of centrally mediated hypercortisolism. In euthymic bipolar patients maintained on lithium, a blunted ACTH response to CRH predicted subsequent relapse into depression [355]. Drug effects of lithium and tricyclics do not significantly interfere with detecting group differences if they fail to treat the depression [42,355].

Depression-related disruptions of cognitive function have been observed in multiple domains [125]. Depression-related deficits in cognitive function include alterations in memory, attention, and executive functioning in areas including working memory and set shifting that may correlate with psychosis [18,233], endogenous features [294], or narrowly defined melancholia using the CORE and Newcastle scales [19,20,222].

Moreover, hypercortisolemia found in severe depression in association with neurotoxicity and atrophy of the hippocampus and other limbic-related structures and selective atrophy of the hippocampus occurs in Cushing's disease [300]. Hippocampal dysfunction may be in part the etiological mechanism in cognitive disturbances involving memory in severe depression [29]. Cumulative cognitive effects of recurrent [154] or lengthy depressive episodes may result in dysfunction of frontostriatal and mesiotemporal/hippocampal circuits [125]. Animal stress model implicate the hippocampus in glucocorticoid-induced abnormalities. Rubinow et al. [291] observed a significant positive relationship between mean urinary free cortisol levels of depressed patients and errors on the Halstead Category Test. Other studies have reported DST nonsuppressors have greater cognitive

deficits than those with normal receptor sensitivity [55,369]. Newcomer et al. [241] found exogenous glucocorticoids impair declarative memory and Wolkowitz et al. [373] found that DST nonsuppression or exogenous glucocorticoids predicted errors of commission on the Wallach Memory Recognition Test. Hippocampal volume loss in depression correlates with declarative memory impairment and the duration of past depression, and plausibly is glucocorticoid-mediated [49]. As well, impairment of working memory after glucocorticoids suggests prefrontal cortex involvement [183]. Prefrontal cortex dysfuntion is reflected in executive dysfunction in severe depression and may predict nonresponse to SSRIs or other standard treatments [103,206]. Thus treatment of hypercortisolism or other factors resulting in cognitive dysfunction in depression may facilitate treatment response.

In 11 studies of antiglucocorticoid agents reviewed by Wolkowitz and Reus, 67% of the treated patients had a clinically meaningful antidepressant response. Earlier studies used ketoconazole, metyrapone, or aminoglutethimide [374]. Interestingly, an early indicator of response may be improved cognitive function [374]. Subjects tended to have sustained improvement even after drug discontinuation [374] and it is suggested that antiglucocorticoid treatment may be "resetting" the HPA axis. Some studies have suggested no relationship to baseline cortisol and response to antiglucocorticoid treatment [119], whereas others have noted considerable relationship. In a double-blind case study, Anand et al. [9] noted a close relationship between decrease in cortisol and Hamilton depression rating scale scores. Wolkowitz also reported significantly reduced 4 PM cortisol in an open study of ketoconazole and a correlation between treatment response and baseline cortisol in a double-blind controlled trial [374,375]. Ravaris et al. treated a bipolar type II patient with ketoconazole that was added to lithium and phenelzine. Improvement correlated in this case with a decrease in urinary free cortisol levels [279]. Brown et al. report improvement in 3 of 6 treatment-resistant bipolar depressed patients. Response occurred in inpatients receiving ketoconazole 400 to 800 mg, although they did not detect a change in serum cortisol levels [54]. Recently, DeBattista and colleagues [85] reported that intravenous administration of a moderate dose of hydrocortisone produced a robust and rapid improvement in Hamilton depression scale scores in depressed patients. Mifepristone (Mifeprex™) is FDA indicated for the medical termination of intrauterine pregnancy and has potential antineoplastic properties [160]. Soon after its synthesis in 1980, the steroid compound mifepristone or RU38486 (17-hydroxy-11b (4-dimethylaminophenyl)-17(1-propynyl) oestra-4,9-dien-3-one) was found to act as a competitive, specific, high-affinity antagonist of progesterone and glucocorticoid receptor-2 and has weak antiandrogenic activity (product labeling). The antiglucocorticoid effect of RU486 is dose dependent and becomes apparent for single doses of 4 mg/kg [59]. It has been demonstrated that after 8 days of treatment with 200 mg of mif per day, the adrenocortical and pituitary reserves are preserved. Mifepristone also attenuates retention of immobility response in the forced swim test, a paradigm sometimes associated with antidepressant activity [88]. Murphy et al. reported that four treatment-refractory patients with chronic severe depression were given RU486 (200 mg/day) for periods up to 8 weeks. One of the patients was diagnosed as bipolar and she improved significantly after 9 days of therapy with mifepristone (3.9 mg/kg): her Hamilton depression rating scale score fell from pretreatment average of 31 to 6 [229]. A number of studies indicate that mifepristone acts quickly on psychotic depressive symptoms and can be effective in treatment periods of 7 days or less. In an early report, the psychiatric symptoms of a patient with co-morbid Cushing's syndrome and psychotic depression were resolved within 2 weeks of treatment with doses as high as 140 mg per day [242]. Lower doses of 400 to 800 mg per day rapidly reversed psychotic

depression in two patients with Cushing's syndrome resulting from metastatic adrenal cancer within 48 h [352].

2. Hypothalamic-Pituitary-Thyroid Axis

Patients with hypothyroidism can appear indistinguishable from those patients suffering from major depression. There is a substantial overlap of symptoms in both conditions, including depressed mood, cognitive impairment, and neurovegetative symptoms. For this reason, the hypothalamic-pituitary-thyroid (HPT) axis has received intense study. It is estimated that only 20 to 30% of patients with depression have discernible thyroid dysfunction. In two studies, the CFS concentration of thyrotropin-releasing hormone (TRH) was reported to be increased in depressed patients compared to neurological and nondepressed controls [25,158]. In contrast, the study by Roy and colleagues [288] did not support this finding. This finding appears to be specific to patients with depression as increased concentrations of CSF TRH were not observed in patients with Alzheimer's disease, alcoholism, or anxiety disorders. The HPT function in depression has also been examined by way of the TRH stimulation test. In this test, plasma TRH concentrations are measured at baseline and at 30-min intervals after a challenge dose of TRH. A series of studies have found that between 25 and 30% of depressed patients have a blunted TSH response to TRH challenge [148,272]. This finding is not due to a primary thyroid disorder because these depressed patients are reported to be euthyroid at the time of the assessment. A modified version of the TRH test has been developed to specifically assess the night-time TSH response to TRH challenge [104]. In this test, the subject receives a TRH challenge at 8:00 AM, with subsequent blood sampling for TSH measure and a second TRH challenge at 11:00 PM, with subsequent TSH determination. It has been reported that this test displays a 95% specificity and 89% sensitivity for the diagnosis of major depression.

3. Hypothalamic-Pituitary-Ovarian Axis

Changes in the hypothalamic-pituitary-ovarian (HPO) axis have been reported to be associated with depression. A recent study found that the mean plasma estradiol level was 30% lower in the follicular phase in women with depression than in their matched controls. In addition, the half-life of luteinizing hormone was found to be significantly shorter in women with depression than in their matched controls in both the follicular and luteal phases [380]. Estradiol has effects on multiple brain systems including memory, synaptic density, and the neurotransmitter systems of serotonin and norepinephrine [208,320]. In addition, some open-label studies have reported beneficial effects with estrogen as an adjunct to treatment of major depression [183,273], although the results of placebo-controlled trials are mixed.

E. Neurodegeneration in Mood Disorders?

Recent evidence suggests that neurodegeneration may be involved in the pathophysiology of major depression. The astroglial peptide S-100B plays a role in axonal growth and synaptogenesis during development and synaptic remodeling. Increased CSF and plasma levels of S-100B were detected after traumatic brain injury, toxic or ischemic brain damage in multiple sclerosis, Alzheimer's disease, and schizophrenia [128,365]. Rothermundt and colleagues [285] reported that patients suffering from melancholic depression showed significantly increased S-100B levels compared to healthy controls while nonmelancholic patients demonstrated normal levels.

F. Vascular Disease and Increased Platelet Aggregability in Major Depression

Increasing evidence suggests that depression constitutes an independent risk factor for cardiovascular morbidity and mortality. The vascular depression hypothesis proposes that late-life depression is associated with vascular disease [4,165]. Many of these findings are summarized in the neuroimaging section of this chapter. Briefly, this hypothesis is based on the strong associations between depression and vascular disease and MRI findings in depressed subjects of elevated rates of deep white matter hyperintensities, which may be vascular in origin [250]. Intercellular adhesion molecule-1 (ICAM-1) is a vascular marker of inflammation, and its expression is increased by ischemia. Recently, Thomas and colleagues [345] reported that ICAM-1 was significantly higher in the postmortem tissue of the depressed subjects' dorsolateral prefrontal cortex compared to comparison subjects.

Markowitz and colleagues [199] reported increased platelet secretion after in vitro stimulation with collagen along with decreased platelet serotonin receptor density in depressed patients. Lederbogen and colleagues [173] reported that major depression is associated with increased platelet aggregability, which seems to persist even under a marked improvement in depressive symptomatology. This effect has been reported to contribute to the increased cardiovascular morbidity in depressed patients.

G. Pineal Function Circadian Rhythm

Several studies have suggested the possible role of the circadian pacemaker in depression [329,351,370]. Partly supporting this theory is the fact that, in the melancholic type of depression, patients typically feel worse in the morning and have early morning awakenings [329,351,370]. The suprachiasmatic nucleus is the circadian pacemaker of the mammalian brain, responsible for maintaining and coordinating different bodily functions including sleep-wake cycles, body temperature, and hormonal rhythms [337], and has been reported to be functionally impaired in depression [381]. Zhou and colleagues [381] suggested that, in depressed patients, both the synthesis and release of arginine vasopressin in the suprachiasmatic nucleus is reduced, resulting in an impaired functional ability.

H. Immunological Function

Recent studies have suggested that major depression is accompanied by significant activation in immune-inflammatory markers. Several studies of depressed patients have shown an increased number of peripheral blood mononuclear cells such as leukocytes, monocytes, $CD4^+$, T helper cells, an increased serum concentration of soluble interleukin-2 receptor, and an increased polymorphonuclear elastase in serum [87,190,326]. There is evidence that the activation of immune cells is related to overproduction of reaction oxygen species (ROS) [363]. ROS are highly reactive chemical species with an unpaired electron, and their formation is catalyzed by transition metals like iron, manganese, and copper. It has been suggested that the activation of immune-inflammatory processes, increase in monoamine catabolism, and abnormalities in lipid compound may cause overproduction of ROS and, in turn, antioxidative enzyme activities and lipid peroxidation. Bilici and colleagues [41] reported that major depressed patients, especially melancholic patients, had higher antioxidative enzyme activities and lipid peroxidation levels than those of healthy controls. In addition, the authors reported that after 3 months of treatment with SSRIs, antioxidative

enzyme activities and lipid peroxidation levels of patients were significantly decreased to normal levels.

IV. CONCLUSIONS

Preclinical and clinical studies over the past 40 years have attempted to uncover pathophysiology of major depressive disorder first by uncovering the specific defects in these neurotransmitter systems by using a variety of biochemical and neuroendocrine strategies. Although such investigations have provided useful information, they have been of limited value in elucidating the pathogenesis of these disorders. It is clear from these studies that depression is much more than increasing intrasynaptic levels of biogenic amines. More recently, research into the pathophysiology and treatment of major depressive disorders has focused on intracellular signaling pathways. The pathophysiology of major depressive disorders remains unknown but probably involves a complex interaction and overlap of multiple systems including neurotransmitter, endocrine, and immune systems and cellular signaling pathways.

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Animal Models of Subtypes of Depression

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I. INTRODUCTION

Animal models of depression are used both as screening tests to discover and develop novel antidepressant drug therapies, and as simulations for investigating various aspects of the neurobiology of depressive illness, including the neuropharmacological mechanisms mediating the effects of antidepressant treatments. These different functions of animal models have different and, to some extent, conflicting requirements.

A simulation of depression aims to mimic aspects of the clinical situation and should embody a degree of complexity to permit investigation of the validity of the model. In addition, if a model is to be used to investigate antidepressant actions, a slow onset comparable to the clinical time course is highly desirable, and the model should therefore exhibit differences (either in direction of response, or in response magnitude) between single (acute) and repeated (chronic or continuous) treatment regimes. By contrast, the only essential requirement for antidepressant screening tests is that they make accurate predictions of antidepressant activity. For practical reasons, they should also be cheap, robust, reliable, and easy to use [63,292], and for all of these subsidiary reasons, a screening test should in principle be as simple as possible. However, the view that such screening tests should also respond acutely has changed during the last decade, in line with the drive from the clinic to identify rapid-onset antidepressant treatments. By necessity, this ap-

proach involves the assessment of drug action associated with chronic/continuous drug treatment regimes and an acute response is thus of little value. Appropriate screening tests to be used relatively early during drug development should therefore have the ability to identify the time course of drug action associated with repeated treatment schedules.

The present chapter is concerned primarily with evaluating the suitability of the available animal models as research tools, and we therefore focus initially on issues relating to the validity of the available models as simulations of depression. In line with current thinking, our assessment of the validity of animal models of depression addresses the three dimensions of predictive, face, and construct validity [286,292]. The concept of predictive validity implies that manipulations known to influence the pathological state should have similar effects in the model. Face validity refers to a phenomenological similarity between the model and the disorder being modeled. Construct validity implies that the model has a sound theoretical rationale. While some reviewers have advocated the primacy of one or the other of these approaches, we favor a balanced approach in which a view of the validity of a model is formed only after considering all three sources of evidence. We therefore begin by reviewing briefly the three sets of validation criteria as they apply to animal models of depression.

II. PREDICTIVE VALIDITY

In practice, the predictive validity of animal models of depression is determined solely by their response to antidepressant drugs. A valid test should be sensitive and specific: it should respond to effective antidepressant treatments (true positive effects), including electroconvulsive shock (ECS), and should fail to respond to ineffective agents (true negative effects). A model with high predictive validity should therefore maximize identification of both true positives and true negatives, but should minimize identification of false positives and false negatives. Furthermore, positive responses should occur at behaviorally selective doses (i.e., those which do not generally disrupt behavior nor induce motor impairment) that are within or close to the clinical range, and should be demonstrable with a range of structurally diverse compounds. It should be recognized that no animal model has a 100% prediction rate, although some complex experimental paradigms have approached this level of predictive ability. Part of the problem lies not so much with the preclinical model but with several gray areas in the clinical literature where it is not known whether certain drugs (e.g., anticholinergics) possess antidepressant activity or not. It is generally agreed that the most effective treatment for depressive illness is electroconvulsive therapy (ECT). A suitable starting point to test the validity of an animal model should therefore be to demonstrate a positive response to repeated ECS. Failure to respond appropriately to ECS would severely question the predictive validity of the model.

About 30% of depressed patients fail to respond to antidepressant drug treatment while the response rate for ECT is slightly higher. The occurrence of refractory patients probably reflects the heterogeneity of depressive illness. Nevertheless, a model that did not respond to the benchmark tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), or the recently developed serotonin-noradrenaline reuptake inhibitors (SNRIs), would not be taken seriously. This situation is unlikely to change in the absence of well-established therapies for TCA-resistant depression.

Beyond the clinically established antidepressant treatments—TCAs, monoamine oxidase inhibitors (MAOIs), SSRIs, SNRIs, and some atypical antidepressants (e.g., mianserin)—are a wide range of newer compounds for which antidepressant activity has been

claimed, ranging from pharmacologically selective compounds that are probably effective (such as α_2-adrenoceptor antagonists; ligands for serotonin (5-hydroxytryptamine; 5HT) receptor subtypes including $5HT_{1A}$-receptor ligands that possess agonist activity [and partial agonists), ligands for $5HT_{2A}$ and $5HT_{2C}$-receptor subtypes, and *n*-methyl-d-aspartate (NMDA) receptor ligands], through preparations of mixed pharmacological activity (SSRI with selective $5HT_{1A}$-receptor antagonist activity) to compounds of uncertain status (e.g., phosphodiesterase inhibitors; calcium antagonists; anticonvulsants). Even in the case of the SSRIs, whose antidepressant efficacy is beyond doubt, it is still uncertain whether their spectrum of activity is identical to that of the TCAs. Therefore, while a well-rounded description of an animal model of depression should include an account of its pharmacological profile broader than that provided by its response to traditional antidepressants, the contribution of these newer compounds to validation is limited.

To some extent, similar uncertainties exist in relation to drugs that are ineffective as antidepressants. The false positives most commonly encountered in animal models of depression are psychomotor stimulants, anticholinergics, and opiates. However, prior to the development of antidepressants, drugs from all three of these classes were regularly prescribed for the relief of depression [287]. Indeed, certain opiates (e.g., buprenorphine) have antidepressant activity [75], but anticholinergics and stimulants have never been properly assessed, as their use was discontinued prior to the introduction of blind clinical trials. Again, the uncertain status of these compounds to some extent undermines their value as definitive standards of negative control.

Finally, the response to antidepressant drugs is insufficient to define an animal model of depression. Some antidepressants are active in some animal models of anxiety and panic following chronic treatment [31,86] and, indeed, are increasingly seen as the drugs of choice in many forms of anxiety. It is therefore crucial for establishing the predictive validity of an animal model of depression to demonstrate that the model does not respond to benzodiazepines.

III. FACE VALIDITY

To assess face validity, the extent of similarity between the model and the disorder is examined, on as wide as possible a range of symptoms and signs. However, not all symptoms of a psychiatric condition carry equal weight and for an animal model to be valid, a resemblance to the clinically defined core symptoms of the disorder carries more weight than a resemblance to any subsidiary symptoms [1]. The latest edition of the Diagnostics and Statistics Manual (DSM-IV) provides a framework within which to assess these similarities. However, not all of the clinical symptoms of depression can be modeled in animals; symptoms conveyed by subjective verbal report (e.g., excessive guilt, feelings of worthlessness, suicide ideation) are in principle excluded [286,291].

A DSM-IV diagnosis of major depression requires the presence of a least one of two core symptoms: loss of interest or pleasure (anhedonia) and depressed mood. Of these two core symptoms, anhedonia can be modeled in animals, but depressed mood cannot. In assessing the face validity of animal models of depression, anhedonia therefore assumes a central position. The problem here, however, is that anhedonia is also a core symptom of psychosis. Drug-induced reversal of anhedonia in animal models, while highly encouraging, should thus be considered in relation to DSM-IV criteria for both depressive illness and schizophrenia.

The subsidiary symptoms of depression in DSM-IV that are amenable to modeling

in animals include psychomotor changes, fatigue or loss of energy (which might be modeled as decreased persistence), and disturbances of sleep or food intake. Interestingly, psychomotor activity, sleep, and appetite may be increased or decreased, and diametrically opposite changes in locomotion have both been cited in support of the validity of a model. This lack of precision, together with the fact that the clinical phenomena of psychomotor retardation and agitation (which are considerably more complex than gross changes in locomotor activity) may even coexist [203], suggests that simulations in which a change in locomotor activity is the major, or only, behavioral feature should not be taken too seriously. Unfortunately, it is precisely these behaviors that feature most prominently in many animal models of depression [293].

Because the pharmacotherapy of depression typically requires chronic drug treatment, the validity of an animal model is called into question by an acute response to conventional (e.g., tricyclic) antidepressant treatment. There is some evidence of very early antidepressant responses in clinical studies designed explicitly to detect them [88, 289], but, even so, repeated treatment is required for a full response. Acute treatment refers to a single bolus dose of drug and any acute treatment effects are usually observed within minutes or hours following the bolus dose. Multiple acute doses may be administered, over a short period, simply to increase levels of drug in the biophase without evoking secondary adaptive changes in neurotransmitter mechanisms. In contrast, chronic treatment refers to repeated bolus doses administered for extended periods (days, weeks, months). Chronic treatment effects may be associated with drug-induced adaptive changes in neurotransmitter receptor-mediated systems, rather than increased drug levels in the plasma. Thus, in comparison to chronic treatment effects, an acute response in an animal model may be opposite, orthogonal (due to side effects, like sedation), or absent. Regardless of how it responds to acute antidepressant treatment, to be valid, an animal model of depression must respond to chronic treatment. Furthermore, since tolerance does not occur to the clinical effects of antidepressants, the response in the model must be maintained until the cessation of antidepressant treatment. This test has not been universally applied to animal models of depression but, in general, those models to which the test has been applied have passed it, though tolerance to antidepressant effects has been reported in some behavioral paradigms [62,204].

IV. CONSTRUCT VALIDITY

An evaluation of the theoretical rationale of an animal model requires a means of bringing the theoretical accounts of both the disorder itself and the disordered behavior exhibited by the model into alignment. However, any evaluation of animal models of depression is intrinsically limited by the rudimentary state of the various pathological theories of depression. Indeed, there is little in the extensive literature describing neurochemical abnormalities or biochemical markers reportedly associated with depression that can be usefully employed to provide a theoretical standard against which to validate animal models. Even the most basic questions of whether the level of activity in monoaminergic systems is elevated or decreased in depression were and remain controversial [287].

Similar problems arise in relation to modeling the etiology of depression. It is now clear that a variety of different factors are implicated in the etiology of depression: psychological factors include undesirable life events, chronic mild stress, adverse childhood experiences, and personality traits such as introversion and impulsiveness; biological factors include genetic influences and a variety of physical illnesses and medications [4,5,287].

However, for most of these factors, there is little theoretical understanding of the processes by which they influence the physiological processes underlying mood. In certain cases, the immediate precipitant of a depression may be clearly identified: for example, seasonal affective disorder (SAD) and postpartum depression. More usually, the pathogenesis of depression is better understood as the result of an accumulation of a number of different risk factors [4,9,38]. This point has been largely overlooked in the construction of animal models of depression, which in general have assumed a single causal factor. This may be counterproductive, since few of the identified etiological factors appear sufficiently potent to precipitate depression in an otherwise risk-free individual.

Although attempts to assess the theoretical rationale of animal models are limited by this lack of theoretical structure at the clinical level, a number of generalizations are possible. The major group of animal models of depression is based on responses to stressors of various kinds, and are usually justified by reference to the role of stressful life events in the etiology of depression [38,39,156]. However, if very severe acute stress is used [284], the relevance of these procedures to depression, rather than, for example, post-traumatic stress disorder, is questionable. Furthermore, the adverse consequences of life events endure for a prolonged period of 6 to 12 months, in part by exacerbating ongoing life difficulties [37,39]. Thus, life events should not be viewed as acute stressors (indeed, in the case of bereavement, a diagnosis of depression is explicitly excluded during the period of acute loss); from this perspective, it may be more appropriate to use chronic stress regimes, rather than acute stressors, to model the etiological role of life events. Other factors have been identified that confer a long-lasting vulnerability to depression, in particular, an inadequate level of social support, which to a large extent arises from inadequate socialization [9,37–39,290]. From these starting points, a number of animal models of depression have been developed that are based on the adverse effects of social isolation. However, with the exception of some of the primate studies (see below), these models have largely ignored both the complexity of childhood social deprivation phenomena and the mediation of their effects through later social relationships.

Although assessment of the theoretical rationale of animal models of depression is limited by the paucity of theory, construct validity can also be evaluated at the level of constructs—that is, whether the behavioral phenomena are correctly described. This approach is best exemplified by the extensive experimental analysis of whether learned helplessness is an appropriate term to describe the impairments of escape learning that follow exposure to inescapable electric shock [247]. The term learned helplessness implies that the animals perform poorly because they have learned that their responses are ineffective in controlling their environment [247]. However, inescapable shock has a variety of other, simpler effects, which could also explain many of the behavioral impairments, such as decreased locomotor activity [11,101] and analgesia [151]. In addition, while inescapable shock results in cognitive impairment [115], such behavioral effects arise from impairment at the level of attentional processing rather than helplessness [181]. Thus, there are many reasons to question whether the learned helplessness procedure does in fact produce helplessness.

Anhedonia is another construct that has been subjected to this kind of experimental attention. In this case, the question is whether the animals fail to perform rewarded behavior because they are insensitive to rewards or for some other reason. This question has been most extensively investigated in relation to the effects of chronic sequential exposure to a variety of mild stressors—chronic mild stress (CMS). CMS has been shown to cause an antidepressant-reversible decrease in the consumption of dilute sucrose solutions, which

is hypothesized to reflect a decrease in the reward value of the sucrose [294,297]. Initial studies showed that, in contrast to the effect on dilute solutions of sucrose or saccharin, CMS did not decrease the intake of plain water, food pellets, or concentrated sucrose solutions; thus the effects are not simply nonspecific changes in consummatory behavior [198]. Some studies have suggested that decreases in sucrose intake reflect loss of body weight [172], but there is ample evidence [294] to reject this suggestion, including the fact that the effects are seen in the absence of body weight changes. Furthermore, the effects of CMS are not confined to consummatory behaviors. CMS also attenuated or abolished place preferences established using a variety of natural or drug rewards. However, drug-induced place aversions were unaffected by CMS. Thus, the effect of CMS on place conditioning cannot be explained by nonspecific motivational impairments or a failure of associative learning [294]. Finally, CMS has also been shown to cause an increase in the threshold for brain stimulation reward [196]. Together, these results support the position that CMS causes a generalized decrease in sensitivity to rewards and that, under appropriate experimental conditions, decreases in sucrose intake provide a simple means of detecting this anhedonia.

These examples of successful experimental analyses of construct validity contrast sharply with a third example, the behavioral despair test [226]. These term was introduced to describe the immobility seen in rats or mice forced to swim in a confined space, based on the assumption that when the animals adopted an immobile posture, they had despaired of escaping. Unfortunately, this interpretation is not susceptible to experimental analysis. Consequently, although the test continues to be very widely used, the name behavioral despair has been largely abandoned and replaced by the theoretically neutral term the forced swim test.

V. MODELS OF DSM-IV MELANCHOLIA

It has frequently been remarked [84] that the approach adopted in successive editions of the Diagnostic and Statistical Manual (DSM), which identifies psychiatric disorders by the presence of a sufficient number of symptoms from a longer list, results in very different clinical presentations receiving the same diagnosis. However, discussion of the heterogeneity of depression is several decades older than the DSM system. It has been recognized since the 1920s that there are at least two forms of depression, but only one of them has enjoyed general agreement as to its nature [287]. This is the syndrome that has been known, variously, as autonomous depression, endogenous depression, endogenomorphic depression, and, more recently, melancholia. Because a consensus exist as to its existence and symptom profile, melancholia has emerged as the major subtype of depression, and its definition in DSM-IV (major depressive episode with melancholic features) is somewhat more restrictive than that of the parent syndrome, major depressive disorder.

In previous reviews, animal models of depression have been classified according to their level of validity [286] or their inducing conditions [291]. An attractive alternative classification could be based upon the subtype of depression modeled. The art of modeling has not yet progressed to the point where this is entirely feasible. However, a number of models include anhedonia as one of their features. In DSM-IV, anhedonia is the defining feature of melancholia as well as the only core symptom of major depression that is amenable to modeling in animals. Anhedonia may be readily modeled as decreased sensitivity to rewards, though, as noted above, this construct is considerably more complex than a simple decrease in the performance of a rewarded behavior. In relation to the other symp-

toms that can be modeled, the diagnosis of melancholia specifies the direction of changes in appetite and sleep (decreased), although, as in the diagnosis of major depressive disorder, psychomotor changes could be in either direction (retardation or agitation). In many empirical studies, psychomotor retardation has emerged as the symptom most characteristic of melancholia, while psychomotor agitation tends to be more closely associated with psychotic features such as delusions of guilt [203]. Nevertheless, there is considerable overlap between these two groups of symptoms, and agitated melancholias are not uncommon. Unlike nonmelancholic depressions, melancholia is characterized by a decrease in the latency to enter the first period of rapid eye movement (REM) sleep [141] and an increased activity of the hypothalamic-pituitary-adrenal (HPA) system usually detected by the dexamethasone suppression test (DST) [45]. In a valid simulation of melancholia, these biological markers would be expected to coexist alongside decreased sensitivity to rewards. However, the specificity of both of these markers for melancholia is less than originally claimed.

The first section of this review considers those models in which anhedonia has been demonstrated. Also included are the forced-swim and tail-suspension tests, in which anhedonia has not been demonstrated, but which appear to be conceptually related to the learned helplessness model.

A. Acute Stress: The Learned Helplessness Model

As noted earlier, the learned helplessness paradigm is based on the observation that animals exposed to uncontrollable stress (usually electric shocks) are subsequently impaired in learning to escape shock, an effect that is not seen in animals exposed to comparable, or indeed identical, patterns of controllable shock. The protective effect of control appears to result from lower levels of fear [114], which raises important questions about the relationship between depression and anxiety. Seligman [247] proposed that exposure to uncontrollable stress provides the basis, in animals as in people, for learning that stress is uncontrollable (helplessness), and that this learning has a number of debilitating consequences, including depression. However, this interpretation has been the subject of considerable controversy, in both the human [2,3] and animal literature [181,182,284], and is probably incorrect as previously stated [288,296]. Nevertheless, the learning difficulties that follow exposure to uncontrollable shock are reversed with reasonable selectivity by multiple acute, subchronic (3–5 days) treatment with TCAs or atypical antidepressants [249]. SSRIs also appear to be effective, but only within a limited dose range [171], as well as a number of other potential antidepressants such as $5HT_{1A}$-receptor agonists [100] and β-adrenoceptor agonists [169], although some of these effects have been disputed [49], as well as extracts of St. John's wort (*hypericum perforatum*) [93,140]. Interestingly, chronic, but not acute, treatment with lithium prevents the development of learned helplessness in rodents [263]. There are a few false positives, including the $5HT_2$-receptor antagonist, methysergide [41], *p*-chlorophenylalanine (*p*CPA) [74], and piracetam [47], while neuroleptics, stimulants, sedatives, and anxiolytics are generally ineffective [228,249].

The learned helplessness paradigm shows many symptomatic parallels to major depression—so much so that it has been suggested that rodents subjected to uncontrollable shock could meet DSM diagnostic criteria [284]. However, the learned helplessness paradigm is implemented in a variety of different ways in different laboratories, and the version of the paradigm giving rise to the broadest range of symptoms [284] uses extremely high shock levels (4–6 mA), which are of doubtful relevance to depression. Furthermore, the

effects of this regime largely dissipate within 2 to 3 days, and the effects of antidepressant pretreatment have not been studied using this procedure. The effects of antidepressants have typically been studied using shocks of a considerably lower intensity (1.0–1.5 mA), which cause a far less pervasive pattern of behavioral impairment.

Paradoxically, the effects of milder shock intensities are of longer duration, making it possible to interpose multiple acute (subchronic) drug treatment (typically for 3–5 days) between the initial uncontrollable shock session and the learning test. Furthermore, the duration of learned helplessness may be prolonged indefinitely by repeatedly exposing the subjects to the environment in which inescapable shock had occurred [162], which strengthens the face validity of this model of depression. However, a further problem is that different components of the learned helplessness syndrome may be related to different aspects of the induction procedure. For example, a long-term (7 weeks) suppression of home-cage locomotor activity has been reported following a single shock session [68], but this effect is unrelated to shock controllability and so is clearly distinct from other learned helplessness phenomena [303]. It is clear that the learned helplessness paradigm should more correctly be considered as a mixture of paradigms, and care should be taken in generalizing conclusions between them.

Among the consequences of low-intensity, uncontrollable (but not controllable) shock is a poor performance of rewarded behavior, which, as noted above, may be of particular relevance to melancholia. One manifestation of this effect is a long-lasting decrease in responding for brain stimulation reward (intracranial self-stimulation; ICSS), in mice, which is specific to certain electrode placement and therefore suggests a subsensitivity within part of the brain mechanism of reward, rather than, for example, a motor impairment [305,306]. Normal sensitivity to ICSS reward was restored by chronic, but not acute, treatment with TCAs [304,305]. Interestingly, a long-lasting anhedonia is seen only if the animals are tested in the immediate aftermath of stress; otherwise the effect dissipates rapidly [305]. A related observation is that mild stressors, which are without effect in normal animals, may reinstate behavioral deficits resulting from an initial exposure to severe stress [10]. These studies suggest that it may be possible to develop conditioning models to explain how the risk of depression is elevated for several months in the aftermath of a stressful life event.

Another important observation is that uncontrollable electric shock has variable behavioral effects (most of which are antidepressant-reversible) in different inbred mouse strains. To take an extreme example, in the C57BL/67 strain, uncontrollable shock severely impaired subsequent learning to escape shock, but had no effect on responding ICSS, while the DBA/2J strain showed exactly the opposite pattern of deficits [248,306]. These studies may provide a starting point from which to investigate the physiological mechanisms underlying individual differences in responses to stress.

Vollmayr and Henn [280] have recently described a procedure in which mild shocks induce learned helplessness in only some of the subjects, which may mimic the variable human predisposition for depressive illness. This procedure has been used as the basis for a selective breeding program, which has produced a congenital learned helplessness (cLH) and a congenital non-learned helplessness strain. An impressive recent neuroimaging study of cLH rats reported that metabolism was decreased in dorsal frontal, medial orbital, and anterior cingulated cortex, but increased in the subgenual region of the cingulated cortex [250]; these exact changes have been described in depressed patients [72,173]. However, there are also anomalies: in particular, cLH rats show a decreased andrenocortical response to stress [127]. There is also uncertainty as to just what is being selected in

the cLH breeding program. cLH animals have been shown to exhibit stress-induced analgesia [127], raising the possibility that an increase in pain threshold might provide a very simple explanation of their escape-learning impairments. And given the separation of shock-induced escape deficits and anhedonia in inbred mouse strains [248,306], there is no reason to assume that the cLH strain, which was bred by selecting on the basis of shock-induced escape deficits, would also exhibit shock-induced anhedonia.

There is a relatively extensive literature dealing with the neurochemical bases of stress-induced motor inactivation [161,287]. Briefly, the debilitating effect of uncontrollable stress on later performance may be reversed by agonists at μ-opioid, dopamine (DA), α_1- and β-adrenergic receptors, or by anticholinergic drugs; conversely, helplessness may be simulated pharmacologically by drugs that reduce DA and/or noradrenaline (NA) function, or by drugs that increase cholinergic function [10,25–27]. Any influence of dopamine neurotransmission on learned helplessness appears to be mediated by the D1 dopamine receptor [91,92]. Furthermore, reduced catecholamine neurotransmission, either by inhibition of catecholamine synthesis with α-methyl-ρ-tyrosine [66] or by treatment with α_1- and β-adrenoceptor antagonists (as well as the opiate receptor antagonist naloxone) has been shown to block the therapeutic effect of TCAs [170]. Similarly, blockade of NMDA receptor-mediated neurotransmission also blocks TCA-induced reversal of learned helplessness [179]. Gamma-aminobutyric acid (GABA) neurotransmission also seems to be involved in learned helplessness behavior. Thus a long-term increase in $GABA_B$ neurotransmission is associated with exacerbation of learned helplessness, while the action of antidepressant drugs in this model is associated with a long-term reduction in $GABA_B$ neurotransmission [200,201]. In contrast, studies of the role of 5HT in learned helplessness are inconsistent [291]. Some 5HT-receptor agonists may reverse learned helplessness, while 5HT lesions did not prevent the action of TCAs in this model [258]. The induction of behavioral depression/learned helplessness may be regulated, at least in part, by serotonergic input into the hippocampal CA3 subfield. Papolos et al. [214] have shown that intracerebroventricular administration of an antisense oligonucleotide to the $5HT_{2A}$-receptor reduced receptor density in the CA3 area and induced learned helplessness behavior.

B. The Forced-Swim Test

In the forced-swim test, rats or mice are forced to swim in a confined space. While mice are subjected to a single swim test following a single drug administration, rats are generally subjected to two tests, usually spaced 24 h apart, to generate increased immobility scores. The onset of immobility exhibited by rats in the second test is delayed by pretreatment with a wide variety of antidepressants, usually administered in a multiple acute (subacute) treatment schedule consisting of three injections over the time period between the two swim sessions. The fact that the forced-swim test responds to acute drug treatment has been a frequent source of criticism. Small antidepressant effects may be present after a single high dose. Larger effects are observed after multiple acute treatments at lower doses, but these changes may simply reflect an elevation of brain drug concentrations.

Effective treatments include TCAs, MAOIs, most atypical antidepressants, the selective NA reuptake inhibitor, reboxetine [54], $5HT_{1A}$, $5HT_{1B}$, and $5HT_{2C}$-receptor agonists [207,59], extracts of St. John's wort (*hypericum perforatum*) [43], ECS, and REM sleep deprivation [224,228,33]. The test is not usually sensitive to SSRIs [32,33,55], although activity of these compounds has sometimes been reported, and this appears to reflect a subtle technical change, an increase in the water depth [59,272]. There have also been a

variety of other negative reports; nevertheless, some 90% of clinically active antidepressants are active in the forced-swim test, with the proportion rising in studies using chronic drug treatment [33,228]. It has been suggested that both serotonergic and noradrenergic systems may be involved in mediating antidepressant-induced reductions in immobility; serotonergic-mediated effects result from increased swimming time while noradrenergic-mediated effects reflect increased climbing behavior [69].

Reduced immobility scores have also been observed following treatment with neurosteroids [234], neuropeptide-Y [260], and NMDA-receptor antagonists [213,271]. The effect of NMDA-receptor antagonists is to reduce calmodulin-mediated activation of nitric oxide (NO) synthase. Likewise, NO synthase inhibitors have been reported to be as effective in reducing immobility as imipramine [103]. In rat studies, selective antagonists for subtypes of the cholecystokinin (CCK) receptor have been shown to reduce immobility scores. However, these positive effects are dependent on time of drug administration. Thus, the CCK_A antagonist, devazepide, is only effective when given before the conditioning pretest [108], while the CCK_B antagonist, L-365,260, is effective when given immediately prior to the retest [107]. These observations suggest a role for CCK in behavioral adaptations to acute stress.

On the negative side, while the test successfully discriminates antidepressants from neuroleptics and anxiolytics [225], false positives have been reported for stimulants, convulsants, anticholinergics, antihistamines, pentobarbital, opiates, a variety of brain peptides (see above), and a number of other drugs [33,228]. Some false-positive responses have been reported to disappear if chronic drug treatment is used or the duration of the test is prolonged [123,128], but the generality of these effects has not been established.

While the predictive validity of the forced-swim test may be better than has sometimes been assumed, its face and construct validity are minimal. The only symptomatic resemblance to depression is an inability or reluctance to maintain effort. Interestingly, positive responses to antidepressant drug treatment (i.e., reduced immobility) are only observed if subjects are forced to swim in water at least 10 °C below core body temperature (P.J. Mitchell, personal observations) and consequently develop severe hypothermia. If mice are forced to swim in water maintained at body temperature, then immobility still occurs, but the response to antidepressant treatment is abolished. These observations clearly implicate the importance of severe cold stress and the consequent induction of hypothermia in this model, which further weakens the parallel with depression.

The theoretical rationale of this test derives entirely from its supposed relationship to learned helplessness. However, the relationship between the two models is unclear. Prior inescapable, but not escapable, shock has been found to increase immobility in the forced-swim test [205,283], but in view of the consistent findings of depressed motor activity following inescapable shock [10], it would be surprising if this were not the case. The reciprocal finding has not been demonstrated: forced swimming did not impair subsequent escape performance in a shock-avoidance task in which performance deficits are typically seen following inescapable shock [206].

Nevertheless, the two tests do seem to share similar physiological substrates. Immobility in the swim test is also reversed by stimulating $5HT_{1A}$, $5HT_{1B}$, and $5HT_{2C}$ receptors [59,207,272], DA or α_1-adrenergic receptors, or by anticholinergics, and potentiated by treatments that decrease the activation of DA (e.g., amisulpride) [216], or NA systems or increase cholinergic transmission. Similarly, the therapeutic actions of antidepressants in this model are blocked by DA-receptor antagonists and by treatments that reduce NA function, including neurotoxic destruction of the ascending NA pathways and α_1-adreno-

ceptor antagonists. As in the learned helplessness test, impairment of 5HT transmission neither increases immobility nor blocks its reversal by antidepressants [33,291].

C. The Tail-Suspension Test

A number of variants of the forced-swim test have been proposed. One of these, the tail-suspension test, has been claimed to be ethically superior to the forced-swim test, as stress levels appear to be reduced [227,259]. In this model, mice suspended by the tail show a temporal pattern of struggling followed by immobility, similar to that seen in the forced-swim test. Antidepressants, at strikingly low doses, have been shown to increase the duration of mobility and also, in an automated version of the test, to increase the power of the movements (although the latter effect is rather less convincing). Effective agents include TCAs, MAOIs, and atypical antidepressants; the latter include mianserin, the selective NA reuptake inhibitor, reboxetine [302], extracts of St. John's wort (*Hypericum perforatum*) [43], the NMDA-receptor antagonist, MK-801 [213], and, significantly, some (but by no means all) 5HT uptake inhibitors [65,90,222,264], which are usually ineffective in the forced-swim test (see above). Immobility was also reduced by stimulant drugs, but was potentiated by neuroleptics or anxiolytics [227,259].

While it is tempting to see the tail-suspension test as a more sensitive version of the forced-swim test, there are subtle differences between them that are not at present understood but involve significant mediation by a noradrenergic receptor-mediated mechanism(s) [83]. In particular, $5HT_{1A}$-receptor agonists are active in the forced-swim test, but are ineffective in the tail-suspension test [228], whereas the mixed $5HT_{1A/1B}$-receptor agonist, RU 24969, was active in the latter. Like the forced-swim test, the face and construct validity of the tail-suspension test are minimal.

D. Chronic Stress Models

Repeated presentation of the same stressor usually leads to adaptation. However, adaptation can be prevented by presenting a variety of stressors in an unpredictable sequence. Katz and colleagues showed that 3 weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle, and a variety of other stressors caused a decrease in the activating effect of acute stress in an open field test. However, the activating effect of acute stress was maintained in animals receiving daily antidepressant treatment during the chronic stress period; the effects of administering antidepressants after exposure to stress have not been studied in this model. A variety of antidepressant drugs, as well as ECS, were found to prevent the effect of chronic stress, but the MAOI tranylcypromine was ineffective. Various nonantidepressants failed to prevent the effect of stress. In addition to causing changes in open field activity, chronic stress also increased plasma corticosteroid levels. This effect showed the same spectrum of pharmacological sensitivity, with the exception that an anticholinergic was also effective [122,291]. Similar effects have also been reported in mice; corticosteroid levels and the response to an acute stress were normalized by TCAs, but not by the SSRI, fluoxetine [255].

A further effect observed in rats after chronic stress was a failure to increase fluid consumption when saccharine was added to the drinking water, suggesting that chronic stress might cause anhedonia; this deficit was partially restored by imipramine [121]. The chronic stress model has been used very little since the original series of publications, in part because the levels of severity employed raise serious ethical problems. However, a

variant of the model has been devised in an attempt to achieve the same endpoints as the chronic stress model, but in a more ethically acceptable manner.

1. Chronic Mild Stress

The CMS procedure involves relatively continuous exposure of rats or mice to a variety of mild stressors, such as periods of food and water deprivation, small temperature reductions, changes of cagemates, and other similarly innocuous manipulations. Over a period of weeks of chronic exposure to the mild stress regime, rats gradually reduced their consumption of a preferred dilute sucrose solution and, in untreated animals, this deficit persisted for several weeks following the cessation of stress [297,299]. As discussed in Section IV, a variety of studies have been performed to confirm that these effects reflect a generalized insensitivity to reward; in particular, chronic mild stress also impairs responsiveness to reward as assessed by different methods, including suppression of place preference conditioning [217–219] and increased threshold for ICSS [196]. Studies of the effects of the individual elements of the chronic mild stress regime revealed that one element, social housing (in animals usually housed individually), was particularly potent, but no individual element (including social housing) was neither necessary to induce anhedonia nor sufficient to maintain anhedonia for a prolonged period [198].

Antidepressant treatment has no effect on sucrose consumption or ICSS threshold in nonstressed animals, but following the reduction of sucrose intake by stress, normal behavior was gradually restored by chronic treatment (2–5 weeks) with a wide variety of antidepressants, including TCAs, SSRIs, a specific NA reuptake inhibitor, MAO-A inhibitors, atypical antidepressants such as mianserin, buspirone, and amisulpride, and ECS. Also effective were some agents of uncertain antidepressant status, such as antihistaminic and anticholinergic drugs. Ineffective drugs include the anxiolytic chlordiazepoxide, various neuroleptics, amphetamine, and morphine [294,295]. Fluoxetine, maprotiline, and mianserin (but not chlordiazepoxide) were also found to restore the rewarding properties of food, as assessed in the place-conditioning paradigm [48,199]. In contrast to the extensive array of drugs correctly classified in CMS experiments, very few false positives or false negatives have been reported.

The reversal of an established behavioral deficit during the continued presence of the stressor is an important feature of this model: if, as seems likely, chronic stress does play a role in the etiology of melancholia [9,194,239], its continued presence during antidepressant therapy would usually be the norm. Also important is the extended time course of antidepressant action, which makes it feasible to detect rapid onset of action. Many novel agents have been identified as potential antidepressants using the CMS procedure, and these include some that appear to act more rapidly than TCAs or SSRIs, including the D2/D3 antagonist amisulpride [216], the glycine antagonist 1-aminocyclopropanecarboxylic acid (ACPC) [215], the $5HT_{1A}$-receptor agonist BIMT-17 [64], and the SNRI venlafaxine (M. Papp, personal communication). Potentiation of antidepressant action by lithium and pindolol has also been reported [252,253]. In at least three instances (the D2/D3 agonist pramipexole, the corticosterone synthesis inhibitor ketoconazole, and the catechol-O-methyl transferase (COMT) inhibitor tolcapone), the antidepressant effect of these compounds was demonstrated in the clinic only after these actions had been predicted in the CMS model [294].

In addition to decreasing responsiveness to rewards, CMS also causes the appearance of many other symptoms of major depressive disorder. Behavioral changes in animals exposed to CMS include decreases in sexual, aggressive, and investigative behaviors, and

decreases in locomotor activity. These are seen during the dark phase of the light–dark cycle, which is the rat's active period; electroencephalogram (EEG) measures of active waking are also decreased during the dark phase. In contrast, CMS did not cause the appearance of an anxious profile in two animal models of anxiety, the elevated plus-maze and the social interaction test, suggesting that the behavioral changes are specific for depression. Animals exposed to CMS show an advanced phase shift of diurnal rhythms, diurnal variation, with symptoms worse at the start of the dark (active) phase, and a variety of sleep disorders characteristic of depression, including decreased REM sleep latency, an increased number of REM sleep episodes, and more fragmented sleep patterns. They also gain weight more slowly, leading to a relative loss of body weight, and show signs of increased activity in the HPA axis, including adrenal hypertrophy and corticosterone hypersecretion. Abnormalities have also been detected in the immune system, including an increase in serum, decreases in thymus weight, natural killer-cell activity and reactivity to T-cell mitogens, and an increase in acute-phase proteins that was reversed by chronic antidepressant treatment. Taken together with the generalized decrease in responsiveness to rewards, these parallels to the symptoms of depression and, in particular, to melancholia, are both extensive and comprehensive [294]. Indeed, it is arguable that the only symptoms of depression that have not been demonstrated in animals exposed to CMS are those uniquely human symptoms that are only accessible to verbal enquiry. Even without these symptoms, a rat exposed to CMS could, in principle, legitimately attract a DSM-IV diagnosis of either major depressive disorder or major depressive disorder with melancholic features.

Studies of the neural basis of the CMS-induced anhedonia have focused primarily on the mesolimbic DA system. The behavioral changes in animals subjected to CMS are accompanied by a decrease in D2/D3-receptor binding and D2-mRNA expression in the nucleus accumbens, and a pronounced functional subsensitivity to the rewarding and locomotor stimulant effects of the D2/D3 agonist quinpirole administered systemically or within the nucleus accumbens. All of these effects are also reversed by chronic antidepressant treatment [73,295]. In other studies, animals successfully treated with antidepressants were treated acutely with D2/D3 receptor antagonists, at low doses that were without effect in nonstressed animals or in untreated stressed animals. This treatment reversed the effects of a wide variety of antidepressants on rewarded behavior [295]. These data argue strongly that an increase in D2/D3-receptor responsiveness may be responsible for the therapeutic action of antidepressants in this model [295]. A similar reversal of SSRI action by the D2 antagonist sulpiride has been observed in a clinical study, as predicted from the CSM data [294].

A recent CMS study suggests that a neural mechanism could mediate the negative information-processing bias characteristic of depression [22]. In this study [70], DA release in nucleus accumbens and prefrontal cortex was monitored by microdialysis following exposure to a palatable reward and a stressor (tail pinch). CMS markedly inhibited DA release in response to rewards, but potentiated responses to the stressor; both of these effects of CMS were reversed by chronic treatment with the TCA desipramine (DMI).

While the CMS model has a great many positive features, and is probably the most valid animal model of depression currently available, a major drawback is that the model has proven extremely difficult to implement reliably, and while many laboratories have succeeded in doing so, many others have not. The reasons for this lack of reliability have been extensively debated [294], but are as yet unresolved.

2. Stress Induction and Mild Stress Maintenance

Tagliamonte and colleagues have described a model that represents a cross between learned helplessness and CMS. In this procedure, an altered state is first induced by acute exposure to a session of moderately intense inescapable tail shock, and then maintained, apparently indefinitely, by exposure to milder stressors (brief restraint, a small number of shocks, or exposure to the inescapable shock apparatus), presented at 2-day intervals. This treatment resulted in an impairment of shock escape learning, as in learned helplessness, and a failure to learn a simple maze task reinforced by a highly palatable food (vanilla sugar), as in CMS. Both of these deficits were prevented by chronic pretreatment with TCAs or fluoxetine [67,97]. The stress procedure also decreased basal levels of extracellular DA in the nucleus accumbens shell, and this change also was prevented by chronic TCA treatment [94].

We noted earlier (Sec. IV) that the effect of severe life events on precipitating depression was best characterized as an acute severe stress followed by a chronic increase in mild stress. Stress induction followed by mild stress maintenance exactly parallels these processes and is the only animal model of depression to do so. This procedure could potentially provide a more reliable and robust alternative to the CMS procedure, but few data are currently available. In particular, while antidepressants have been shown to prevent the development of behavioral abnormalities, they have not been shown to reverse an established deficit. Also, the portability of the procedure to other laboratories has not been demonstrated.

E. Withdrawal from Chronic Psychomotor Stimulants

A number of studies have reported that responding for ICSS was reduced in the days following withdrawal from chronic amphetamine treatment. In these studies, amphetamine was administered to rats for between 4 and 10 days, typically using several administrations each day, at increasing doses [19,129,145,251]. The threshold for ICSS was elevated following amphetamine withdrawal, confirming that the rate reduction reflects a subsensitivity to brain stimulation reward rather than a depression of motor activity [46,146]. After 14 days of amphetamine treatment, the decreased sensitivity to ICSS did not recover during 18 days of further testing [146], reflecting subsensitivity of reward systems rather than simple depression of motor activity. In a single pharmacological study, this effect was alleviated by 2 days of imipramine or amitriptyline treatment, and with continued treatment, normal responding was restored [130].

In a variant of this procedure, animals self-administer cocaine, rather than being administered amphetamine [131]. In these experiments, the threshold for brain stimulation reward, administered through electrodes in the posterior lateral hypothalamus, was obtained using a discrete trial procedure [133], which is sensitive to changes in reward value, but not to changes in motor performance [167]. Following 24 h of cocaine self-administration, ICSS thresholds were elevated for several hours [131], indicating that cocaine withdrawal induced a state of anhedonia. Acute administration of the DA receptor agonist, bromocriptine, restored ICSS thresholds to normal [166]. Only one conventional antidepressant has been tested in this procedure: repeated administration of the TCA, DMI, was reported to shorten the duration of post-cocaine anhedonia [168].

It has frequently been assumed that stimulant drug treatment is a form of stress, since in many respects stimulant treatment and stress appear to be interchangeable [12, 229]. Indeed, withdrawal from chronic cocaine treatment in rats is associated with intense

anxiety-related behavior and extrahypothalamic-limbic corticotropin-releasing hormone (CRH) hypersecretion [242]. The similarities between stress and stimulant withdrawal are thought to arise from the fact that, like stimulant drugs, stressors activate the mesolimbic and mesocortical DA projections [30]. Independent of a relationship to stress, there is an obvious parallel between the effects of stimulant withdrawal and the depressions that frequently follow the cessation of chronic stimulant use [282], although in the animal model the time course is rather more compressed.

As the stimulant withdrawal models are based exclusively upon changes in ICSS behavior, their construct validity depends largely on the assumption that brain stimulation reward activates natural reward pathways [111]. Although early studies suggested that ICSS had unusual properties compared to natural rewards (e.g., rapid extinction), it was later recognized that these properties derive from differences in the experimental procedures typically employed (such as the delay of reinforcement); when such extraneous factors are equated, ICSS appears very comparable to a high-incentive natural reward presented under conditions of low drive [98]. These parallels, together with the observation that responding for ICSS performance is influenced by many of the factors that control responding for natural rewards, have justified the assumption that the ICSS electrode stimulates directly the neural substrates that are activated indirectly by natural rewards [111]. A degree of caution is required, because although people implanted with ICSS electrodes report a variety of pleasurable sensations, they also report other reasons for stimulating, such as curiosity [14,270]. Nevertheless, the commonality of anatomical substrate between ICSS and other types of reward supports the use of this procedure as an animal model of hedonic behavior [131,301]. However, the relationship of drug-induced depression to major depressive disorder is uncertain, and validity of this model is therefore questionable.

F. Social Dominance Models

Parallels have often been noted between depressive and submissive behaviors [95,230], and a number of laboratories have attempted to model depression by using animals of low social rank. Subordinates, and those who have lost status as a result of defeat in social conflict situations, are at greater risk for psychopathology. Biological similarities between defeated animals and human depression have frequently been noted [99,106,268], and there is considerable evidence that depressed individuals see themselves as inferior and behave submissively [99]. Indeed, social skills training, of which assertiveness training represents a major component, is used clinically as a psychotherapy for depression, which has been found to be equally effective as TCA treatment [109]. Like the chronic mild stress procedure, animal models based on social dominance employ realistic inducing conditions that are of particular ecological relevance.

1. Social Defeat

Social defeat is a potent stressor, and repeated defeat is a form of chronic stress, which is associated with a decrease in aggressive behavior [6]. A single social defeat has been reported to produce a gradual, but long-lasting, increase in immobility in the forced-swim test, which was prevented by chronic treatment with clomipramine [132]. A similar model has been developed in submissive C57BL/6J mice: a single defeat by a dominant male mouse of the same strain was reported to cause a gradual increase over weeks in passive behavior in response to a mild stressor, which could be antagonized by clomipramine; defeated animals also had higher immobility times in the forced-swim test [134]. In a

chronic version of a similar procedure, mice were housed in social contact, but were physically separated except for one daily 3-min encounter. Again, increased immobility in the forced-swim test was observed in repeatedly defeated animals, and this effect was prevented by chronic treatment with imipramine, although in this study imipramine did not normalize social behavior in the defeated animals [139].

In a modified rat model, defeat of dominant pair-housed rats by rats of a different, more aggressive, strain resulted in the loss of dominant status relative to their previously submissive partners, which was restored by chronic imipramine treatment [300]. This loss of status was accompanied by the abolition of morphine-induced place conditioning, most likely reflecting a decrease in hedonic tone [58]. This suggests that it may be appropriate to view submission models potentially as models of melancholia. However, more evidence is needed for any firm conclusion.

In a modified mouse social defeat paradigm, subordinate mice subjected to repeated social defeat showed reduced growth compared to dominant subjects, together with citalopram-sensitive anxiogenic-like behavior [124,125]. The defeated mice exhibited a maintained increase in both core temperature and circulating corticosterone levels indicative of chronic stress, although social defeat had no effect on either ethanol consumption or immobility time measured in the forced-swim test. Similarly, a single social defeat of Lewis rats resulted in hypophagia and weight loss, together with increased measures of anxiety, and these effects were reduced following acute fluoxetine treatment [24].

Overall, these various studies suggest that social defeat may in principle provide a valid and ecologically sound model of depression. However, a variety of procedures have been used and, at present, the data are not entirely consistent.

2. Social Hierarchy

A related procedure is based on the observation that rats housed in closed groups develop a social hierarchy and the relative social position of each group member may be identified by assessing each individual's level of success during agonistic encounters with other group members. If rats are housed in triads, then a social hierarchy consisting of a dominant, subdominant, and subordinate develops. Two-week chronic administration of either clomipramine or mianserin to the subdominant animal results in an increase in that subject's rank position at the expense of the level of dominance enjoyed by the dominant group member [188]. The increase in the social position of the antidepressant-treated subdominant rat is likely to be related to increased assertiveness expressed during social encounters. An attractive feature of this model is that daily assessment of social structure allows the time course of antidepressant-induced elevation of social position to be determined. However, not all features of subordinate animals are necessarily of relevance to depression, and the relationship between social dominance and social competition is potentially problematic. In group-housed rats competing for limited access to a high-incentive reward, the performance of subordinate animals was improved by acute or chronic anxiolytic treatment [96,118], suggesting that the social competition test is relevant to anxiety rather than depression. Consistent with this view is the observation that chronic treatment with *m*-chlorophenylpiperazine (*m*CPP), a major metabolite of the antidepressant trazodone which also possesses antidepressant activity, failed to increase the performance of subdominant rats in a social competition test [193] at a dose previously shown to increase the aggressive behavior of resident rats in a resident-intruder test (see below) [192]. However, the benzodiazepine anxiolytic diazepam, which improves performance in a social competition test, did not increase aggressive behavior in dyadic encounters, after either

acute or chronic administration [189]. Further studies will be necessary to confirm this ineffectiveness of anxiolytics in social dominance tests.

3. The Resident-Intruder Test

Antidepressant treatment has consistently been shown to have profound effects on rat, but not necessarily mouse, social and agonistic behavior. While acute treatment with pharmacologically disparate antidepressant drugs (including TCAs, MAOIs, SSRIs, SNRIs, 5-HT_{1A}-receptor agonists and partial agonists, $5HT_{2C}$-receptor agonists) commonly reduces the aggressive behavior of resident rats when confronted with an unknown conspecific intruder, chronic antidepressant treatment (including repeated ECS) increases such aggressive behavior [51,52,183–187,189–192]. These observations are consistent with the view that aggression is the only type of rodent social behavior consistently increased following chronic treatment with antidepressants [85].

The fact that chronic antidepressant treatment increases aggressive behavior appears at first sight to be incompatible with the use of SSRIs in the clinical treatment of impulsive aggression [53,76,82]. However, this is a clinical, rather than an experimental, paradox, since clinically, antidepressants both increase aggression in submissive depressed individuals (manifest as a reversal of intropunitive aggression and/or impaired sociability: [71,119,231] and decrease pathological aggression [112,275]). A resolution of this paradox may be that antidepressant treatment increases assertiveness, since this would increase low levels of social dominance while at the same time decreasing high levels of physical aggression. Hence, the ability of chronic antidepressant treatment to increase aggression in rats may reflect the increased assertiveness and associated externalization of emotions expressed during recovery from depressive illness. Such increased assertive/aggressive behavior is consistent with the effects of such treatment in the social hierarchy model (see above). However, face validity of the resident-intruder model is reduced by the fact that the test involves normal unmanipulated animals; nondepressed people do not respond to antidepressant treatment. Interestingly, and in contrast to the rat studies, the aggressive behavior of male mice in murine resident-intruder studies is particularly sensitive to anxiolytic, rather than antidepressant, drug activity [159].

By programming daily dyadic encounters, the resident-intruder paradigm can be used to compare the rate of onset of antidepressant-induced elevation in aggression between antidepressant treatments, and to assess the utility of potential adjuvant treatment to accelerate antidepressant-related changes in rodent behavior. Indeed, the first published studies to demonstrate the ability of a selective $5HT_{1A}$-receptor antagonist (WAY-100635) to accelerate time-dependent antidepressant-induced behavioral changes used the resident-intruder test [191].

G. Neonatal Antidepressant Treatment

Neonatal treatment of rat pups with the TCA clomipramine has been reported to cause a spectrum of symptoms reminiscent of depression, including decreases in sexual and aggressive behavior, a shortening of REM sleep latency, and subsensitivity to ICSS. Treated animals were also hyperactive in some tests [278,279]. Animals were typically tested when mature, at ages that varied between tests. Most of the behavioral and sleep abnormalities were present on first testing at approximately 3 months; however, the ICSS abnormalities were absent at 4 to 5 months, but present at 6 to 8 months. ICSS was not tested in older animals, but some other abnormalities appeared to normalize at around 11 months [278].

These effects are probably not specific to clomipramine, since neonatal treatment with DMI, zimeldine, or the SSRI, Lu 10-134-C, also increases immobility in the forced-swim test in adult rats [102,110,276], while neonatal treatment with the SSRI citalopram similarly reduces adult aggressive behavior [163]. Zimeldine, like clomipramine but not DMI, also reduces or disrupts adult rat sleep patterns by shortening the duration of REM sleep bouts [87]. In contrast, neonatal treatment with scopolamine, a cholinergic receptor antagonist, suppressed REM sleep, as observed with neonatal clomipramine treatment but, unlike clomipramine, facilitated adult male sexual behavior. These results suggest that the neonatal clomipramine-induced reduction in male sexual behavior is not due to early REM sleep deprivation [277].

As yet, information concerning the pharmacological responsiveness of this model is minimal: some effects of imipramine and REM sleep deprivation have been reported, but the numbers of subjects tested were too low to allow reliable conclusions to be drawn [278]. While this model appears to have good face validity in terms of the range of symptoms displayed and the insidous onset of anhedonia, the mechanisms by which neonatal antidepressant treatment has adverse effects in mature animals are unknown. In particular, it is unclear what, if any, is the relationship between neonatal antidepressant treatment and the etiology of depression in humans. The extent to which the symptoms can be reversed by antidepressants is uncertain. Nevertheless, these studies raise the disconcerting possibility that a breast-fed infant could develop a susceptibility to melancholia by ingesting TCAs prescribed to the nursing mother for the relief of postpartum depression.

1. Perinatal Stress

Possibly related to the neonatal antidepressant treatment model are models based on prenatal and neonatal stress. Anhedonia has not been demonstrated in these models, and indeed their relevance to depression, as opposed to other forms of psychopathology, is far from established, but some endocrinological data suggest a possible relevance to melancholia.

Prenatally stressed (PS) rats (where the dam is subjected to repeated foot shock during the early stages of pregnancy) exhibit elevated activity of the HPA axis and defensive behavior before weaning, and the heightened defensive behavior, as well as exaggerated behavioral, physiological, and neuroendocrine responses to stressful stimuli, persist into adulthood [262,281,285]. Current behavioral data suggest similarities between the behavioral profiles of PS rats and the anxiogenic changes in behavior induced by yohimbine and idazoxan [285]; these data indicate high levels of anxiety in PS rats. However, female, but not male, PS mice exhibit an antidepressant-reversible increase in immobility time in the forced-swim test, indicating that PS might induced a gender-dependent increase in the risk of depression-like behavior [7,8].

Neonatal stress in nonhuman primates has been shown to induce hypersecretion of CRH [57] and abnormal social behavior in adulthood, indicative of enhanced response to stressors [50]. Similarly, neonatal stress in rat pups, achieved by maternal separation, also increases CRH levels in adulthood [142].

H. Selective Breeding for Muscarinic Hypersensitivity

The Flinders Sensitive Line (FSL) rat is the result of selective breeding for sensitivity to the hypothermic effect of cholinergic agonists and is based on the hypothesis that central cholinergic systems are important in depression since increased cholinergic sensitivity has

been reported in depressed patients. Although bred for cholinergic hypersensitivity, FSL animals also show a number of other pharmacological abnormalities, including serotonergic hypersensitivity and dopaminergic hyposensitivity [210]. Relative to the control Flinders Resistant Line, FSL animals have a number of characteristics reminiscent of depression, including cholinergic supersensitivity, increased REM sleep, and reduced locomotor activity, which is further pronounced following electric footshock [209]. FSL animals also show greater immobility in the forced-swim test [210]. This behavior was normalized by chronic treatment with a high dose of imipramine, and partly reversed by DMI or sertraline, but was not restored by chronic amphetamine or scopolamine [208,211,244]. FSL rats also exhibit a greater vulnerability to the suppressive effect of chronic mild stress on responsiveness to sweet reward [232,233], but behave normally in the elevated plus maze, a putative animal model of anxiety [208].

While these observations are consistent with a depressive diathesis and greater behavioral responsiveness to stress, other data are not. FSL rats have markedly elevated levels of 5HT, NA, and DA in specific brain areas that are normalized during chronic treatment with DMI [307,308], and hypothalamic levels of CRH and circulating levels of adrenocorticotrophic hormone (ACTH) are lower in FSL rats [212], indicating reduced HPA axis activity, in contrast to the increased HPA axis activity observed in depression. Similarly, acute and chronic treatments with nicotine, a nonantidepressant, both exhibit an antidepressant-like effect on the behavior of FSL rats in the forced-swim test [266], which was blocked by prior treatment with the nicotinic receptor antagonist, mecamylamine [267]. It should be noted that the major pharmacological evidence supporting the FSL strain as a model of depression derives from studies using the forced-swim test, which is itself of questionable validity.

VI. MODELS OF OTHER SUBTYPES OF DEPRESSION

Little attention has been paid to the question of subtypes of depression in the development of animal models, which have tended to focus either on an undifferentiated depressive state or on melancholia.

Unlike melancholia, no consensus exists that nonmelancholic depression is a homogeneous entity, and this issue has been extensively debated. Within the nonmelancholic spectrum, two syndromes have been described that are of interest in the present context. One of these is not recognized in DSM-IV, but has features that suggest it might represent a coherent biological entity. This is a form of depression in which central 5HT activity is decreased, as indicated by low concentrations of the 5HT metabolite 5-hydroxyindole acetic acid (5HIAA) in cerebrospinal fluid. These depressions are characterized by high levels of anxiety and agitation, and Van Praag [273] has suggested that these symptoms are primary, with depressed mood as a secondary response to a failure to cope with the consequences. It has been suggested that some animal models of depression which are characterized by high levels of locomotor activity and/or aggression, may represent models of this subtype, and these are described below.

A second nonmelancholic subtype of depression is delusional depression (previously known as psychotic depression). Delusional depression (DSM-IV major depressive episode with psychotic features) is difficult to translate into behavioral terms, being differentiated from nondelusional depression only by a greater association with psychomotor agitation [203]. Delusional depressions are pharmacologically distinct in that they respond to ECT or combinations of TCAs with neuroleptics, but not to TCAs alone [202]. The

development of an animal model that responds to ECS but not to TCAs has not yet been achieved.

Bipolar disorder is another well-defined diagnostic category for which there are no animal models. There are a number of animal models of mania [160], but the alteration of depressive-like and manic-like behavior in an animal model has not yet been systematically addressed. Indeed, the episodic nature of unipolar depression (e.g., brief recurrent depression) also remains to be explored in animal models.

A. Social Separation

The presumed etiological role in depression of loss events, and particularly, loss of a loved one, has led to the development of a number of animal models of depression based on separation phenomena.

Although the evolutionary proximity of primates has led some authors to consider primate separation models to be of particular importance [78], they have produced remarkably little of value. Precisely because of their evolutionary proximity, the use of primates sacrifices many of the advantages of animal models, such as the easing of ethical constraints, and the possibility of testing adequately sized groups of subjects.

1. Neonatal Isolation

The most familiar of these models involve nonhuman primates, either infants isolated from their parents, or juveniles isolated from their peer group. The separation response consists of an initial stage of protest, characterized by agitation, sleeplessness, and distress calls, followed by despair, characterized by a decrease in activity, appetite, play and social interaction, and the assumption of a hunched posture and sad facial expression [105,261]. These symptoms are strikingly similar to those of anaclitic depression in institutionalized children [238]. However, while parental loss in childhood, and bereavement in adults, are implicated in the etiology of depression, they also increase the risk of a variety of other psychiatric and nonpsychiatric disorders [40,245]. The nature of the separation response is sensitive to the environment in which the experiments are carried out [235,261], and the incidence of depressive behaviors may in some experiments be as low as 15% [150].

The few pharmacological studies using these models have not been impressive. Very few published studies have attempted to use antidepressant treatments to modify primate separation behavior, and because of the expense of using primates, the size of experimental groups in most studies has usually been too small to provide reliable data. Chronic treatment with DMI [113], imipramine [261], oxaprotiline [177], and ECS [149] have been reported to reverse some, but not all, of the effects of separation in monkeys. Trifluoperazine, amphetamine, and diazepam did not affect responses to social isolation in chimpanzees [180,269], but some therapeutic effects of chlorpromazine were seen in rhesus monkeys [178]. Depressive-like behaviors in singly housed rhesus monkeys are associated with decreased concentrations of NA in the cerebrospinal fluid, with relatively little effect on DA or 5HT [138]. Following a return to social housing, NA levels normalize, but the animals remain hypersensitive to pharmacological challenges of the NA system [136,137].

In fact, separation phenomena of protest followed by despair are present to some extent in many other species, including cats, dogs, rodents, and precocial birds [120,176], and several of these phenomena have also been used as the basis of the development of animal models of depression. One of these, the reactivation of distress calling in 1-week-

old chicks, appears to perform relatively well as an antidepressant screening test [144]. The vocalizations of guinea pig pups separated from their mothers also respond to antidepressants, and this test was used successfully to detect antidepressant-like activity of the substance P neurokinin-1 (NK1) receptor antagonists [240].

2. Adult Isolation

Chronic (4–6 weeks) isolation of adult rats has been found to cause a disruption of cooperative social behavior [23] reminiscent of the poor social performance of depressed people [148]. In a single study of the effects of antidepressant treatment, the impairment of social cooperation in isolated rats was reversed by chronic treatment with either imipramine or fluoxetine, and the effect of imipramine in this model was abolished by the 5HT antagonist metergoline [298].

B. Olfactory Bulbectomy

The other major animal model of depression is based on the destruction of the olfactory bulb in rats, which disrupts the limbic-hypothalamic axis. Bulbectomized (OB) animals display a variety of behavioral changes, including irritability, hyperactivity, and impairment of passive avoidance learning [44]. Recent observations suggest that OB rats exhibit increased reactivity together with a reduced rate of habituation to novel stimuli [164] and changes in the immune system and NA, DA, 5HT, GABA, cholinergic, and glutaminergic neurotransmitter systems [126]. They also show an elevation of circulating corticosteriod levels (as do stressed animals), which appears to be an increased corticosteroid response to stress rather than an increase in basal levels [36]. These changes are reversed by antidepressants, including compounds with $5HT_{1A}$-receptor agonist activity [34,175], SNRIs [174], and selective NA reuptake inhibitors [104] in addition to the TCAs. The most specific antidepressant effect is the reversal of the passive avoidance learning deficit [44,274], although attenuation of hyperactivity in the open field is nowadays the most commonly used behavioral predictor of antidepressant activity [126]. However, while all TCA and atypical antidepressants appear to be effective in this test, MAOIs are not [116]. While repeated administration of TCAs is necessary to normalize behavior in this model, a limited number of studies suggest that some SSRIs act after acute treatment [117,157]. Recent studies suggest that the OB rat model of depression is insensitive to the potential rapid-onset antidepressant action induced by concomitant treatment with $5HT_{1A}$-receptor antagonists [60,61]. The implication that the model involves primarily serotonergic mechanisms is supported by the observation that the effects of subchronic treatment with imipramine and mianserin were reverse by acute adminstration of the $5HT_2$-receptor antagonist metergoline [35].

Although pharmacologically specific for antidepressants, the face validity of the olfactory bulbectomy model appears slight. Unlike the stress models, the bulbectomized rat resembles an agitated hyposerotonergic, rather than a retarded, depression [158], but beyond hyperactivity, it is difficult to discern any further points of behavioral resemblance. Indeed, increased irritability and aggression, together with exaggerated reactivity to auditory and tactile stimulation, may reflect additional septal damage. Nevertheless, bulbectomized rats do resemble depressed humans on a surprisingly wide range of peripheral neurochemical and immunological markers, such as abnormalities of platelet 5HT transport and neutrophil phagocytosis [147].

C. Waiting Behavior

To the extent that they appear to involve serotonergic mechanisms, two final models may be related to olfactory bulbectomy. These are two paradigms in which antidepressants increase the ability of rats to withhold responses.

In one paradigm (differential reinforcement of low rate–72 s; DRL–72 s), premature responding delays the delivery of reward; the ability of drugs to improve performance in this model has been claimed to be an efficient antidepressant screening test [246], although this has been disputed [223]. In the second paradigm, a larger reward may be earned by waiting; again, the improvement of performance in this model appears to be a specific property of a wide range of antidepressants [28]. Drugs that possess $5HT_{1A}$-receptor agonist activity are antidepressant-like in both of these waiting paradigms [13,29,135,236], and in the DRL-72 test, antidepressant effects were blocked by 5HT-receptor antagonist [165]. The relevance of these behaviors to depression is unclear. In a different schedule designed to examine accurate estimation of time intervals, acute treatment with antidepressant drugs (imipramine, clomipramine, and zimeldine) failed to modify timing accuracy [20], suggesting that improvement in performance on DRL schedules, including SSRIs [256], is most likey due to a reduced rate of lever pressing rather than improved timing. It has been reported that antidepressant effects in the DRL vary considerably between rats of different strains, or rats of the same strain obtained from different suppliers [15], and this may account for the discrepancies between different laboratories [223,246].

Clinical evidence strongly suggests that similar disorders of 5HT function cut across diagnostic boundaries and are expressed as pathologically impulsive behavior rather than as any particular disorder [257,290]. Indeed, mice lacking the $5HT_{1B}$ receptor ($5HT_{1B}$ knock-out mice) exhibit more impulsive aggressive behavior, drink more ethanol, and acquire cocaine self-administration faster than wild-type mice [42,243], which reflects (in part at least) reduced 5HT function [77], and may provide an animal with addiction and motor impulsivity. In vervet monkeys (*Cercopithecus aethiops sabaeus*), there appears to be a clear inverse relationship between serotonin turnover and social impulsivity [80]. The inability to withhold responses in the two rodent waiting models may constitute instances of impulsive behavior and, as such, the effect of antidepressants in these paradigms may not relate specifically to depression.

VII. GENOMIC MODELS

In this review, we have focused on behaviorally defined animal models of depression, and exclude from consideration a number of older tests, now little used, based on interaction between antidepressants and a range of other pharmacological agents, such as reserpine, tetrabenazine, amphetamine, yohombine, apomorphine, and clonidine. Generally speaking, the predictive validity of these tests is poor, and their face and construct validity as simulations of depression is minimal or zero. Although still in use, this is largely confined to mechanistic studies.

Of greater current interest, a number of genomic models have recently been examined for their possible relevance to depression. Although their success is as yet limited, we review them here as potentially the shape of things to come.

A. HPA Transgenic

In depressive illness, the secretion of CRH from the paraventricular nucleus of the hypothalamus is under the control of a glucocorticoid receptor-mediated inhibitory feedback system. Conditions of extreme, prolonged stress cause reduced sensitivity of the glucocorticoid receptor, thereby reducing the effectiveness of the feedback loop, resulting in hypercortisolemia. A transgenic mouse strain expressing glucocorticoid receptor antisense has been described which exhibits dysfunction of the HPA axis in a manner similar to that seen in depressive illness [195,220]. Chronic treatment with antidepressants reduces plasma cortisol and ACTH levels [195,221] by increasing glucocorticoid-receptor mRNA expression [17], with a subsequent elevation of glucocorticoid-receptor activity resulting in increased sensitivity of the HPA axis to glucocorticoid negative feedback [18]. However, while behavioral and neuroendocrine responses to stress are modified in transgenic mice [81,155], the direction of these changes is inconsistent with a model of depressive illness: relative to wild-type controls, transgenic mice exhibit reduced immobility in the forced-swim test and less anxiety-related behavior on the elevated plus maze but increased aggressive behavior [21,195]. All of these changes are in the direction opposite to those expected.

B. 5HT Transporter Knockout

The serotonin reuptake transporter (5HTT) is pivotal in the inactivation and control of serotonergic neurotransmission, and blockade of the serotonin transporter is the principal target of the TCA, SSRI, and SNRI drugs. Investigations of subjects that lack the serotonergic transporter, while not a model of depression, may provide insight into the adaptive mechanisms associated with a permanent lack of serotonin reuptake. Recent studies suggest that 5HTT knock-out mice show regional differences from wild-type mice in terms of $5HT_{1A}$-receptor protein and mRNA expression (reduced in dorsal raphe, hypothalamus, amygdala, and septum, but increased in hippocampus [79,143,153], that are associated with reduced functional sensitivity to the hypothermic effects of the $5HT_{1A}$-receptor agonist 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT) [152]. Similarly, $5HT_{2A}$-receptor density is also reduced in 5HTT knock-out mice [237]. Interestingly, the desensitization of $5HT_{1A}$ autoreceptors in 5HTT knock-out mice is further enhanced by exposure to stressful conditions [143].

C. CRH Receptor Subtype Knockouts

CRH is a critical coordinator of the HPA axis and subtypes of CRH receptors, CRH-R1 and CRH-R2, are found throughout the central nervous system and peripheral tissue. CRH has higher affinity for the CRH-R1 receptor, while urocortin (a CRH-related peptide) exhibits 40-fold selectivity for the CRH-R2 receptor. CRH-R1 knockout mice exhibit anxiolytic-like behavior, which may be due to impaired spatial recognition memory [56], together with characteristic responses to stress [265] indicative of disrupted HPA axis [254]. In contrast, CRH-R2 knockout mice exhibit increased anxiety-like behavior and are hypersensitive to stress [16].

D. Tachykinin Receptor Knockout

The NK1 receptor is expressed in brain areas associated with the control and management of depressive illness, anxiety, stress, and sensitivity to the rewarding properties of food and

drugs of abuse. Indeed, antagonists at the NK1 receptor have been suggested as potential antidepressant drugs with a novel mode of action [89,240,241]. The NK1-receptor knock-out mouse is not an animal model of depression. Rather, these mice show behavioral changes similar to those elicited by antidepressants in normal mice, including a decrease in neonatal vocalization following maternal separation, decreased aggressive behavior in the resident-intruder test, and decreased immobility in the forced-swim and tail-suspension tests [241]. NK1-receptor knock-out mice also exhibit a loss of the rewarding properties of morphine, together with reduced physical response to opiate withdrawal, but their response to cocaine is unchanged [197], suggesting that this may reflect a specific interaction with opioid systems, rather than a general effect on brain reward mechanisms.

VIII. OBSERVATIONS

Animal models of depression have traditionally been defined primarily by their responsiveness to antidepressant drugs, and, consequently, this term encompasses a range of different procedures that are difficult to subsume within a simple conceptual structure. Previous reviews [291] have suggested that a broad distinction can be made between two groups of models, which is preserved in the present review. There are various, partially overlapping, ways of defining the two groups: by etiology, symptomatology, and physiological basis. Thus, one group of models, here defined as models of anhedonia (although this is not demonstrated for the forced-swim test or submissive behavior models) are brought about largely by stress (although this is difficult to argue in the case of the neonatal antidepressant model), and reflect primarily impairments of catecholaminergic transmission (to the extent that the physiological substrates are known). The second group (other models) may involve aspects of impulsive behavior, may be related to social isolation, and may reflect impairment primarily of 5HT. The tentative nature of the generalizations is all too obvious. However, they do provide hypotheses that bring animal models into contact in traditional monoamine theories of depression, and suggest ways in which those theories might be tested and amplified [287,291]. It does seem important that the diversity of depressive symptomatology is matched by a similar diversity in the phenomenology expressed in animal models, and this consideration may justify the continued use of models that have little face or construct validity.

It was noted earlier that while the etiology of depression probably involves multiple factors, animal models have tended to be unifactorial. An interesting trend is that symptoms characteristic, or at least reminiscent, of depression may be generated in animal models in various ways. Thus, anhedonia is subject to genetic influences (strain differences in the anhedonic effects of uncontrollable shock or chronic mild stress) [232,304]; epigenetic influences (neonatal antidepressant treatment) [278]; acute severe stress (uncontrollable shock) [304]; chronic mild stress [299]; and psychomotor stimulant withdrawal [131]. Similarly, immobility time in the forced-swim test is increased by genetic influences (strain differences) [210]; epigenetic influences (neonatal antidepressant treatment) [110]; acute stress (defeat or uncontrollable shock) [134,284]; and chronic stress (defeat) [139]. Studies of combinations of etiological factors are clearly warranted: an understanding of the manner in which different factors (in particular, predisposing and precipitating factors) interact to generate depression-like symptoms in animals models would be of considerable interest to both basic researchers and clinicians.

It remains the case that the greatest immediate contribution of animal models of depression is likely to be in relation to the elucidation of the mechanisms of action of

antidepressant drugs. Thirty years of clinical experience have given rise to two widely accepted axioms: that only rarely do antidepressants cause discernible clinical improvement within the first 2 weeks of treatment and that they are devoid of mood-elevating effects in normal human subjects. The clinical requirement for chronic treatment regimes has led to a considerable literature describing the effects of chronic antidepressant administration in normal animals, and numerous changes in pre- and/or postsynaptic receptor function have been reported in a variety of systems. These descriptive studies are an essential first step toward establishing mechanisms of antidepressant action. However, the inability to determine which of the many effects of antidepressants are responsible for their therapeutic actions constitutes a fundamental limitation of this approach, which has not been widely recognized. The development of chronic animal models, in which an abnormal state is induced and maintained for a prolonged period during which therapy can be administered, provides a powerful methodology for investigating these problems.

Although they have as yet made little impact, it is to be expected that genomic models will play a significant role in these developments. However, it will be important not to lose sight of the fact that genetic and genomic models are mostly of relevance to the predisposition to depression rather than to precipitation of depressive episode. Studies in which valid and realistic models of predisposition to depression are combined with valid and realistic precipitation models are awaited with interest.

Finally, although this review has not been concerned with the problem of screening for antidepressant activity, it is clear that some of the models discussed could profitably be incorporated into antidepressant screening programs. It has traditionally been a major consideration in the design of antidepressant screening tests that they respond to acute or subacute drug administration. As a direct consequence, they are incapable, by virtue of their design, of responding to the major current challenge of discovering new antidepressants that have a shorter onset of action. By contrast, it is implicit in their protracted time course that chronic realistic animal models do have the capacity to detect a rapidly acting novel antidepressant. In fact, most pharmaceutical companies have now abandoned the high volume, random screening approach in favor of the development of a small number of compounds specifically designed to meet predetermined pharmacological criteria. In such a program, the place of behavioral screening methods shifts from the discovery phase to the development phase, and the logistical disadvantages of using complex, chronic models are small relative to the costs of testing an ineffective drug in the clinic.

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21

Pathogenesis of Depression: Reconsideration of Neurotransmitter Data by Depletion Paradigms

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I. INTRODUCTION

Considerable evidence is available in the literature that supports the idea that brain monoamine systems play a key role in the pathogenesis of affective disorders, in particular depression. These hypotheses have primarily taken the form of proposing abnormal regulation in serotonin (5HT) [1], and the catecholamines norepinephrine [2,3], and dopamine [4] in the disorder. 5HT is known to be involved in the regulation of a wide variety of functions, including mood, anxiety, aggression, sleep, appetite, and sexual function, although it has to be acknowledged that the precise details of the mechanisms involved are not fully understood. There are many data available showing that, besides 5HT, both dopamine and norepinephrine also are involved in the pathogenesis of depression and its treatment. Brain catecholamines are thought to be involved in the regulation of mood, anxiety, appetite motivation, rewards, and attention to novel environmental stimuli and most patients with depression show disturbances in these functions.

The situation regarding how to evaluate the potential role of monoamines in depression has been hampered by the fact that, until recently, it has not been possible to measure

brain 5HT and catecholamines directly, which means that researchers had to rely on indirect evidence for the involvement of these transmitter systems in the pathogenesis of depression and their role in antidepressant treatment modalities. Over the past years, neurotransmitter depletion paradigms have provided another means of examining the systems involved and have become important tools in investigating the role of monoamines in depression and its pharmacological and nonpharmacological treatments. The purpose of this chapter is to present biochemical and behavioral data concerning the role of monoamine systems in the pathogenesis of depression and antidepressant treatment modalities.

II. BASIC CONCEPTS

A. Catecholamine Depletion

Of various methods employed for modifying the functions of sympathetic nervous systems, a unique and successful one has been the inhibition of catecholamine biosynthesis by α-methyl-para-tyrosine (AMPT) [5,6]. AMPT decreases norepinephrine and dopamine levels via inhibition of tyrosine hydroxylase, a rate-limiting enzyme in the synthesis of both transmitters [7]. AMPT is adequately absorbed from the gastrointestinal tract and the degree of inhibition achieved in humans approximates the values, which could be predicted from the plasma levels of the drug [8]. Originally, the drug was used to successfully treat pheochromocytoma, whereas in cases with essential hypertension the drug was not successful. In clinical investigations, AMPT (in dosages ranging from 1 to 4 g/day) leads to profound decreases in urinary excretion of catecholamine metabolites and cerebrospinal fluid levels of the dopamine metabolite homovanillic acid (HVA), with no change in the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA) [5,6,9]. A maximal reduction of catecholamine metabolites during AMPT treatment occurs after 2 to 3 days of treatment [8,10].

In the initial studies unrelated to depression, researchers noted the central nervous system effects of AMPT, and reported sedation (generally mild, but to varying degrees), anxiety, agitated depression, and changes in sleep pattern after withdrawal of the medication. In addition to these changes in mood and mental activity, adverse effects related to neuromuscular activity appeared in some patients. The symptoms included mild tremor of the hands and, in the most severe form, gross tremor of the hands and legs unaffected by rest or movement. Other side effects are diarrhea, galactorrhea, crystalluria and urolithiasis, and decreased salivation. As with mood changes, all these effects disappeared rapidly with cessation or reduction of drug dose. Altogether, balancing the risks of toxicity versus therapeutic benefit, it seemed reasonable to continue to utilize AMPT for therapeutic purposes only in specific cases of patients with malignant pheochromocytoma. In contrast, in carefully conducted clinical trials including medically healthy subjects, manipulations of central catecholamines with AMPT offer a unique opportunity to identify transmitter systems that are believed to play a role in depression, and to further understand brain structures and transmitter systems being involved in the effects of antidepressants.

B. Tryptophan Depletion

The aim of tryptophan depletion is to lower brain 5HT by depleting its precursor tryptophan. Most of the tryptophan in plasma is protein bound, with only about 5% being left free and available for transport across the blood–brain barrier by an active protein shuttle for which five other large amino acids (valine, leucine, isoleucine, phenylalanine, and

tyrosine) also compete. Once in the brain, the initial step in the biosynthesis of 5HT is the conversion of L-tryptophan to 5-hydroxytryptophan, a reaction catalyzed by the rate-limiting enzyme tryptophan hydroxylase [11]. The Michaelis constant for tryptophan hydroxylase is higher than tryptophan concentration in the brain, suggesting that under physiological conditions the activity of this enzyme is limited by the availability of the substrate [12]. Animal studies have shown that the synthesis and content of 5HT in rat brain vary in parallel with brain tryptophan concentrations [13]. Moreover, it has been shown that increase in brain tryptophan concentration raises 5HT release in vitro [14,15] and in vivo [16,17], although some studies disagree with these findings [18,19]. In summary, the concentration of brain 5HT depends upon the availability of its precursor tryptophan.

Preclinical data show that the acute administration of a tryptophan-free amino acid mixture of essential amino acids produces a rapid and substantial decrease in plasma tryptophan levels, associated with a decrease in brain tryptophan, brain 5HT, and 5HIAA levels in rats [20]. Studies in humans show profound decreases of plasma tryptophan levels [21,22], and cerebrospinal fluid levels of 5HIAA [23,24] after oral administration of an amino acid mixture without tryptophan. A positron emission tomography study of humans showing a marked lowering of brain 5HT synthesis induced by tryptophan depletion confirms the assumption that brain serotonergic activity is transiently reduced during tryptophan depletion [25]. However, it has to be acknowledged that uptake of alpha-methyl L-tryptophan is not clearly established as a reliable indicator of 5HT synthesis.

Two mechanisms are responsible for the transient decrease in brain 5HT activity during tryptophan depletion: (1) the amino acid mixture (Table 1) given to the subjects during tryptophan depletion stimulates protein synthesis in the liver, which uses up plasma tryptophan, and (2) the amino acid mixture contains large amounts of other neutral amino

Table 1 Amino Acids Used for Tryptophan Depletion Versus Sham Depletion

	Makes one beverage
L-Alanine	5.5 g
Glycine	3.2 g
L-Histidine	3.2 g
L-Isoleucine	8.0 g
L-Leucine	13.5 g
L-Lysine	11.0 g
L-Phenylalanine	5.7g
L-Proline	12.2 g
L-Serine	6.9 g
L-Threonine	6.9 g
L-Tyrosine	6.9 g
L-Valine	8.9 g
L-Methionine	12.0 g
L-Arginine	19.6 g
L-Cysteine	10.8 g
	During sham depletion the beverage contains additional amino acid
L-Tryptophan	2.3 g

acids, which compete with tryptophan for transport across the blood–brain barrier and thus restrict uptake of tryptophan into the brain. Both mechanisms lead to the rapid and substantial, albeit transient, reduced synaptic availability of 5HT in the brain. The value of a depletion paradigm depends on whether the method is reliable, reversible, and specific. All three issues have been addressed in several studies and have shown that the tryptophan depletion method fulfills all three requirements for a meaningful research tool [26–29]. In particular, studies in monkeys [30] and humans [31] showed that tryptophan depletion did not change the metabolism of other neurotransmitters, whereas levels of tryptophan and 5HIAA in plasma and cerebrospinal fluid, respectively, were lowered. Thus, if the effects of tryptophan depletion can be attributed to changes in a transmitter system in the brain, it is probably 5HT systems that are affected.

III. CATECHOLAMINE DEPLETION STUDIES

A. Healthy Subjects

In order to evaluate the role of catecholamines in regulation of mood, anxiety, alertness, and its potential role in the pathogenesis of psychiatric disorders, it is important to study the effects of catecholamine depletion in healthy volunteers. Treatment with AMPT has been shown to induce pronounced increases in sleepiness and mild increases in negative mood and anxiety when administered to healthy male controls [32]. Noteworthy is the fact that replacement of catecholamine stores with L-dopa reverses the effects of catecholamine depletion, and is associated with a more rapid recovery from AMPT effects than when subjects are treated with AMPT alone. Another study of the same group comparing the psychological effects of AMPT alone versus AMPT plus 40.5 h of total sleep deprivation [33] suggests that catecholamines may be involved in mood changes during sleep deprivation. Combined treatment with AMPT and sleep deprivation led to significant increases in negative mood, whereas no treatment alone produced consistent mood changes. With the use of a different methodology to deplete catecholamines, by administering an amino acid mixture deficient of phenylalanine and tyrosine, Leyton and colleagues [34] induced lowered mood and energy and increased irritability scores in a group of healthy women. Interestingly, the behavioral changes induced by catecholamine depletion were similar to those found during tryptophan depletion in the same group of subjects. In both conditions, the behavioral effects were more pronounced following exposure to aversive psychological events. Another study suggests that acutely decreased catecholamine transmission may disrupt mechanisms mediating alcohol self-administration [35]. Together, these studies support the role of catecholamines in a variety of human behaviors and also suggest that disturbed catecholamine transmission may predispose humans to various psychiatric disorders, including depression and anxiety.

B. Depression

The role of norepinephrine and dopamine in the pathogenesis of depression and in the mechanisms of action of antidepressant drugs has been the subject of intensive research during the past decades. A comprehensive overview of catecholaminergic function in depression is beyond the scope of this chapter, which is to show the biochemical and behavioral effects of catecholamine depletion in depressed patients in different states of their illness, and the implications for our understanding of the pathogenesis of depression and its treatment. In the initial studies performed by Bunney and colleagues at the National Institute of Health [10] demonstrated the antimanic effects of AMPT in a small group of

manic patients, whereas three of four unipolar psychotic depressed patients showed an increase in depression on AMPT. However, small sample sizes and the lack of a control or placebo group limit the interpretation of these initial findings. More recent studies addressing these methodological shortcomings showed that drug-free, untreated, symptomatic depressed patients had significant increased visual analogue ratings of "tired," and decreased ratings of "energetic." However, there was no alteration in mood reported by the authors [36]. This finding suggests that a simple norepinephrine or dopamine deficiency hypothesis is insufficient to explain the neurobiological basis of depression. Rather, the role of noradrenergic systems needs to be considered in relation to many other neurobiological factors that may be involved in the pathogenesis of depression. An alternative explanation could be that, during a depressive state, the catecholamine systems are already maximally dysfunctional and thus further manipulations do not worsen the condition of the patient.

Studies of the effects of AMPT on patients remitted on antidepressants or remitted off medications provided further insight into the pathogenesis of depression and mechanisms of action of different antidepressants (Table 2). Depressed patients in remission and on noradrenergic antidepressants (desipramine and mazindol) experienced a depressive relapse during catecholamine depletion, whereas those patients whose remissions were maintained with the selective 5HT reuptake inhibitors (SSRIs) fluoxetine and sertraline remained well during catecholamine depletion [37]. The administration of AMPT induced core symptoms of depression, such as depressed mood, loss of interest, anhedonia, cognitive disturbances, and sense of worthlessness and failure. Another study reported the recurrence of depressive symptoms induced by catecholamine depletion in a group of patients with seasonal affective disorder remitted on light therapy [31]. These findings disagree with a small study of three depressed patients with a favorable treatment response to imipramine [38]. However, besides the fact that the sample size of that study was small and there was no control situation, it has to be considered that imipramine is a potent 5HT reuptake inhibitor as well as a norepinephrine reuptake inhibitor. Findings that catecholamine depletion disrupts the antidepressant effects of catecholaminergic, but not serotonergic, antidepressants, whereas tryptophan depletion reverses the antidepressant effects of serotonergic antidepressants, but not catecholaminergic antidepressants [39], argue against a single monoamine-related mechanism of action of antidepressants. However, such studies suggest that enhanced catecholamine function is important to maintain response to noradrenergic antidepressants, and enhanced serotonergic function is important to maintain response to serotonergic antidepressants.

State-related changes in catecholamine function have been described in depressed subjects, whereas enduring abnormalities have been less reliably identified. A recent catecholamine depletion study in fully remitted, medication-free, formerly depressed subjects showed a depressive relapse in these subjects during catecholamine depletion, but not during sham depletion [40]. The authors argue that this depressive relapse may represent a reliable marker for a history of depression. Further studies are needed to clarify the importance of this finding.

IV. TRYPTOPHAN DEPLETION

A. Healthy Subjects

Studies of tryptophan depletion in healthy subjects have shown inconsistent results. Healthy male subjects with their baseline ratings of depression in the upper normal range

Table 2 Monoamine Depletion Studies in Patients with Depression

Structure	Study design	Ref.	Subjects (*n*)	Intervention	Outcome
A. Untreated depressed patients					
Untreated, symptomatic depressed patients	Double-blind, placebo-controlled, balanced cross-over study	57	43	TD vs. SD	Bimodal mood response one day after TD: 37% of patients show decrease of HDRS total score, 23% increase of HDRS score
Untreated symptomatic depressed patients	Double-blind, placebo-controlled, balanced cross-over study	36	17	AMPT vs. diphenhydramine	No exacerbation of depressive syndrome
Untreated, symptomatic depressed patients	Double-blind, placebo-controlled, balanced cross-over study	56	11	TD vs. SD	No exacerbation of depressive syndrome
Untreated, symptomatic depressed patients, mCPP Infusion during depletion	Double-blind, placebo-controlled, balanced cross-over study	55	22	TD vs. SD	Cortisol response to i.v. mCPP greater during TD than SD
Untreated, symptomatic depressed patients, TRP-Infusion at maximum point of depletion	Double-blind, placebo-controlled, balanced cross-over study	54	38	TD vs. SD	Depressive symptoms decreased after i.v. TRP following TD, not SD

B. Remitted depressed patients on treatment					
Responders to imipramine	Open label	38	8	AMPT vs. PCPA	Transient depressive relapse after PCPA
Remitted depressed patients on SSRI citalopram	Double-blind, placebo-controlled, parallel-group design	58	20	TD vs. SD	Transient depressive relapse in 5/12 patients after TD, not SD
Remitted depressed patients on SSRIs	Double-blind, placebo-controlled, balanced cross-over study	59	21	TD vs. SD	Transient depressive relapse in 7 patients after TD, not SD
Remitted depressed patients in antidepressant-induced remission	Double-blind, placebo-controlled, balanced cross-over study	27	21	TD vs. SD	Transient depressive relapse in 14 of 21 after TD
Fluoxetin- or desipramin-responders	Double-blind, placebo-controlled, balanced cross-over study	39	30	TD vs. SD	8/15 FLU responders relapsed, 1/15 DMI responders relapsed after TD, not SD
Sleep deprivation responders	Double-blind, placebo-controlled, balanced parallel-group design	31	30	TD vs. SD	TD did not induce depressive relapse, but prevented relapse after recovery night
Light therapy-induced remission >14 days	Double-blind, placebo-controlled, balanced cross-over study	21, 67	10 12	TD vs. SD	Transient depressive relapse

Table 2 Continued

Structure	Study design	Ref.	Subjects (*n*)	Intervention	Outcome
C. Remitted depressed patients off treatment					
Fully remitted drug-free patients	Double-blind, placebo-controlled, balanced cross-over study	40	14	TD vs. SD	Transient depressive relapse
Patients fully remitted, off therapy during summer	Double-blind, placebo-controlled, balanced cross-over study	63	12	TD vs. SD	Transient depressive relapse
Remitted drug-free patients	Double-blind, controlled, balanced cross-over study	64	24 (including 12 healthy controls)	TD vs. SD	Transient depressive relapse HC<P
Remitted drug-free patients	Double-blind, placebo-controlled, balanced cross-over study	65	15 Women	TD vs. SD	Transient depressive relapse after TD, not SD
Patients fully remitted, off therapy during summer (SAD)	Double-blind, placebo-controlled, balanced cross-over study, including healthy controls	67	22 (including 10 healthy controls)	TD vs. SD	No deterioration of condition in patients and controls
Patients fully remitted, off therapy	Double-blind, placebo-controlled, balanced cross-over study	66	14	TD	No exacerbation of depressive syndrome

TD, tryptophan depletion; SD, sham depletion; CD, catecholamine depletion; AMPT, α-methyl-para-tyrosine.

exhibit a transient worsening of their mood during tryptophan depletion, although never amounting to clinical depression [22,28]. In contrast, healthy male subjects who were euthymic at baseline and who were rigorously screened for any psychiatric or somatic illness remained unaffected by tryptophan depletion [41]. Healthy male controls with a multigenerational family history for major affective disorders reported a greater reduction in mood induced by tryptophan depletion than healthy controls without a positive family history [42]. Tryptophan depletion studies in female subjects with no personal history of depression showed an increased risk of developing depressive symptoms during tryptophan depletion. In some tryptophan depletion studies in healthy female controls, family histories of affective disorders were assessed and were positive [43], negative [26,43], and in one study unknown [44]. These studies consistently showed an increased risk for an exacerbation of depressive symptoms during tryptophan depletion with more pronounced effects when the family history is positive. However, another study reported no mood-lowering effects of tryptophan depletion in healthy females with a negative family history of depression [45], and several studies report no mood-lowering effects of tryptophan depletion in healthy female subjects [46,47].

Other studies have focused on the memory and cognitive effects of tryptophan depletion and showed that tryptophan depletion impairs long-term memory formation and interferes with the process of memory consolidation [48,49]. Tryptophan depletion did not affect other measures of frontal functioning. Sleep disturbances represent another key symptom of depressive disorders and thus it has also been of interest whether tryptophan depletion induces sleep abnormalities including changes in sleep continuity or architecture. Studies reported reduced REM latency after tryptophan depletion [50,51], but one study disagrees with this finding [46]. Another field of interest is whether reduced 5HT activity during tryptophan depletion induces changes in aggressive behavior since reduced brain serotonergic activity is believed to play a role in aggression or suicidal behavior. A recent study in humans shows that tryptophan depletion indeed induces a rise in ratings of aggression in subjects with high-trait aggression but has little effect on those with low-trait aggression [52]. Acute ethanol consumption may be associated with a decrease in tryptophan availability, and may induce aggressive behavior in susceptible individuals [53].

Behavioral responses to tryptophan depletion in healthy subjects show a high variability. There are subgroups of subjects who appear to be at a greater risk of developing depressive symptoms during tryptophan depletion. Possible explanations include a positive family history of depression, gender, and possibly high, albeit not reaching the levels of clinical depression, baseline ratings of depression. An intriguing finding is the association between the serotonin transporter gene promoter polymorphism (5HTTLPR) and the behavioral responses to tryptophan depletion in a group of healthy female subjects with and without family history of depression [53a]. The study showed subjects with the short allele of the promoter region at increased risk of developing depressive symptoms during tryptophan depletion relative to subjects with the long allele. Future studies must confirm the relevance of this initial finding and will answer the clinically and scientifically relevant question of whether the short allele of the 5HTTLPR polymorphism is associated with an increased risk of developing depression, as was hypothesized by the authors.

B. Depression

To test the hypothesis that decreased 5HT function is associated with depression, several studies were performed, including untreated, symptomatic depressed patients prior to initi-

ation of antidepressant treatment [54–57]. It was hypothesized that tryptophan depletion would lead to an exacerbation of the depressive syndrome.

However, the results of these studies were somewhat unexpected. It was consistently shown that tryptophan depletion did not exacerbate depressive symptoms in these subjects. In two studies [54,57], some patients showed an improvement in their condition on the day after tryptophan depletion. The failure to aggravate depression by depleting brain 5HT can be explained by the fact that brain 5HT function is already maximally dysfunctional in depressed patients and thus further lowering of 5HT activity has no greater effects on depressive symptoms. Alternatively, it can be hypothesized that disturbed 5HT function does not explain the biological basis of depression, and that there is no direct relationship between severity of depressive symptoms and brain 5HT function. A possible explanation for the improvement in symptoms the day after tryptophan depletion is an upregulation of postsynaptic 5HT receptors because of the decreased release of 5HT at the synapse during tryptophan depletion. Typically, 5HT levels are restored the day after tryptophan depletion and the net effect is an enhancement of brain 5HT function, resulting in an improvement of the patient's condition.

Intensive research using tryptophan depletion has been done during the past years to study the role of 5HT in the mechanism of action of antidepressant drugs, and nonpharmacological treatments for depression, such as light therapy and sleep deprivation. The hypothesis of these studies was that antidepressant treatments lead to an enhancement of brain 5HT function and that tryptophan depletion will disrupt the antidepressant effects. This has now been tested in multiple studies and researchers found that tryptophan depletion indeed reverses the antidepressant effect of antidepressant treatments, in particular that of SSRIs [27,39,58,59]. The depressive symptoms evoked by tryptophan depletion were often similar to those experienced by the patient during a depressive episode. The behavioral responses to tryptophan depletion were substantially more pronounced in subjects who had been successfully treated with SSRIs relative to responses in subjects who were treated with noradrenergic antidepressants [37,39]. This finding, and the finding that catecholamine depletion predominantly induces a depressive relapse in subjects treated with noradrenergic antidepressants, suggests that enhanced serotonergic or noradrenergic transmission is necessary to maintain the antidepressant responses to SSRIs or noradrenergic agents, respectively. Another issue that affected the depressive relapse after tryptophan depletion was the length of the remitted state of the patient. Patients who were recently remitted were at higher risk of developing depressive symptoms during tryptophan depletion than those being remitted for a longer period. This suggests that antidepressants induce biological changes in the neuron that make the subjects less vulnerable to acute changes in brain 5HT function.

Nonpharmacological, albeit biologically based, treatments for depression include light therapy and sleep deprivation. Light therapy is the treatment of first choice for patients suffering from seasonal depression during fall and winter and has been shown to be effective in nonseasonal depression when given in conjunction with other antidepressant treatment modalities [60]. Tryptophan depletion [31,61] and catecholamine depletion [31] reversed the antidepressant effects of light therapy, suggesting that both transmitter systems are involved in the mechanism of action of light therapy. Several lines of evidence suggest that sleep deprivation exerts its antidepressant effects by enhancing serotonergic transmission. To test this hypothesis, patients who responded to a single night of total sleep deprivation underwent tryptophan depletion or sham depletion [62]. It was expected that tryptophan depletion would reverse the antidepressant effects of sleep deprivation.

Unexpectedly, tryptophan depletion did not reverse the antidepressant effects of sleep deprivation, but rather prevented the otherwise naturally occurring depressive relapse after the recovery night. The authors concluded that it seems to be unlikely that serotonin alone mediates the antidepressant effects of sleep deprivation; however, serotonergic mechanisms may play an important role.

The effects of tryptophan depletion in formerly depressed, fully remitted patients off medication is of particular interest for understanding the role of 5HT in the pathogenesis of the disorder. It might be expected that these subjects are specifically vulnerable to the depressogenic effects of tryptophan depletion. The majority of the studies [63–65] reported a depressive relapse induced by tryptophan depletion whereas subjects remained well during sham depletion. However, two studies found no exacerbation of depression during tryptophan depletion [66,67]. These discrepancies may be explained by the differing length of remission among the different studies as well as by differing study populations. The depressive relapse induced by tryptophan depletion in remitted patients off therapy suggests that these subjects remain vulnerable to changes in 5HT function. Interestingly, one study showed that the majority of subjects who relapsed during tryptophan depletion experienced further depressive episodes in the near future [68]. Thus, tryptophan depletion may be capable of predicting the future course of depression.

V. SUMMARY

The data obtained from monoamine depletion studies provided us with further insight into the neurobiological systems involved in the pathogenesis of depression and also into the mechanisms of action of pharmacological and some nonpharmacological treatment modalities, such as light therapy and sleep deprivation. It became obvious that monoamines do not have a direct effect on regulating mood, but rather have a modulator role on other neurobiological systems that have to be identified. However, adequate function of monoamines is necessary to achieve and maintain an antidepressant response in depressed patients on and off medications. Thus far, tryptophan depletion has been a research tool, and most probably this will not change in the near future. Future reasearch should further increase our knowledge about the changes in transmitter function and its implications on other neurobiological systems induced by tryptophan depletion. This may become of clinical relevance since preliminary evidence suggests that, at least in a subgroup of depressed patients, tryptophan depletion is capable of inducing a transient, albeit clinically significant, improvement in depressive symptoms.

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22

Effects of Antidepressants on Specific Neurotransmitters: Are Such Effects Relevant to Therapeutic Actions?

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I. INTRODUCTION

Depression is a growing burden, both in terms of quality of life of the patient and his or her family and economic burden to society. Current projections indicate that the global burden of depression will rank second only to ischemic heart disease by 2020 [69a], while the current costs of depression are comparable to those of other major illnesses such as cancer, AIDS, and coronary heart disease [42]. An additional factor that adds to the long-term clinical and economic impact of depression is its recurrence and chronicity. Yet despite the adverse effects of depression on the patient and on society, for over 40 years effective antidepressants have been available that can reduce the burden of the condition. Indeed it has been calculated that of those depressed patients who seek treatment, less than half are prescribed antidepressants and, of these, only about 25% are adequately treated [50]. This situation has led to the concept of the iceburg of depression, which implies that only a minority of patients in the community suffering from the condition are properly diagnosed and even fewer treated with an appropriate antidepressant in an adequate dose for an adequate length of time. Consensus guidelines are available that recommend the appropriate dose and length of treatment necessary [11].

The advent of better tolerated antidepressants, such as the selective serotonin reuptake inhibitors (SSRIs) and a host of second-generation antidepressants, combined with numerous educational programs to increase the awareness of clinicians and the general public of the burden of depression, has had only very limited impact on the diagnosis and

treatment of the condition [27]. The reasons for this unfortunate state of affairs are numerous and complex [66], but the perception that the efficacy of antidepressants is limited and that their side effects are frequent and unacceptable to the patient undoubtedly contribute to poor compliance and treatment failure.

The purpose of this chapter is to consider some of the pharmacological factors that may contribute to the limitations in the efficacy of antidepressants and how these issues are being addressed by the application of new research arising from studies of the etiology of depression and how antidepressants may act on subcellular mechanisms within the neuron.

II. BIOLOGICAL MARKERS OF DEPRESSION

Over the past 10 years, a number of biological changes have been reported to occur fairly consistently in untreated patients with major depression. These are summarized in Table 1. Such markers may have not only a diagnostic value but also a means to monitor treatment response. These markers may be divided into five main areas, namely those involving changes in the neuroendocrine axis, in serotonin neurotransmission, in the sleep architecture, in brain structure, and, more recently, in the expression of genes that regulate neurotransmitter function. These will be briefly considered.

A. Changes in the Neuroendocrine Axis

It is well established that the hypothalamic–pituitary–adrenal (HPA) axis is hyperactive in patients suffering from major depression, particularly those with melancholic and/or psychotic features [18,57,89]. The predominant abnormalities in the dysfunction of the HPA axis in such patients include hypercortisolemia, increased 24-h urinary excretion of cortisol, resistance to the suppression of cortisol by dexamethasone, a blunted adrenocorticotropic hormone (ACTH) response to corticotropic releasing factor (CRF), and hypersecretion of CRF. Most of these changes in the HPA axis are normalized following the effective treatment of the depressed patient and would therefore appear to be state markers of the condition. Other factors that indicate that the HPA axis is hyperactive in depression include an enlargement of the adrenal glands [87] and a hypersecretion of arginine vasopressin (AVP) by the pituitary gland [26]. It is also of interest that an increase in the size of the pituitary gland has been reported in a small group of patients with major depression [51], which lends support to the view that a hypersecretion of CRF is a major factor in causing these changes in the HPA axis.

1. Role of CRF in Depression

It is widely accepted that stress acts as a major trigger factor in depression in genetically vulnerable individuals. CRF, together with AVP, plays a major role in the physiological and behavioral responses to stress. For example, in experimental studies it has been shown that the central administration of CRF produces symptoms such as anorexia, loss of libido, disruption of the sleep profile, and changes in general activity that simulate those seen in patients with major depression [102]. CRF produces its physiological effects by stimulating CRF1 and CRF 2 receptors. These receptors are members of the G-protein-coupled family, which stimulates intracellular adenylate cyclase activity. Both types of CRF receptor occur in the brain, but it would appear that the CRF1 receptors are particularly involved in mediating the central components of the stress response and also the main symptoms

Table 1 Some Biological Markers Found in Depressed Patients

Changes in the neuroendocrine axis
A. Evidence of HPA axis hyperactivity
Increased urinary free cortisol
Dexamethasone nonsuppression
Blunted ACTH response to CRF challenge
Elevated concentration of CRF in the CSF
Hypertrophy of the adrenal and pituitary glands
B. Changes in the HPT axis
Blunted TSH response to TRF
Elevated concentration of TRH in the CSF
Autoimmune thyroiditis
C. Changes in somatostatin
Decreased concentration of somatostatin in the CSF
Changes in serotonin neurotransmission
A. Reduced concentration of 5HIAA in the CSF of suicide attempters
B. Reduced brain and platelet affinity of serotonin transporter for 3H-serotonin
C. Increased density of 5HT receptors on platelet membrane
D. Decreased platelet aggregation induced by serotonin
E. Decreased free plasma tryptophan
Changes in other neurotransmitters
A. Increased platelet α_2-adrenoceptors and lymphocyte beta-adrenoceptors
B. Increased functional activity of the cholinergic system
C. Reduced concentration of HVA in the CSF
Changes in the sleep architecture
A. Reduced REM latency
B. Decreased delta-wave sleep
C. Redistribution of REM sleep during the night phase
Changes in brain structure
A. Presence of subcortical and periventricular hyperintensities
B. Decreased hippocampal and temporal lobe volumes
C. Reduction in neurogenesis and increased apoptosis (?)
Changes in immune system
A. Evidence of immune activation as shown by increased plasma proinflammatory cytokines and acute phase proteins
B. Decrease in T-cell replication and natural killer cell activity

of depression that arise as a consequence of an increase in HPA axis activity [1]. In addition to its role in activating the HPA axis, CRF also activates receptors located on the raphe and locus coeruleus, the main serotonergic and noradrenergic cell bodies in the brain, circuits long postulated to be involved in the etiology of anxiety and depression.

While there has been much attention directed toward the role of CRF as the primary regulator of the HPA axis, more recently attention has been directed toward the role of

AVP, a peptide that is known to act synergistically with CRF on the adenohypophysis to elevate the plasma ACTH concentration. Dinan et al. [26] have shown that whereas the CRF-induced elevation of ACTH was blunted in patients with major depression, the CRF plus AVP response was unchanged in these patients. They interpret this as evidence in favor of a decreased sensitivity of the CRF1 receptor, but an increase in the activity of the vasopressin V1b receptor in depression, which implies that there is a switch in the regulation of the HPA axis in which the vasopressin pathway become hyperactive while the CRF pathway is hypoactive. This contributes to the hypercortisolemia that characterizes depression.

In addition to the changes in CRF and AVP receptor function in depression, there is also evidence that the glucocorticoid receptor feedback mechanism is dysfunctional. Two types of glucocorticoid receptor have been identified in the brain, namely, type 1 (mineralocorticoid) and type 2 (glucocorticoid) receptors. These receptors have different affinities for glucocorticoids, the former having a high affinity for the steroids that is important in the regulation of the basal secretion of cortisol and the latter having a low affinity for the steroids, therefore regulating the stress-induced hypersecretion of cortisol. There is evidence that in depression or following chronic stress, the glucocorticoid type 2 receptors are desensitized and therefore less likely to respond to feedback inhibition following the elevation in the plasma cortisol concentration. Both types of receptor are widely distributed in the brain, particularly in those regions assumed to be important in depression.

Given the close association between major depression and hypercortisolemia, it is not unreasonable to speculate that the changes in central neurotransmitter function that are assumed to underlie depression are secondary to those occurring in the HPA axis [25]. This hypothesis is supported by the observation that glucocorticoid synthesis inhibitors, such as ketoconazole, and receptor antagonists, such as metyrapone, exhibit antidepressant activity presumably by normalizing the inhibitory feedback mechanism thereby leading to a reduction in circulating glucocorticoids [108]. Another approach to the treatment of depression has been to block the hypersecretion of CRF by the administration of CRF1 receptor antagonists. This approach has led to the development of a series of CRF1 receptor antagonists that exhibit anxiolytic activity in rodent models of stress, depressions, and anxiety [75].

2. Changes in the Hypothalamic–Pituitary–Thyroid–Axis in Depression

Prange et al [85] first reported that approximately 25% of patients with major depression but with normal plasma concentrations of thyroid stimulating hormone (TSH) and thyroid hormones showed a blunted TSH response following the administration of thyrotropin stimulating hormone (TRH). This observation has been widely replicated. While the mechanism underlying such changes is uncertain, it has been shown that the cerebrospinal fluid concentration of TRH is significantly elevated in depressed patients [5], which suggests that TRH is hypersecreted. This could contribute to the hyperplasia of the pituitary gland, which has been detected in patients with major depression.

3. Changes in Growth Hormone Secretion in Depression: Involvement of Somatostatin

Somatostatin (SRIF) inhibits the release of growth hormone (GH) in addition to TSH, prolactin, cholecystokinin, and vasoactive intestinal peptide (VIP). Several studies have shown that the CSF concentration of SRIF is reduced in depressed patients, a change that

has been shown to correlate with the dexamethasone nonsuppressor status of the patients [29,38]. These changes in SRIF thus correlate with those seen in the HPA axis. Although the precise role of SRIF in depression is uncertain, it has been hypothesized that the peptide may play a role in cognitive function in addition to its regulatory effect on the HPA axis. It is well known that cognitive dysfunction frequently occurs in patients suffering from chronic depression [21] and other major psychiatric conditions.

4. Changes in Serotonergic Neurotransmission in Depression

Interest in the role of serotonin in mood disorders stems from the early clinical studies that demonstrated that the concentration of the main serotonin metabolite 5-hydroxyindolecetic acid (5HIAA) was reduced in the CSF of depressed patients who had attempted violent suicide [2] and similar changes were reported to occur in depressed patients who had successfully committed suicide [105]. The decrease in the functional availability of serotonin that such findings suggest may be linked to increased impulsivity, which is a feature not only of suicidal behavior but also of alcoholism, compulsive gambling, and aggression.

A number of investigators have confirmed the original observation that the number of 3H-imipramine binding sites on the platelet membrane is reduced in untreated depressed patients [10,81,103], while others have reported that a reduction in the number of ^{3}H-imipramine binding sites occur in the cortex and hypothalamus of suicide victims who were depressed at the time of their death [83]. As the decrease in the imipramine binding sites does not apparently change following effective antidepressant treatment, it can be concluded that such a parameter may be a suitable trait marker for depression. More recently, the specific ligand for the serotonin transporter 3H-paroxetine has been used and, at least in some studies [63], has also been shown to exhibit reduced binding in depressed patients.

Platelets are, like neurons, of ectodermal origin and contain enzymes such as enolase that are otherwise restricted to neurons. In addition, platelets contain storage vesicles for serotonin from which the amine is released by a calcium-dependent mechanism. An energy-dependent transport site for the uptake of serotonin also occurs on the platelet membrane, the structures of which are identical to those that found in the brain. A detailed discussion of the importance of platelets in psychiatric research has been the subject of a recent review [56]. The platelet membrane also contains both $5HT_2$ and α_2-adrenoceptors that are functionally involved in platelet aggregation; there is evidence that the densities of these receptors are increased in patients with depression and return to control values following effective treatment [13,14,45,46]. The transport of ^{3}H–5HT into the platelet is significantly reduced in untreated patients with depression, but largely returns to normal following effective treatment [54]. Thus it appears that the changes in adrenoceptor and serotonin receptor densities and serotonin transport are state markers of depression and occur regardless of the nature of the antidepressant treatment.

The function of the $5HT_2$ receptor appears to be subnormal in depression, as shown by a diminished aggregatory response to serotonin in vitro [14]; the function normalizes following effective antidepressant treatment. Conversely, the density of $5HT_2$ receptors on the platelet membrane is increased in the untreated depressed patient which suggests that the link between the receptor and the second messenger system is malfunctional, presumably at the level of the G-protein. While it is uncertain how these changes in platelet serotonin function are reflected in the brain, there is evidence from studies in which the changes in serotonin transport in platelets and neurons was compared in a bulbectomized

rat model of depression that the neuronal transporter is also defective [15]. These changes could be a reflection of the hypercortisolemia that is a feature not only of the depressed patient but also of the olfactory bulbectomized rat. There is also evidence that the changes could be due to low-molecular-weight plasma factors that impede the serotonin transporter prior to effective antidepressant treatment [74a]. There is evidence, however, to support the view that changes in the mobility of calcium within the platelet are key factors in determining the abnormal responsiveness of the platelets to agonists such as serotonin and thrombin [33].

Patients undergoing treatment with SSRI antidepressants have been shown to relapse rapidly when given an amino acid–containing drink that is deficient in tryptophan [22]. The onset of the symptoms of depression is rapid (within 24 h) and it has been proposed that the lowering of the mood of the patients is due to a reduction in brain serotonin synthesis. This view is supported by experimental studies in which rats, given a tryptophan-free fluid, show a reduced release of serotonin from the frontal cortex. Delgado et al. [22] also showed that those depressed patients who had responded to the noradrenaline reuptake inhibitor desipramine did not relapse when given the tryptophan-free drink but did relapse when given the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine. These findings raise the question of whether depressed patients may have a deficit in noradrenaline or serotonin that may account for the differential response to the serotonin- or noradrenaline-depleting agents. However, this would conflict with the widely observed situation where most classes of antidepressants are effective regardless of their presumed specificity for the noradrenergic or serotonergic systems.

5. Changes in Sleep Architecture in Depression

The development of all-night electroencephalographic (EEG) monitoring has enabled investigators to objectively and precisely assess the degree of sleep disturbance in depressed patients. From such studies it has been established that there are four main types of abnormality found in depressed patients. These are a shortened rapid eye movement (REM) sleep latency, increased REM activity, reduced delta slow-wave sleep, and a disturbance in the overall continuity of sleep [52]. Sleep EEG studies may become an important area for understanding the neurobiology of depression in the future. For example, the sleep EEG at baseline and following the acute administration of a tricyclic antidepressant may predict the response of the patient to antidepressant therapy. Similarly the change in REM sleep latency may help to identify those patients who are at risk for early and more frequent recurrences of depression following effective treatment. These aspects have been reviewed by Buysse and Kupfer [16]. The detection of REM sleep abnormalities in depressed patients and in their biological relatives may be a useful means of examining the familial and genetic aspects of the condition in the future [20].

6. Changes in Brain Structure in Depression

McDaniel and Nemeroff [61] briefly reviewed the literature that indicated that changes in brain structure frequently occur in patients with major depression. Thus enlarged ventricles, particularly lateral ventricles, have been identified by CAT and MRI imaging techniques and there is some evidence that the cerebellar vermis is smaller in patients with mood disorders than in healthy controls. In addition, there is evidence that there is a higher rate of subcortical and periventricular hyperintensities in elderly depressed patients.

However, changes in brain structure also occur in other psychiatric conditions and

the precise relevance of the structural changes seen in depressed patients to the symptoms exhibited and to their response to treatment is unknown.

Recently, attention has been directed to changes in hippocampal structure in depression. Thus several studies have shown that chronic stress or the administration of glucocorticoids causes neuronal atrophy, reduced neurogenesis, and cell death in experimental animals. In brain imaging studies of patients with depression it has been demonstrated that hippocampal volume is reduced [9,90]. In addition, in patients with Cushing's disease or adrenal hypertrophy, a reduction in the hippocampal volume has been reported which is reversed following the reduction in the circulating glucocorticoid levels. In the elderly depressive, the changes in hippocampal volume have also been shown to correlate with deficits in memory [58a]. Such findings lend support to the hypothesis that the changes in brain structure seen in depression are correlated with hypercortisolemia, although inflammatory processes involving such cytokines as interleukin 1, 2 and tumor necrosis factor (TNF)-alpha may also play an important role. With regard to atrophic changes in the prefrontal cortex, brain-imaging studies have demonstrated that both the structure and the blood flow to the area is reduced in depressed patients [30]. In addition, both the number of neurons and the glia in the prefrontal cortex and the orbitofrontal cortex have been shown to be reduced [86]. These structural changes in brain regions concerned with memory and cognition may be of importance in understanding the cause of such symptoms in depression.

7. Polymorphisms of the Enzymes Involved in Monoamine Synthesis as Possible Factors in Depression

In humans, it has been estimated that there are approximately 100,000 genes, each of which has a characteristic pattern of expression in different cells and tissues. The individual differences, polymorphisms, are useful genetic markers but may also represent pathological mutations at the level of the single gene. These mutations are unique, necessary, and sufficient to engender a hereditary disease. So far, however, there is no evidence that such a mutation occurs in any of the affective disorders. In addition to these rare mutations, most common variations at the genomic level that are responsible for the uniqueness of each individual are not pathological but they may intervene in the regulation of gene expression. As a consequence, they may interact with environmental factors, shape quantitative traits, and thereby contribute to a pathological change. Thus, depression, genes may increase the risk whereby the disorder is expressed.

In depression, the monoamine neurotransmitters are thought to play a crucial role and twin, adoption, and family segregation analyses have established that there is a strong, albeit complex, genetic component to the disorder. Thus the enzymes that control the synthesis of these neurotransmitters may be strong candidate genes. In summary, it has been shown that the tryptophan hydroxylase gene is associated with impulsivity and violent suicide and that some investigators have identified an allelic form (the U-allele) that correlates with aggression and anger-related personality traits in some individuals [10]. In another study of patients in Finland, two polymorphic forms of the tryptophan hydroxylase gene have been correlated to suicidality [72]. In regard to tyrosine hydroxlase, there is conflicting evidence in the literature regarding the link between different polymorphic forms of this enzyme and depression in patients with bipolar disorder [65]. No evidence of a linkage between the DOPA-decarboxylase and the dopamine-beta-hydroxylase genes and psychiatric disease have so far been found. Clearly this is an important area for future research into the role that abnormal allelic forms of the enzymes and receptors that are involved in neurotransmission may play in depression.

III. EFFICACY OF ANTIDEPRESSANTS AND LIMITATIONS OF CLINICAL TRIALS IN ASSESSING EFFICACY

The efficacy of antidepressants in the treatment of depression has been well established in randomized controlled trials [37,99]. Such studies have demonstrated that taking the antidepressant at an effective dose for an adequate period reduces the number and severity of the acute symptoms, improves the ability of the patient to function, and minimizes the risk of relapse and recurrence. In major depression, pooled data from meta-analyses of clinical trials have demonstrated similar efficacy in all classes of antidepressants and superiority to placebo [3]. However, the conditions under which clinical trials are conducted differ considerably from clinical practice. Typically such trials take place in highly selected populations treated under controlled and optimal conditions, with treatment determined by clear study protocol. This type of study reduces the potential for bias and ensures that any differences detected are attributable to the drugs under investigation. In contrast, treatment conditions in general practice do not involve patient selection or randomization in addition to variations in the behavior of prescribers and patients and in the systems of health care in which the treatment is delivered [95]. Thus the efficacy of randomized controlled trials, while important in the selection of an antidepressant, is a reflection of the efficacy of the drug under standardized and optimal conditions. A more useful concept has been termed "real world efficacy," and concerns the use of the antidepressant to ensure that the desired outcome of treatment can be achieved [66]. This approach may lead to an overestimation of the efficacy of an antidepressant as demonstrated by clinical trials. Thus the widely quoted efficacy of antidepressants in achieving 60% response after 6 to 8 weeks of treatment, with placebo treatment achieving a 30% response, may be overoptimistic in the "real world" situation. A discussion of the importance of efficacy of antidepressants versus their effectiveness in clinical practice has been the subject of a recent review [28].

While a detailed discussion of the differences between the efficacy and effectiveness of antidepressants is beyond the scope of this chapter, a brief summary will be given since it impacts upon the differences between various classes of antidepressants in current use. For example, the tolerability of an antidepressant would be anticipated to influence both the adherence of the patient to treatment, the duration of treatment, and the consequent outcome of treatment. Thus the selective serotonin uptake inhibitors (SSRIs) are generally considered to be better tolerated with an improved adverse effect profile when compared to the older tricyclic antidepressants [23,68]. This is confirmed in studies where the percentage of patients who discontinue treatment has been evaluated [69]. However, it is now apparent that differences arise between antidepressants of the same class. For example, early studies showed that the pharmacological differences between the SSRIs were small and none has been shown to be consistently better tolerated [36,53]. However, following their extensive use, differences have been shown in their side effect profiles. Thus paroxetine has the highest incidence of anticholinergic side effects associated with its antimuscarinic activity at normal therapeutic doses [24], while citalopram has been associated with some sedation at higher therapeutic doses because of its slight affinity to histamine-1 receptors [36]. Fluoxetine has been reported to produce an increase in the frequency of anxiety-related symptoms including nervousness and agitation [24,60], together with weight loss and anorexia [85a]. Conversely, weight gain has been associated with paroxetine and citalopram [8,34]. However, based on data from clinical practice, the incidence of adverse events has been reported to be the highest with fluvoxamine [59], particu-

larly in terms of gastrointestinal events, although paroxetine and sertraline have also been reported to cause a higher frequency of such side effects than occurs with citalopram and fluoxetine. Sexual dysfunction is a common side effect of all the SSRIs but appears to be more frequent after paroxetine and sertraline [59]. This summary serves to illustrate the differences between controlled clinical trial data and that obtained in "real world" settings. Whereas the former show no significant differences between the SSRIs, community-based studies demonstrate important differences between them that could have a bearing on compliance and therefore efficacy.

A. Limitation of Clinical Trials of Antidepressants

A typical trial of an antidepressant consists of groups of 50 to 60 patients, including a placebo-treated arm and a reference drug, usually amitriptyline or imipramine, assigned to patients in a second arm. Such trials usually last 6 to 8 weeks even though the recommended duration of treatment in clinical practice is at least 6 months. Most clinical protocols do not provide justification for the sample size or even specify the statistical power. As the proportion of nonresponders to antidepressant treatment ranges from 20 to 30%, and the low statistical power for showing any differences between treatments together with the usual exclusion of noncompliant subjects from the final analysis, it is unlikely that any absolute difference would emerge between a new and a standard antidepressant. In practice, this means that virtually all clinical trials only show equivalence between a new and a standard antidepressant and little difference in clinical efficacy. It has been estimated that the sample size that would be required in order to show a difference of 5% between two drugs, assuming 20% nonresponders to treatment, would be between 1250 and 2380 patients per arm of the trial [11]. This implies that the widely held view that all antidepressants, regardless of their presumed mechanism of action, are only effective in about 70% of depressed patients, is more a reflection of the limited design of clinical trials than the real potential differences.

IV. HOW DO ANTIDEPRESSANTS WORK? THE LIMITATIONS OF THE AMINE HYPOTHESIS

It is widely accepted that, despite the success of antidepressants in treating the symptoms of major depression for the past 40 years, little advance has been made in improving the efficacy or time of onset of such treatments. Undoubtedly there are many reasons for this but an important reason may rest with the assumption that as all effective antidepressants modulate the function of noradrenaline and/or serotonin in the brain, the biogenic amines probably play a definitive role in their mechanism of action. This is the basis of the monoamine hypothesis of depression that has played an important role in the development of antidepressants and in interpreting the pathological basis of depression over the past 40 years. However, in recent years, evidence has emerged that challenges the amine hypothesis and links the therapeutic effects of antidepressants to the subcellular changes that occur distal to the neurotransmitter receptor. In addition, other approaches have considered the role of excitatory neurotransmitters such as glutamate and inflammatory mediators such as the proinflammatory cytokines in the etiology of depression and in the mechanism of action of antidepressants. Such approaches may serve not only to enrich our understanding of depression but also to facilitate in the development of novel approaches to its treatment. These possibilities will now be discussed.

A. The Action of Antidepressants on Subcellular Processes

Studies on the action of stress and antidepressants suggest that changes in the basic mechanisms that underlie neural plasticity are involved in the etiology and treatment of depression. Thus exposure to stress, which is a major trigger factor in precipitating depression, opposes the mechanisms that underlie neural plasticity involved in learning and memory. Conversely, antidepressant treatments induce changes in signal transduction and gene expression that could either block the effect of stress or induce the appropriate response in neuroplasticity.

In the past, research into the mechanism of action of antidepressants has focused on the proximal actions of antidepressants (such as effects on amine reuptake and intracellular metabolism) or on the adaptive changes in noradrenergic or serotonergic receptors. Such adaptive changes include the "downregulation" of beta-adrenoceptors, $5HT_2$, and $5HT_7$ receptors [19,31]. Such discoveries lead to the monoamine hypothesis of depression becoming the monoamine receptor adaptation hypothesis. However, this hypothesis has its limitations. For example, some beta-adrenoceptor agonists have been shown to exhibit antidepressant properties whereas it would be anticipated that such drugs would precipitate depression. Furthermore, desensitization of beta receptors by antidepressants occurs within a few days even though it takes several weeks for the drugs to show a therapeutic response. With regard to the changes in the density of $5HT_2$ receptors, not all antidepressants desensitize these receptors. electroconvulsive shock treatment increases the density of $5HT_2$ receptors in rat brain. Thus it seems reasonable to hypothesize that adaptive changes in postsynaptic intracellular sites, rather than in postsynaptic receptors per se, may be critical to the action of all antidepressant treatments.

One signal transduction pathway that is regulated by antidepressant treatment is the cyclic AMP intracellular cascade and recent studies have demonstrated that chronic antidepressant treatment results in the upregulation of this system. Thus it has been shown that antidepressant treatment increases the activation of adenylyl cyclase by the stimulatory G protein, Gs, in limbic regions of the rat brain [78]. In addition, it has been shown that chronic, but not acute, antidepressant treatments increase the activity of camp-dependent protein kinase [70,82]. As such, antidepressant treatments also increase the activity of protein kinase A in nuclear fractions from rat brain, and it appears that gene transcription factors are regulated by these changes. This is supported by the finding that the response of the camp response element binding protein (CREB) is increased by antidepressant treatment [71]. Thus, the expression of CREB mRNA and protein was shown to be upregulated by chronic, but not acute, antidepressant treatments; both SSRI and SNRI antidepressants were found to produce similar responses, which suggests that such changes may be a common action of antidepressants that modulate the noradrenergic and serotonergic systems.

CREB regulates gene transcription by binding to a specific nucleotide sequence, the camp response element (CRE), in the promotor region of the gene. Although CREB may remain bound to DNA under basal conditions, activation of this transcription factor occurs primarily via phosphorylation at Ser 133. Chronic antidepressant treatments have also been shown to increase the phosphorylation of CREB in rat hippocampus, and in several other structures including the cerebral cortex [104]. Thus CREB may act as a common postreceptor target for different classes of antidepressants [31].

In support of the hypothesis that antidepressants activate specific target genes, experimental studies have shown that the regulation of neurotrophic factor gene expression is of particular interest. The nerve growth factor (NGF) family includes brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4. These neurotrophic fac-

tors have been shown to regulate the guidance and survival of neurons in both the immature and the mature brain, where they influence both neuronal firing and synaptic plasticity. These actions are dependent upon the activation of tyrosine kinase receptors (Trk)[88]. Of these Trk receptors, TrkA is selective for nerve growth factor, TrkB for BDNF, and TrkC for neurotrophin-3. Activation by these receptors results in autophosphorylation of several intracellular sites.

The link between these intracellular changes and stress is provided by the observation that immobilization stress in rats causes a decrease in the concentration of BDNF in the hippocampus. This effect is mediated by high concentrations of glucocorticoids [98]. Chronic antidepressant treatments have been shown to increase the expression of BDNF in the hippocampus [71]. This increase in the concentration of BDNF was not found to occur following the chronic administration of psychotropic drugs which lack antidepressant activity. These results suggest that antidepressants block the effect of stress by increasing the synthesis of BDNF. In addition, BDNF has been shown to have antidepressant effects when administered chronically to rats subjected to the forced-swim and learned helplessness models of depression [96]. Thus BDNF may provide a final common pathway for the action of antidepressants.

Chronic stress, glucocorticoid treatment, and prolonged episodes of depression all result in neuronal atrophy and reduced neurogenesis. Thus it has been shown that the volume of the hippocampus is reduced in those suffering from depression or post-traumatic stress disorder [9,90]. In patients with Cushing's disease, these changes are reversed when the corticosteroids return to basal concentrations. Such results lend support to the hypothesis that the hypersecretion of cortisol, which is a common feature of major depression, plays a crucial role in initiating the intracellular changes that result in neuronal atrophy and cell death and has been the subject of a recent review by McEwen and Magarinos [62]. This is supported by the findings that the volume of the prefrontal cortex is decreased, together with a reduction in blood flow, in depressed patients [30]; the number of cells in the prefrontal cortex is also decreased in the postmortem brain of depressed patients [77]. Thus, chronic stress and depression appear to be part of a continuum that ultimately results in premature neuronal death; effective antidepressant treatments appear to block these adverse effects possibly by enhancing the synthesis of neurotrophic factors such as BDNF. This forms the basis of the neurotrophic hypothesis of depression first proposed by Duman and coworkers [31]. The importance of this hypothesis is that it not only proposes a common site of action of the different classes of antidepressants but also convincingly accounts for the delay in the onset of action of these drugs. In addition, the neurotrophic hypothesis suggests novel ways in which antidepressants may be developed by acting on targets that selectively enhance neurotrophic factors in the brain. Regarding the psychopathology of depression, this hypothesis suggests that it is a subtle neurodegenerative disorder and that early diagnosis and vigorous antidepressant treatment is important for preventing the degenerative changes. However, much of the research that has been described above is based on animal studies and clearly needs to be replicated, if possible, in depressed patients. Extrapolation from the results of animal studies to humans must always be treated with caution!

B. The Action of Antidepressants on Nonaminergic Neurotransmitters

Whereas much emphasis has been placed on the monoamine neurotransmitters with respect to the mode of action of antidepressants, little attention has been given to the possible

role of the glutamatergic system which is the primary excitatory pathway in the brain [109]. There is experimental evidence to show that tricyclic antidepressants inhibit the binding of dizolcipine (MK-801) to the ion channel of the main glutamate receptor, the N-methyl-D-aspartate (NMDA) receptor.

These initial studies have been extended to show that both typical and atypical antidepressants have a qualitatively similar effect of inhibiting the binding of dizolcipine to the ion channel of the NMDA receptor [50a]. Whether this is because of the direct action of antidepressants on the ion channel or an indirect effect, possibly by enhancing the action of glycine on the glycine receptor which forms part of the NMDA receptor complex on the cell surface, is uncertain. However, there is evidence that glycine and drugs that directly stimulate the glycine site on the NMDA receptor [74] have antidepressant-like properties in animal models of depression. Thus, the possibility arises that antidepressants selectively enhance glutamatergic activity in the brain by modulating the glycine site. Support for the role of the NMDA receptor system in depression and in the mode of action of antidepressants is provided by studies in suicide victims who suffered from depression. Thus, Nowak et al. [73] and Paul [80] have shown that the high-affinity binding of a selective ligand (3H-CGP-39653) to the glycine site for cortical tissue is reduced in suicide victims; a dysfunctional NMDA receptor complex seems to be involved in the psychopathology of suicide and presumably depression. It is also of interest to note that intravenously administered ketamine, a NMDA receptor antagonist, has been reported to produce a rapid antidepressant response in patients with major depression [6].

The central cholinergic system has also been neglected as a possible site of action, of antidepressants. The anticholinergic activity of tricyclic antidepressants is usually associated with their unacceptable peripheral side effects, which most second-generation drugs lack. However, there is experimental evidence to show that different classes of antidepressants increase the density of muscarinic (MI) receptors in rat brain after chronic administration. This applies regardless of the actions of the drugs on peripheral cholinergic function [32]. These findings provide support for the cholinergic hypothesis of depression. It is of interest that studies in depressed patients have also led to the detection of an abnormality in the central cholinergic system. Thus O'Keane et al. [76] showed that the short-acting anticholinesterase, pyridostigmine, when administered to drug-free depressed patients, caused an enhanced release of growth hormone from the pituitary gland. This suggests that the cholinergic receptors are supersensitive in depressed patients and that one of the actions of antidepressants is to normalize the functioning of these receptors. The mechanism whereby antidepressants bring about these changes is presently unknown but is unlikely to be direct. It is of interest that Janowsky et al. [48] postulated that depression arises as a result of an imbalance between the central cholinergic and noradrenergic systems within the brain. Janowsky postulated that depression arises as a consequence of an overactivity of the cholinergic system, whereas mania is a consequence of an overactivity of the noradrenergic system. Experimental evidence suggests that all antidepressants, regardless of their presumed mode of action, enhance central noradrenergic function. Thus, the cholinergic hypothesis proposes that the central cholinergic system is overactive in depression and that chronic antidepressant treatments reduce this system by enhancing noradrenergic function and/or by enhancing the activity of the glycine site on the NMDA receptor.

In addition to changes in the major excitatory neurotransmitter glutamate there is also evidence that antidepressants modulate the action of the principal inhibitory transmitter, gamma-amino butyric acid (GABA). Over 20 years ago it was shown that the concen-

tration of GABA in the cerebrospinal fluid and plasma from depressed patients is reduced [49,83a]. Experimental studies have also shown that different classes of antidepressants, including ECT, increased the density of GABA-B receptors in the frontal cortex and hippocampus of rat brain [58,84]. Other experimental studies in which several GABAmimetic drugs were tested also showed that they had a similar behavioral profile in the olfactory bulbectomized rat model of depression to standard antidepressants [106]. The possible mechanism whereby antidepressants modulate GABA-B receptor activity is uncertain, but there is evidence that GABA-B receptors may act as heteroceptors on serotonergic terminals in limbic regions of the rat brain [41]. Thus it was shown that the GABamimetic drug progabide increased the head-twitch response induced by serotonin in rodents; the density of cortical $5HT_2$ receptors was also shown to be increased under these conditions. Thus, it would appear that the functional changes in the GABAergic system are closely interrelated to those in the serotonergic and noradrenergic systems.

C. The Link Between the Monoaminergic Systems and the Specificity of Antidepressants

The ability of most classes of antidepressants to modulate beta-adrenoceptors and $5HT_2$ receptors in the frontal cortex of the rat brain following their chronic administration suggests that an important interaction occurs between these two neurotransmitter systems. There is abundant evidence from experimental studies to show

1. Lesions of the central serotonergic tracts prevent the reduction in the density of cortical beta-adrenoceptors that follows the chronic administration of desipramine, a noradrenaline reuptake inhibitor [127].
2. In vitro studies have shown that stimulation of beta-adrenoceptors in cortical slices of rat brain is associated with an increase in the density of $5HT_2$ receptors [92], whereas α_2-adrenoceptor antagonists such as yohimbine can enhance the desensitization (downregulation) of both the beta-adrenoceptors and the $5HT_2$ receptors.
3. The α_2-agonist clonidine causes locomotor hypoactivity in rodents, which is enhanced following lesions of the central serotonergic system [44a]. These investigators also showed that such treatment attenuated the reversal of the clonidine hypoactivity caused by the chronic administration of desipramine. These results suggest that sedrotonin, or a peptide cotransmitter contained within serotonergic neurons [35], modulates noradrenergic function.
4. Gravel and De Montigny [40] showed that lesions of central noradrenergic neurons with 6-hydroxydopamine failed to enhance the responsiveness of hippocampal pyramidal neurons to microiontophoretically applied serotonin without affecting the responsiveness to noradrenaline or acetylcholine. This suggests that the integrity of the noradrenergic system is essential for antidepressants to modulate central serotonergic function.
5. Several studies have investigated the role of serotonin in the regulation of noradrenaline-coupled adenylate cyclase in the rat cortex. Gillespie et al. [39] showed that although in vitro serotonin did not affect the maximum binding of the beta-adrenoceptor antagonist 3H-dihydroalprenolol to cortical tissue, it did abolish the increase in beta-adrenoceptor density, and the increase in isoprenaline-induced cAMP synthesis in cortical membranes following the selective lesions of the serotonergic tracts. These investigators also showed that the changes

in beta-adrenoceptor density were restricted to the low-affinity conformation state for the agonist and that the increase in the density of this subpopulation of receptors occurred approximately 11 days following the lesions of the serotonergic system. From these and other investigations, it appears that the pivotal role of serotonin in the dual regulation of the linked serotonin–noradrenaline beta-adrenoceptor-coupled adenylcyclase system acts at a different site to noradrenaline. Thus noradrenaline regulates the population of beta-adrenoceptors in the antagonist high-affinity state whereas serotonin regulates those in the low-affinity state.

The results of these studies serve to illustrate the interaction that occurs between the serotonergic and noradrenergic systems within the brain and helps to explain the results of behavioral studies in which chronically administered antidepressants, regardless of their specificity for the noradrenergic or serotonergic systems in in vitro studies, or following their acute administration to rats, enhances both noradrenergic and serotonergic function following their chronic administration [43].

In addition to the experimental evidence that the serotonergic and noradrenergic systems are integrally linked, there is also evidence that serotonin can regulate dopamine turnover in the brain. Over 20 years ago it was demonstrated that in depressed patients positive correlation exists between the cerebrospinal fluid concentrations of the major dopamine metabolite homovanillic acid and the main serotonin metabolite 5-hydroxyindole indole acetic acid (5HIAA) [4]. Experimentally it has been shown that stimulation of the serotonergic cell bodies in the median raphe nucleus reduces the firing of the substantia nigra, a main dopaminergic area of the brain. In addition, there is evidence to show that different classes of antidepressants decrease the functional activity of presynaptic dopamine receptors as demonstrated in rats by the antagonism of the locomotor effect of low doses of apomorphine. This effect was only observed following the chronic administration of the drugs [44]. Such findings imply that the effects of antidepressants, despite their apparent selectivity in acute studies and in in vitro preparations, could be equally ascribed to changes in all the monoaminergic systems that are functionally interrelated. Presumably the improved sense of pleasure and reward that effective antidepressant treatments induce is partly attributable to the increase in the functioning of the dopaminergic system in mesocortical regions of the brain.

D. The Modulatory Effects of Antidepressants on Proinflammatory Cytokines

Stress is frequently a trigger factor for depression in vulnerable patients. There is clinical evidence, already referred to above, that the concentration of corticotropin-releasing factor is increased in the CSF of depressed patients which contributes to the hypercortisolemia that usually occurs in major depression. It would therefore be assumed that a sustained increase in the cortisol concentration would result in a suppression of humoral and cellular immunity that could help to explain the susceptibility of depressed patients to an increased risk of cancers, infections, and autoimmune diseases. However, despite the well-established findings that there are profound changes in both humoral and cellular immunity in depression [55,63], there is clear evidence from both depressed patients and the bulbectomized rat model of depression [101] that activation of some aspects of cellular immunity also occurs despite the rise in circulating glucocorticoids. One possible explanation of this paradox is related to the decrease in the sensitivity of the glucocorticoid receptors located

on the immunocytes, a situation that is analogous to the decrease in the sensitivity of the central glucocorticoid receptors to feedback inhibition that occurs in the depressed patient. It is known that chronic antidepressant treatment not only normalizes the functioning of the HPA axis but also the immune changes that occur in depression [79].

Despite the complexity of the interactions that occur between the proinflammatory cytokines, central neurotransmitters, and glucocorticoids, there is growing evidence to support the view that the proinflammatory cytokines play a primary role in the etiology of depression [7]. This forms the basis of the macrophage hypothesis of depression [98]. This hypothesis postulates that the macrophages in both the periphery and in the brain where microglia and astrocytes perform the function of macrophages) are overactive in depression and that many of the key symptoms of the disorder are a consequence of the release of proinflammatory cytokines such as interleukins (IL)-1 and -6 and tumor necrosis factor-alpha (TNF-α). Support for this hypothesis comes from studies of the effects of proinflammatory cytokines and interferon-alpha in nondepressed patients undergoing treatments for certain types of cancer. Such treatments frequently result in depressed mood, anxiety, cognitive impairment, loss of libido, anorexia, disturbed sleep profile and general fatigue, symptoms that are very similar to those occurring in patients with major depression [67]. Such changes would appear to be a consequence of the neurotransmitter and endocrine changes induced by the cytokines rather than a result of the pathological changes induced by the cancer. Research into the immune changes that occur in depressed patients has identified changes in the plasma proinflammatory cytokines, soluble cytokine receptors, and acute phase proteins. Thus Song et al. [100] have reported that the plasma concentrations of the positive acute phase proteins (alpha-1 acid glycoprotein, haptoglobin, and alpha-1 antitrypsin, for example) were raised in the depressed patient while negative acute phase proteins, such as albumin, were decreased. Such changes are a reflection of the actions of IL-1 and IL-6 on the liver. In addition, it has been shown that the complement proteins (C3,C4, and immunoglobulin M) are increased in depressed patients [100]. This evidence implicates an activation of the immune system involving the proinflammatory cytokines and the B cells that are responsible for antibody synthesis. Further evidence that immune activation occurs in depression is provided by the studies of Sluzewska et al. [93], who showed that the plasma concentrations of the proinflammatory cytokines and the soluble IL-6 and IL-2 receptors and IL-1 receptor antagonist, were raised in depressed patients. These changes returned to normal following effective antidepressant treatment [94]. How these alterations in immune function are linked to those in the central neurotransmitters is unclear. But there is evidence that the serum antibody titers to serotonin are increased which, if present in the brain, could impair serotonergic function [91]. In addition, it has been shown that the serium and CSF concentrations of prostaglandins (PG) E1 and E2 are increased in depressed patients. These changes are at least partly initiated by the stimulation of cyclooxygenase activity by the proinflammatory cytokines within the brain [17].

The rise in the concentrations of the prostaglandins may contribute to the central deficit in monoamine neurotransmitter function due to a reduction in the release of monoamines. This, together with the increased release of CRF caused by the proinflammatory cytokines, may provide a link between immune activation, central neurotransmitter dysfunction, and the onset of depression. To date, relatively few studies have examined the effects of antidepressant treatments on these changes. However, there is some evidence that effective antidepressant treatment is correlated with a reduction in the activated immune system, which suggests that many of these changes are state-, rather than trait-,

dependent markers of depression. Thus antidepressants act as immunomodulators that normalize the immune function that characterizes the condition. If the macrophage hypothesis of depression is correct, then it may be argued that antidepressants first reduce the synthesis and release of the proinflammatory cytokines from activated macrophages and related immunocytes. The precise mechanism whereby this occurs is unknown but could involve an antidepressant-induced increase in anti-inflammatory cytokines (such as IL-4-10, and –13) and soluble antagonists (such as IL-1 receptor antagonist) coupled with a reduction in the synthesis of PGE-1 and –2. Maes [64] has reviewed the immunomodulatory effects of antidepressants.

Despite the substantial body of evidence that implicates a disorder of immune function with depression, controversy exists regarding the causal connection between the increase in proinflammatory cytokines, PGEs, and the symptoms of the disorder. However, consideration of these factors is important in stimulating new concepts regarding the etiology of depression and how antidepressants bring about their therapeutic effects.

V. CONCLUSIONS

The precise mechanism of action of antidepressants still remains an enigma. Nevertheless, evidence from both clinical and experimental studies suggests that all effective antidepressants, regardless of their presumed selectivity of action determined by acute experimental studies, ultimately bring about adaptive changes in both monoaminergic and nonaminergic systems. This raises some important and fundamental questions. If, for example, the SSRI antidepressants do not inhibit serotonin reuptake into platelets from depressed patients (despite their proven effect in healthy volunteers and in rat brain), their classification as selective serotonin reuptake inhibitors is a misnomer [47]. Similarly, SNRI antidepressants affect the turnover of serotonin following their chronic administration and also normalize the defective reuptake of ^{3}H serotonin into platelets from depressed patients, thereby losing their presumed selectivity. Such findings lend support to the hypothesis that the neural network of interconnected neurotransmitter systems is malfunctioning in depression and that all antidepressants, by initially acting at different parts of the network, eventually bring the system back to homeostasis. Clearly, the monoamine hypothesis of depression, despite its value in the past, needs to be radically restructured or even replaced to take into account the latest research emanating from molecular biology. The increasingly important contribution of molecular biology to our understanding of the mode of action of antidepressants, and indirectly to the biology of depression, has helped to focus attention on the changes that occur in the secondary and tertiary messenger systems and, ultimately, in gene expression. These intracellular changes only occur following the chronic administration of antidepressants and are independent of the type of antidepressant. From experimental studies, it appears that specific neurotrophic factors provide the final common pathway that leads to changes in synaptic plasticity and improved neuronal contacts.

In addition to the changes in various neurotransmitter systems that occur in depression and following chronic antidepressant treatment, attention has recently focused on the HPA axis and the immune system. The profound changes in these systems that occur in depression probably contribute to those in neurotransmitter function that are directly linked to the behavioral state. Such studies have also served to direct the attention of researchers to a more holistic interpretation of depression rather than restricting studies to the brain alone. This approach may help to explain the increased morbidity and mortality due to infectious disease, heart disease, cancer, and autoimmune diseases that frequently occur

in patients with major depression. The decade of the 1970s was largely devoted to an explanation of the mode of action of antidepressants in terms of their effects on the concentrations of amines and their metabolites. In the 1980s, emphasis switched to the adaptive changes in postsynaptic monoamine receptors, while in the 1990s attention was directed to the link between the postsynaptic receptors and the second and tertiary messenger systems. Perhaps in this present century there will be a shift in emphasis away from the monoamines to the endocrine and immune systems as causal factors in depression and in the mode of action of antidepressants.

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23

Pharmacotherapy of Depression: The Acute and Long-Term Perspective

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I. INTRODUCTION

In the past several decades, we have seen important changes in the way that we view depression. Previously seen as simply an episodic illness, we now appreciate a more longitudinal perspective. For many, depression is a chronic, lifetime illness. This change in perspective has also changed the treatment of depression. Although most research continues to concentrate on the acute treatment of the major depressive episode, a growing body of investigation is taking a more longitudinal view. We have begun a shift that includes the investigation of antidepressant treatment at later stages of the disorder. This chapter will explore part of this shift. This body of research continues to grow, but questions remain unanswered, and these will be examined as well.

II. BASIC CONCEPT

A. Definition of Terms: The Change Points in Depression

Before discussing research on the treatment of depression, we must first define terms. This is particularly important because early research on the longitudinal treatment of depression

was confused by conflicting and contradictory uses of terminology. It was for this reason that a MacArthur Foundation task force [1] recommended that the change points be described with these terms: episode, remission, response, relapse, and recurrence (Fig. 1).

An *episode* can be thought of both conceptually and categorically. Conceptually, it is the acute episode of a disorder: if one is in the condition of having a disorder, one is experiencing an episode. Such a conceptual definition is difficult to demonstrate with reliability, and a categorical definition is necessary to reliably agree on when an episode has started and finished. In general, an episode can be practically defined as having a certain number of symptoms for a certain period of time. It relies more on statistical definitions than any "absolute" gold standard. In choosing such a definition, a decision must be made as to where to place the "bar" for a disorder: whether it should be conservative or liberal, and how much of what type of error to accept. This type of decision making must be made at each change point of the disorder, both in terms of number of symptoms and length of time.

Remission, conceptually, is the point at which an episode ends. It is defined by an accepted period of time in which an individual no longer meets (an agreed upon number of) criteria for the disorder. Remissions can be partial or full. In *partial remission*, an individual still has more than minimal symptoms. *Full remission* is defined as the point at which an individual no longer meets criteria for the disorder and has no more than minimal symptoms.

The terms discussed thus far relate to the natural history of depression and imply no specific intervention. Some terms, however, are related to an intervention. A *response*, for example, is a remission that is the result of a treatment intervention. All responses are really "apparent" responses, because one can never fully establish a direct relationship between treatment and remission. A remission that occurs proximately to an intervention is, most likely, causally related. Similar to a remission, a response can also be full or partial. Again, such terms can be defined by setting a required number of symptoms.

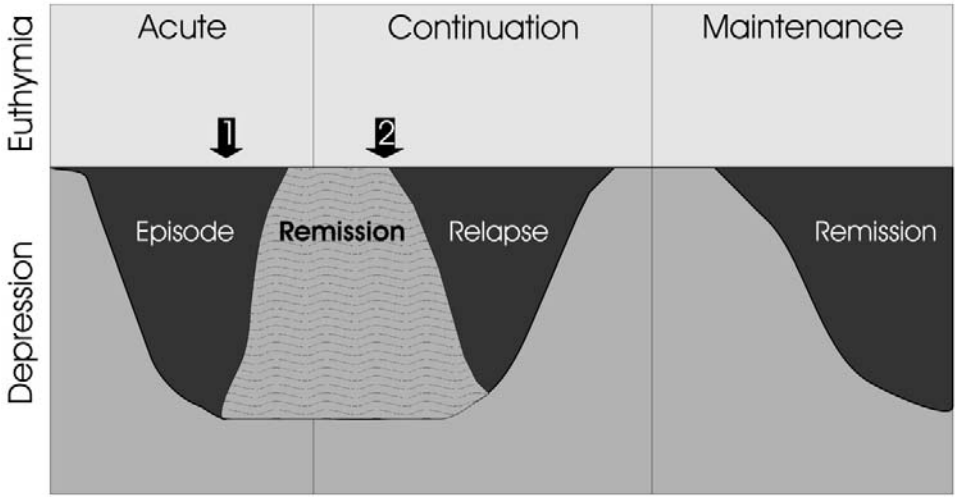

Figure 1 The change points in depression. The arrows denote a hypothetical course of treatment, superimposed on the natural course, beginning during the acute episode (arrow 1) and discontinuing after remission (arrow 2).

A *relapse* is defined as the early return of symptoms following an apparent response. Conventionally, *continuation treatment* means treatment during the initial 4 to 6 months after response. Episodes that occur during this period are relapses.

A *recovery* is defined as a full remission that lasts for a defined period of time. Conceptually, it implies the end of an episode of a disorder, not the disorder per se. An episode that occurs after this period of recovery is called a *recurrence*. Treatment during this period is referred to as *maintenance treatment*.

Differentiating between a relapse and a recurrence can be difficult. During continuation treatment, one expects a relapse to occur soon after the discontinuation of treatment. For a recurrence, a reemergence of symptoms does not necessarily occur soon after stopping treatment; actually, the opposite is often true, and the recurrence of symptoms is not causally related to treatment discontinuation, but rather reflects a new episode of illness altogether.

The introduction of clinically useful biological markers for depression would make it easier to define the beginning and end of an episode of depression. Until such markers are introduced, these definitions represent reasonable working definitions and are used by most of the studies that are reviewed here.

B. Longitudinal Course Modifiers for Depression

The concept of longitudinal change points was added to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) [2]. DSM depressive diagnoses (major depression and dysthymia), and the variety of longitudinal presentations were incorporated in the DSM-IV longitudinal course modifiers (Fig. 2). For these modifiers, major depression was divided based on whether episodes are single or recurrent, whether there is an interepisode recovery, and whether there is concomitant dysthymia in addition to the episodes. Combining these variables yields several possible courses: (1) single episode without antecedent dysthymia; (2) single episode with antecedent dysthymia; (3) recurrent, with full interepisode recovery, with no dysthymic disorder; (4) recurrent, without full interepisode recovery, with no dysthymic disorder; (5) recurrent, with full interepisode recovery, superimposed on dysthymic disorder; and (6) recurrent, without full interepisode recovery, superimposed on dysthymic disorder (i.e., double depression).

The Mood Disorders Field Trial for DSM-IV [3] examined the frequencies of the different possible courses (Fig. 3). Of these courses, several are important from a treatment perspective. Course 1 (single episode without antecedent dysthymia) is the "classic" type of major depression in which there is a single episode with full recovery. As seen in Figure 3, however, this type of depression does not represent the majority of cases of major depression. Also important is recurrent depression, with and without full interepisode recovery (3 and 4); and its treatment will be discussed shortly.

Chronic depression also has various types. There are patients with types of depression who do not fully recover and may go on to develop a chronic condition, such as major depression (Fig. 2). However, there are other chronic depressions that may be less severe than major depression, but still debilitating. The most common is dysthymia, in which the symptoms may be less severe than those for major depression, but still debilitating. Besides dysthymia, there may be various "subsyndromal" depressions in which the level of symptomatology is not sufficient to meet the criteria for major depression or even dysthymia. Though minor, however, the persistence of even a few depressive symptoms

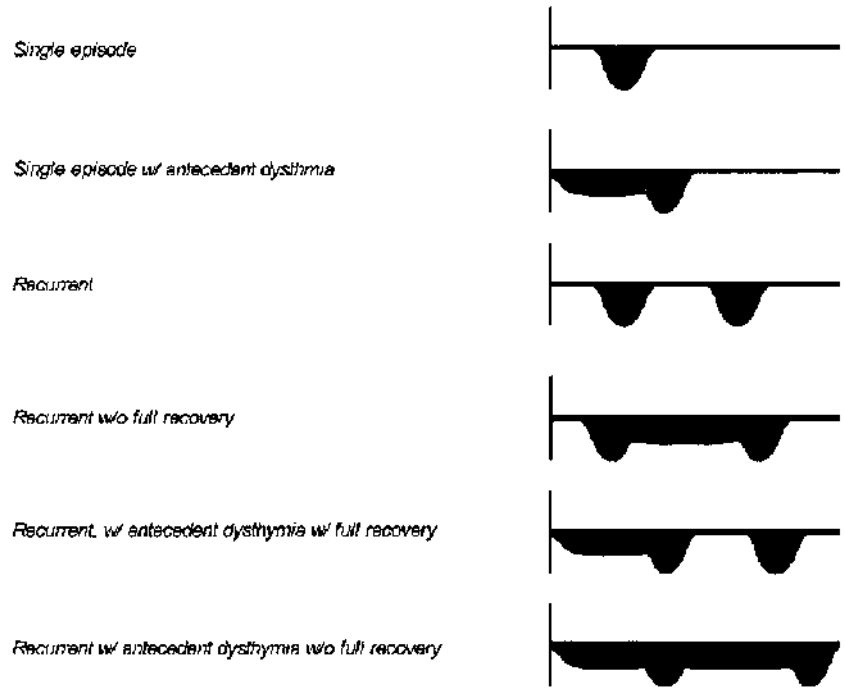

Figure 2 Longitudinal course modifiers. (Adapted from DSM-IV.)

can cause disability, and the longitudinal factors may be as important in considering disability from a mood disorder as cross-sectional factors.

III. THE COURSE AND TREATMENT OF DEPRESSION

A. Acute Depression

1. The Problem of Acute Depression

In the United States, the lifetime prevalence rate of major depression is estimated at 17% [4], which is consistent with several other epidemiological studies conducted in different regions of the world [5,6].

The morbidity and mortality from major depression are comparable to that found in other chronic medical conditions. Wells and colleagues, for the Medical Outcomes Study, found that the disability from depressive symptoms and disorders was comparable with or worse than that for eight major chronic medical disorders [7]. Estimates are that the economic costs associated with major depression in the United States are more than $53 billion per year [8]. The World Health Organization calculated that, in 1990, depression was the most costly of all medical illnesses in the developed countries of the world [9].

2. Treatment of an Acute Episode

A great deal of data exist to support the contention that treatment of depression can shorten the time to recovery. Such data represent the primary justification for the use of antidepres-

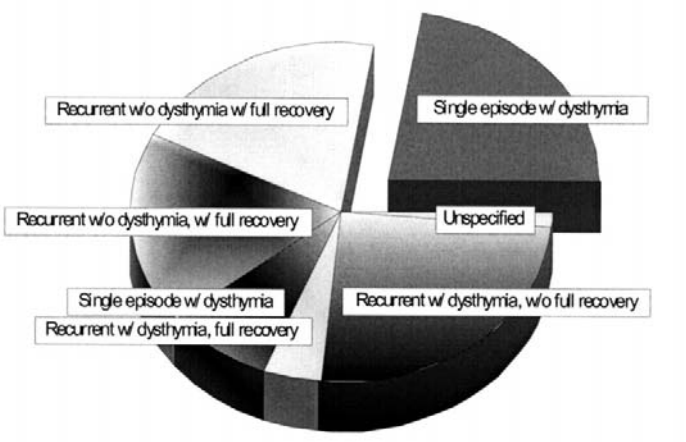

Figure 3 Prevalence of DSM-IV–defined course types. Classic "acute depression" is separated from the rest. (Data from the Mood Disorders Field Trial).

sant medications and several psychotherapies. Though some individual studies occasionally suggest an increased efficacy of one antidepressant compared with others, the weight of evidence suggests that the efficacy of all available antidepressants is approximately the same [10]. Although antidepressants vary greatly in their pharmacokinetic profiles (half-life, time until steady state, and solubility) and differ widely in their potency for various receptors and transporters, their pharmacodynamic effects are surprisingly similar. Thus, all antidepressants show a similar delayed onset of action (approximately 2–6 weeks), and have about the same efficacy rate (about 60–70%) in treating an acute depressive episode. (These efficacy studies will not be reviewed in detail here because they are detailed in all standard reference texts on psychiatry.) There has been considerable success in improving pharmacokinetic profiles, particularly those factors affecting safety and tolerability. However, improving such pharmacodynamic factors as efficacy and time to effect has been more difficult. There is some evidence for newer compounds with unique to multiple modes of action, with preliminary but hopeful evidence of improved efficacy [11] and time to effect [12]. Although research in this area shows some promise, it should be noted that the primary challenge in treating acute episodes of depression is not so much in finding newer and better treatments, but in ensuring that patients receive such treatment. The Collaborative Depression Study (CDS) and other naturalistic studies continue to support the assertion that only a minority of patients with major depression will receive adequate treatment for their disorder [8].

B. Relapse and the Continuation Period

1. The Problem of Relapse

Approximately 20% of patients who recover from major depressive episodes will experience a relapse of symptoms after initial recovery. For some of these patients (again, about 20%), achieving a subsequent remission will be more difficult. For the 141 patients in the CDS who recovered from their index episode of major depression, 22% relapsed within 1 year of follow-up [13]. Factors predicting a relapse included multiple episodes of major depression, older age, and a history of comorbidity with another (non-mood) psychiatric

Table 1 Relapse Rates Versus Placebo: Continuation Studies

Drug	Weeks of treatment	Relapse (drug) %	Relapse (placebo) %	*p* value
Fluoxetine	52	26	57	<0.01
Paroxetine	52	16	43	<0.001
Sertraline	44	13	46	<0.001
Citalopram	24	11	31	<0.05
Mirtazapine	40	19	44	<0.001
Nefazodone	36	17	33	<0.05
Venlafaxine	52	22	55	<0.001

illness. The number of previous episodes of depression was a particularly strong predictor of a relapse. For this relapsed group, the likelihood of remaining depressed for at least a year after a relapse was 22%. Predictors of prolonged time to recovery included a longer length of the index episode, older age, and a lower family income.

2. Treatment of Depression During the Continuation Period

The goal of continuation treatment is to prevent a relapse. Compared with the wealth of data about the treatment of acute depression, the data to guide us in continuation therapy are much more limited. However, for most of the commonly used antidepressants there exists at least one rigorous study to support the use of most of the currently used antidepressants, including the selective serotonin reuptake inhibitors (SSRI) fluoxetine [14], paroxetine [15], sertraline [16], and citalopram [17], as well as venlafaxine [18], nefazodone [19], and mirtazapine [20]. Although the specific methodologies used differ, in general each study randomized patients to receive either the study drug or a placebo after an acute treatment phase. In each study, relapses were much higher among the placebo group (Table 1). Viewed in aggregate, these studies lend reasonable support for the belief that most antidepressants that are effective in the acute period also lessen the risk for relapse during the continuation period.

C. Recurrent Major Depression

1. The Problem of Recurrent Depression

The risk of recurrent depression is surprisingly high: the CDS found a 25 to 40% risk of recurrence after two years [21]. These rates continued to increase over time, which means that patients had a 60% risk of recurrence after 5 years, 75% after 10 years, and 87% after 15 years [20]. Thus, the risk of recurrence increased with time, and there was no clear plateau for this increase. This remained true with subsequent new episodes of depression observed over the period of the study (Fig. 4).

2. Treatment of Recurrent Depression

Few data exist to guide us in the use of antidepressants during the maintenance period. Prien and colleagues [22] reported on a 2-year maintenance study of patients with major depression. In this study, patients successfully treated for acute depression were then randomized into a maintenance treatment phase using imipramine, lithium carbonate, both drugs, or placebo for 2 years beyond acute treatment. Of 150 patients beginning mainte-

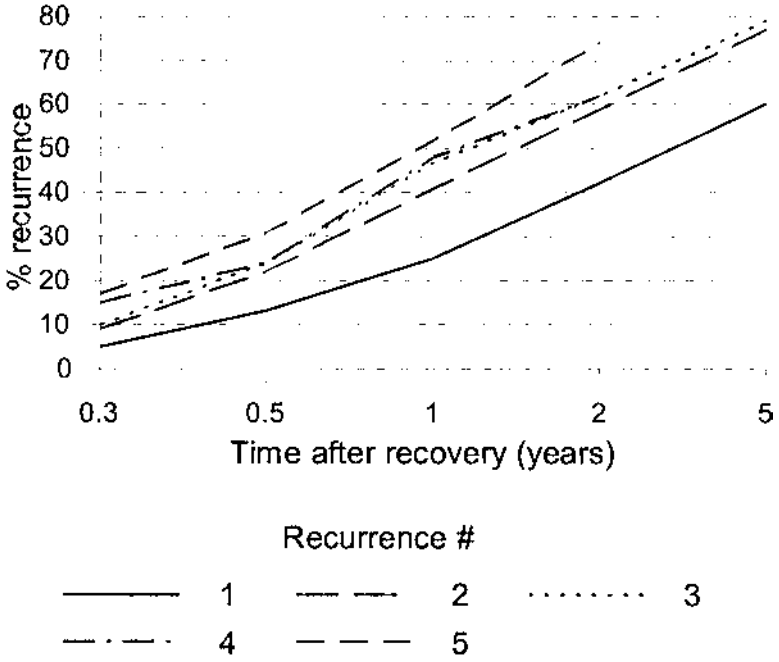

Figure 4 Probability of recurrence of depression following the index and subsequent episodes of depression.

nance treatment, 36% did not have a recurrence of depression during the 2-year maintenance period. The lowest rates of recurrence were in the groups receiving imipramine, or imipramine with lithium. The rate for these groups was 47%, which still seems uncomfortably high (Fig. 5).

A second study of maintenance treatment looked specifically at patients with a history of recurrence. This study, the Pittsburgh Study of Maintenance Treatment for Recurrent Depression [23], involved patients who first were successfully treated for an acute episode of major depression, and then randomized into one of five treatment conditions: (1) monthly interpersonal psychotherapy (IPT) alone; (2) medication clinic and imipramine; (3) monthly IPT and placebo; (4) imipramine and placebo; and (5) imipramine and monthly IPT. Of the 218 patients beginning the 3-year maintenance study, 106 completed it. Patients receiving imipramine, either alone or in combination with interpersonal therapy, had an 80% chance of remaining well for the 3 years of the study (Fig. 6).

This study was then continued for another 2 years, albeit with a much smaller (n= 20) sample of patients [24]. Those who had remained well for the 3 years of the initial study were randomized to receive either continued imipramine or placebo. The imipramine-treated group had a much lower rate of recurrence (10%) compared with the placebo group (approximately 66%).

D. Chronic Major Depression

1. The Problem of Chronic Major Depression

The CDS found that most patients (70%) recovered from an episode of depression by the first year [25]. However, of those patients who did not recover by 1 year, 66% still had

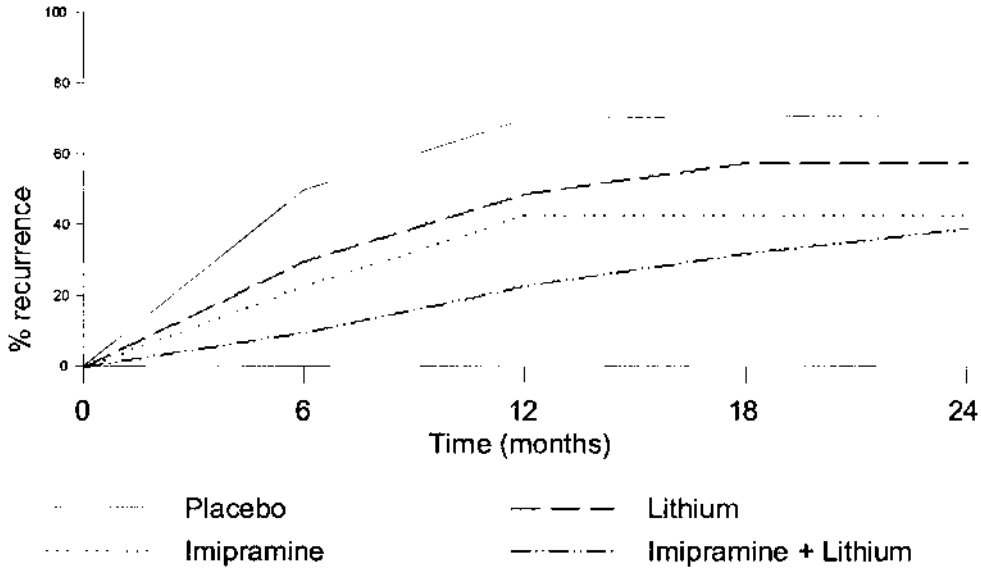

Figure 5 Prien and colleagues: maintenance treatment with imipramine, with or without lithium.

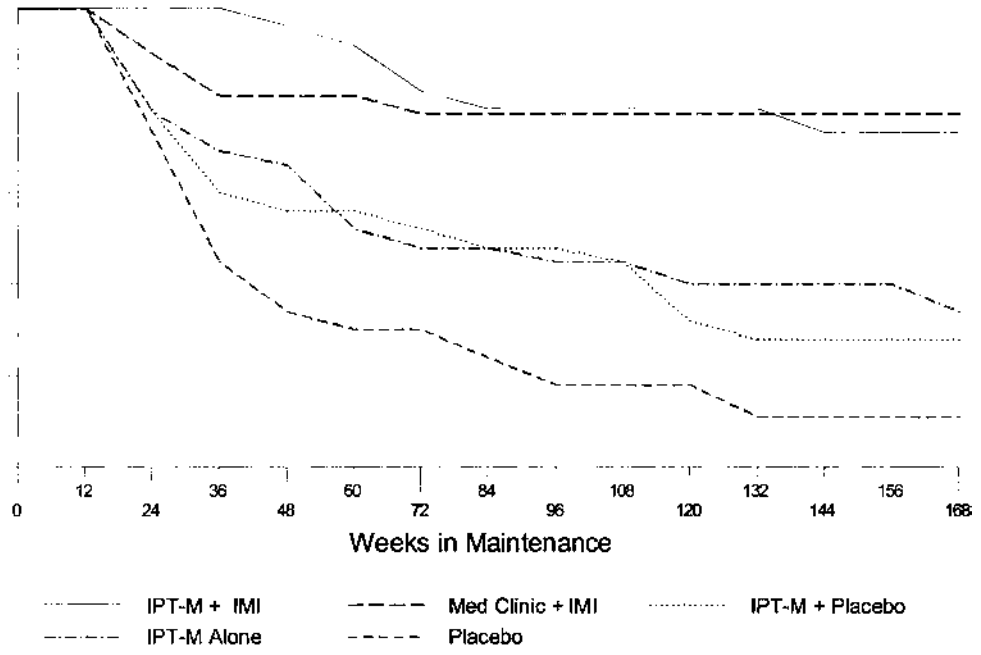

Figure 6 The Pittsburgh Study of Maintenance Treatment for Recurrence.

not recovered by 2 years, 40% by 5 years, and 23% by 10 years. By 15 years, the rate leveled off at about 20% [20]. The CDS observed multiple episodes of depression for a single individual, and these rates were generally true for each individual episode, whether it was the first or the fifth episode of depression (Fig. 7). Thus, there appears to be a cumulative risk for developing chronic depression with multiple episodes.

2. Treatment of Chronic Major Depression

Most data on the treatment of chronic major depression focus on the acute phase of treatment (i.e., the treatment of a depressive episode in patients with a history of chronic major depression). However, these data are still important because most antidepressant studies (particularly studies designed to assess efficacy) specifically exclude patients with chronic depression.

Kocsis and colleagues [26] studied the use of desipramine to treat chronic depression. Their study had three phases: an acute open treatment with desipramine (10 weeks), an open continuation phase (16 weeks), and a randomized placebo-controlled treatment with desipramine for up to 2 years. Only patients successfully treated in the first phase continued on to the open treatment with desipramine. In the acute treatment phase, 41% of patients were full responders and 22% were partial responders. Sixty patients who were either full or partial "remitters" continued onto the next phase. In the continuation phase, the majority of patients (83%) maintained their response status: 7 partial remitters became full remitters; 3 full remitters became partial remitters; and 1 partial remitter relapsed. In the maintenance phase, patients receiving a placebo were four times more likely than the treatment group to have a recurrence of major depression.

A second study by Keller and colleagues used a similar approach of three phases of treatment, with the benefit of a large sample size and the ability to randomize subjects at each phase. The treatment consisted of either sertraline or imipramine [27,28]. Similar to Kocsis's study, only full or partial responders proceeded to the next phase of the study. In the acute phase of treatment, 60% of patients responded to treatment. Although the rates of response were the same for both treatment groups, the sertraline group found the

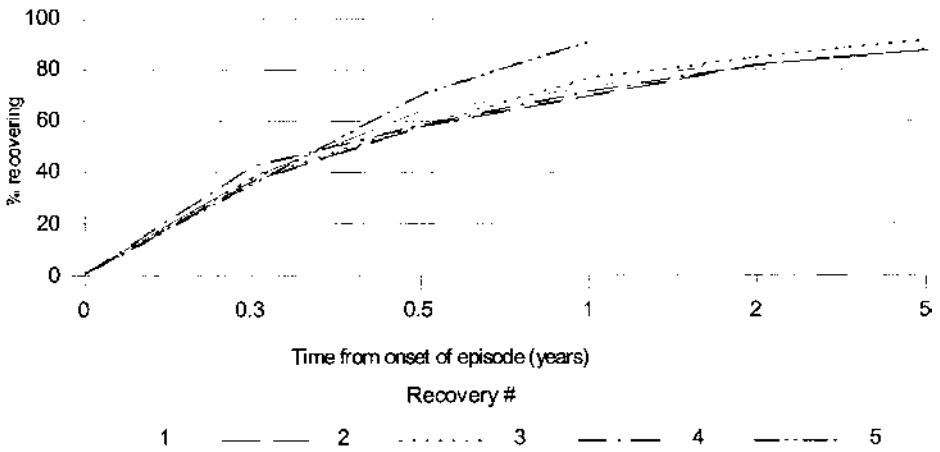

Figure 7 Proportion of subjects recovering from the index and subsequent episodes of depression.

Table 2 Continuation Treatment of Chronic Depression: Acute-Phase Full Responders

	Sertraline	Imipramine	Combined
n	140	88	228
Continuation status			
% discontinued	11	9	11
% nonresponders	12	15	13
% remaining partial responders	17	13	15
% improved to full response	71	73	72

treatment to be more tolerable. Of the patients who had a full response during the acute phase, 72% continued to be full responders during the continuation phase. An additional 15% were partial responders, and 13% did not respond to treatment. Once again, treatment response did not differ between the two treatment groups (Tables 2 and 3). In the maintenance phase, patients receiving a placebo were four times more likely to have a recurrence of depression, a finding similar to that of Kocsis and colleagues.

Another study by Keller [29] has looked at the effect of combined medication and psychotherapy in the treatment of chronic depression, with some surprisingly positive findings. In this study, 681 adults received acute-phase treatment with either 12 weeks of nefazodone treatment, cognitive-behavioral therapy, or both. Approximately 50% of patients in both the medication-alone and psychotherapy-alone groups had at least satisfactory responses to treatment. Most interesting in this study was the fact that the combined treatment (nefazodone and cognitive-behavioral therapy) had a much higher rate of response (73%) than with either treatment alone. Those patients who responded to the acute phase of treatment were then continued for a 52-week continuation phase. The great majority of patients (90%) in the continuation phase maintained their initial response; 90% of those in remission in the acute phase remained responders in the continuation phase. Once again, the combined treatment group fared somewhat better than the monotherapy groups [30].

E. Other Affective Disorders

1. The Problem of Non-Major Depression

In addition to the major forms of depression, there are several minor, but significant, forms of depression, many of which can cause substantial morbidity. One of the most commonly

Table 3 Continuation Treatment of Chronic Depression: Acute-Phase Full Responders

	Sertraline	Imipramine	Combined
N	99	59	158
Continuation status			
% discontinued	19	17	18
% nonresponders	15	29	20
% remaining partial responders	34	27	31
% improved to full response	51	45	49

recognized forms of minor depression is dysthymia, which by definition is a chronic disorder and may occur in 3% of the population [31]. It has a long duration of illness, ranging from 2 to 20 years with a mean of 5 years. In children, dysthymia has an average length of 3 years.

People suffering from dysthymia have an increased risk of major depression. Many patients have both: the concurrent presence of major depression and dysthymia is double depression. Double depression affects perhaps 30% of individuals with major depression [32]. Generally, remissions are not to states of euthymia but rather to dysthymia, and relapses to major depression are perhaps twice as likely for those with double depression than for patients with major depression alone [33].

Other forms of depression are less well understood but are beginning to be recognized more in the United States. Among these conditions is recurrent brief depression (RBD), which describes multiple brief occurrence of depressive symptoms that do not meet the 2-week time criteria for major depression. The prevalence of this disorder may be as high as that for major depression [34]. The lengths of episodes are short, around 3 to 5 days; however, the average number of episodes can be as high as 20 per year. During these brief, but frequent, periods, an individual can meet the full criteria for major depression (except for the time criteria)—thus, for individuals with RBD, a good proportion of the year will be spent coping with the disruption of serious depressive symptoms. Currently, RBD is included in the Appendix of the DSM-IV under Criteria Sets and Axes Provided for Further Study as research criteria.

Similarly, minor depression is described in the Appendix of DSM-IV. This disorder appears to be a subthreshold variant of a major depression, in which individuals have fewer than the required number of depressive symptoms. Otherwise, it appears to be similar in course and outcome to major depression and is probably at least as common [35]. Although minor depression is less severe and impairing than major depression, these patients are often less likely to receive treatment, the assumption being that treatment is either ineffective or not needed for this "less serious" disorder.

2. Treatment of Other Affective Disorders

The treatment of minor and subthreshold forms of depression remains controversial. Because of difficulties in recognition and diagnosis, these disorders have been grossly undertreated. For example, in one study of the least controversial of these diagnoses, dysthymia, only about half of the patients received psychotherapy and even fewer received pharmacotherapy [36].

For many years, pharmacotherapy was not considered a viable treatment for dysthymia and other minor depressions. This was largely due to the conceptualization of dysthymia and other disorders as more akin to personality disorders than to major depression. Thus, there is a paucity of rigorous studies on the pharmacotherapy of dysthymia and other affective disorders that do not meet the criteria for major depression. Most published studies investigate the treatment of dysthymia and show at least modest responses to medication [37]. Overall, randomized controlled studies show efficacy for most available agents, including tricyclic antidepressants [38,39], serotonin reuptake inhibitors [40–43], and such atypical agents as ritanserin [44], moclobemide [45], and amisulpride [46] (Table 4). As with major depression, there are no definitive data to suggest that any one agent is more efficacious that the other. Instead, the bulk of data suggests that any available agent used for treating major depression is likely to be effective for treating dysthymia and other forms of minor depression.

Table 4 Pharmacotherapy of Dysthymia

Drug	Compared to	Study	Duration of study (weeks)
Imipramine	placebo	38	6
	phenelzine, placebo	39	6
Desipramine	placebo	39	6
Fluoxetine	placebo	40,43	8;12
Sertraline	placebo	42	12
	imipramine, placebo	41	12
	imipramine	28	12
Ritanserin	imipramine, placebo	44	7
Moclobemide	placebo	45	4
	imipramine, placebo	48	8
Amisulpride	amineptine, placebo	46	12
	fluoxetine	49	12

IV. SUMMARY

A. Unanswered Questions

The past several decades have changed our view of depression from an acute illness to one with a variety of possible longitudinal courses. This chapter has discussed some of these longitudinal courses, and their implications for the outcome of depression. With a better understanding of the course of depression, it becomes critical to examine the approach to treatment, including the use of pharmacotherapy. The critical issue is the optimal length of treatment for depression. Clinical data and experience suggest that choices for duration of treatment tend to be made arbitrarily. Most treatment guidelines recommend at least 16 to 20 weeks of pharmacotherapy [47]. The rationale for this recommendation is based on data about relapse—most relapses will occur before this time. However, as we have shown, a significant number of relapses still occur after this period, and the question remains whether longer than conventional periods help decrease the rates of relapses in clinical samples. In addition, it remains unclear whether we can generalize across different treatments and whether different agents, or different classes of agents, are more likely to prevent relapses.

Beyond the question of treating an acute episode of depression, one must also consider when to choose longer term maintenance treatment. As we have discussed, as the length of treatment time increases, the available data decreases, and we have surprisingly little data to guide us in treatment decisions beyond 2 years. Currently, the standard recommendation is to offer long-term treatment to patients with histories of recurrent depression. However, this recommendation, as well as suggested lengths of long-term treatment, is based on a small amount of data. Finally, should antidepressant treatment ever be withdrawn from patients with recurrent depression and, if so, in what manner?

Dosing strategies during maintenance treatment are also understudied. In the 1970s and 1980s, it was standard practice to treat at one-half the acute period dose; a decision based largely on data from the Pittsburgh Maintenance Study [23], which found that patients kept at half of the acute dose had a higher recurrence rate during the maintenance period than patients maintained on their original dose. This study used imipramine, and

there is a paucity of data comparing maintenance dosing strategies for other agents; thus, the question of maintaining a patient on acute dosing with newer agents is not answered.

Other treatment questions remain unanswered as well. Anecdotally, most patients, and some clinicians believe that the effects of antidepressants tend to "wear off" over time. No data exist to support this belief, and limited data are available to refute it. In terms of nonpharmacological treatments, electroconvulsive therapy has an important role in treating acute depression and may be useful as a form of maintenance treatment. The use of psychotherapy, either as an adjunct or an alternative to antidepressants, needs more research. A recent study [29], for example, showed dramatic improvements in response when a modified type of cognitive therapy (with elements of interpersonal therapy added) was compared with pharmacotherapy alone.

B. Recommendations

Allowing for limited data, we can generalize from available data to recommend the following:

1. All patients with acute major depression should be considered reasonable candidates for pharmacotherapy. Currently, there is no strong evidence for choosing one medication over another, and treatment recommendations should be made on the basis of tolerability and, when appropriate, cost.
2. All patients with other forms of depression should be treated as suggested in recommendation No. 1. This is particularly true for dysthymia, and may be true for other minor forms of depression.
3. Patients with chronic depression and recurrent depression should receive extended treatment.
4. Maintenance treatment should consist of the same dose of antidepressant as used to achieve acute-phase remission.
5. Although the optimal length of maintenance treatment is not known, decisions about indefinite treatment should be based on risk–benefit analyses, made with the consent of the patient who is entitled to be informed as to the limitations of our knowledge.

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24

Pharmacotherapy of Bipolar Disorder

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I. INTRODUCTION

Bipolar disorder has a high risk of recurrence; it is a disorder that accompanies the affected all their life and therefore necessitates long-term treatment and prophylaxis with mood stabilizers. Bipolar disorder ranks high on the WHO list of leading causes of disability among the global population, higher than schizophrenia [1], for instance. In all cases of prophylactic treatment, "if patients do not take treatment with good medical supervision, they will not stay well" [2]. What is true for lithium might in the long run also apply to the other mood stabilizers, as the chasm between the efficacy of lithium as established in controlled clinical trials and its effectiveness in naturalistic settings could at least in part be explained by lack of compliance among both patients and health providers [3,4].

II. MOOD-STABILIZING SUBSTANCES

A. Lithium

After 50 years of ongoing research, lithium remains the classic mood stabilizer both for the acute manic phase as well as for the prophylaxis of recurrent manic and depressive episodes [5]. Lithium is an element (cation) discovered in the early nineteenth century. However, it took until 1949 for John Cade to introduce lithium in the therapy of manic patients, and until the early 1950s for Schou to revolutionize the treatment of manic-depressive illness [6].

The impact of lithium on the development of psychiatry is unique, reshaping medical, scientific, and popular concepts of severe mental illness [7]. Lithium remains first-line therapy, especially in patients with an episodic course of illness. Besides its mood-

stabilizing effect, lithium seems to exert an antisuicidal and antiaggressive effect [8,9]. It is the only mood stabilizer with a proven effect on the excessive mortality rate of bipolar patients.

Because lithium seems to have several different clinical effects, it is unlikely that any single biochemical aspect is responsible for all of them. One effect of lithium is that it only acts on overactive systems or overstimulated receptors to bring them back to normal state, but does not affect the stable system. It might inhibit components of various neurotransmitter signaling pathways and inositol monophosphatase activity, resulting in depletion of an endogenous source of inositol. Blocking inositol 1,4,5-triphosphate-dependent response has a powerful modulatory influence on neuronal excitability and neurotransmitter release. Nevertheless, lithium also has an effect on cells with an exogenous source of inositol [10]. Taking lithium for a prolonged period may regulate transcriptional factors and in this way modulate gene expression [11].

B. Anticonvulsants

In the 1970s, preliminary data on the possible mood-stabilizing properties of carbamazepine and valproate started to evolve. One hypothesis underlying the use of anticonvulsants in the therapy of bipolar disorder is the concept of "kindling," which has its origin in epileptology. There seems to be a local hypersensitivity in the amygdala precipitating affective episodes. Anticonvulsants like carbamazepine have a membrane-stabilizing effect on this area and seem to decrease sensitivity in the amygdala. This might also be their way of action in bipolar disorder. Valproate is thought to involve a GABAergic mechanism [12] and therefore to have an additional antipanic anxiolytic effect.

Carbamazepine and valproate showed some superiority over lithium in the treatment of atypical courses. In rapid cycling, a very severe course of illness, valproate seems to be the best therapy. Newly introduced anticonvulsants are lamotrigine, gabapentin, and topiramate. Their usefulness in bipolar disorder is still to be proven, although in some cases promising results exist either as monotherapy or in combination with another mood stabilizer [13,14].

C. Other Substances with Mood-Stabilizing Capacity

Calcium channel blockers like verapamil and nimodipine are used in the therapy of bipolar disorder as add-on medication. Nimodipine, in particular, seems to have some merit in the therapy of ultrarapid or ultradian cycling.

Atypical neuroleptics like clozapine, olanzapine, and risperidone are of growing interest in the treatment of bipolar disorder. Some authors claim that they have a mood-stabilizing potency of their own [15,16]. In any case, compared to typical neuroleptics, they have a much lower risk of side effects such as tardive dyskinesia, for which bipolar patients seem to be at greater risk. Thyroid hormones have been used to augment the effect of mood stabilizers, especially in cases of therapy-resistant bipolar depression. In the last years, supraphysiological doses of thyroid hormone have been used as treatment in resistant cases of bipolar disorder. There are some hints that cholinesterase inhibitors carry a mood-stabilizing potency, but more research is still needed.

III. ACUTE TREATMENT OF MANIC, MIXED, AND HYPOMANIC EPISODES

Although 1 to 3% of the general population suffer from bipolar disorder, the severity of this condition is often underestimated. Patients suffering from it have a high risk of

suicide, hospitalizations, rapid cycling, and other complications. Lithium remains the first choice in cases of classic euphoric mania. Valproate can also be seen as a first-line choice in classic mania and can be regarded as the treatment of choice in mixed episodes, dysphoric episodes, and mania in rapid cycling. An alternative in mixed or dysphoric episodes can be either lithium or carbamazepine. For mania in rapid cycling, carbamazepine is the first-line alternative to valproate [17,18].

When rapid stabilization is required in severe mania, valproate is the choice, as therapeutic blood levels can be achieved quickly by means of oral loading-dose strategy (20 mg/kg/day) [19,20]. Because the effect of mood stabilizers can be delayed, it is often necessary to use additional medication in the beginning, especially in patients with psychotic symptoms, insomnia, or agitation. In mania with psychotic symptoms, it may be necessary to add high- or medium-potency antipsychotics. For further sedation, a benzodiazepine or a low-potency antipsychotic may be added. Insomnia can be treated with a benzodiazepine. In this case, a high- or medium-potency antipsychotic is rarely needed.

When there is no or only partial response to the first mood stabilizer after 1 to 3 weeks, the initial mood stabilizer can be changed or a second mood stabilizer can be added. Lithium and valproate seem to be the most reliable combination in such cases [21]. Preferably carbamazepine is combined with lithium [22]. If all above options fail, combining carbamazepine and valproate might be considered, or even a combination of all three.

Drug interactions have to be noted carefully. Carbamazepine induces metabolism of both itself and valproate via the cytochrome P450 3A3/4 system. This may decrease the serum concentration of both drugs and the risk of relapse may increase. On the other hand, valproate may displace protein-bound carbamazepine and in this way increase the level of free carbamazepine, which could result in neurotoxicity.

Comorbidity of bipolar disorder and substance abuse can be found in more than a third of bipolar patients. It might be linked to a poorer course of illness and states of mixed mania. There are some hints that patients with a comorbidity of substance abuse might profit more from a treatment with anticonvulsive mood stabilizers than from lithium [23].

An alternative choice in treatment-resistant severe manic and/or mixed episodes can be electroconvulsive therapy. The concomitant use of benzodiazepines and lithium is very safe and effective. There have been some cases reported where benzodiazepines could replace neuroleptics in the treatment of acute mania. In any case, benzodiazepines decrease the need for neuroleptics and treat comorbid anxiety. The situation may become critical when patients with a history of substance abuse develop a problem with long-term benzodiazepine treatment.

Research now focuses on atypical neuroleptics and their mood-stabilizing properties. They have a greatly reduced liability to cause extrapyramidal syndromes and tardive dyskinesia. Clozapine seems to exert greater antimanic than antidepressive effects [24,25] and it could have some effect on treatment-resistant bipolar disorder [26]. Early clinical experience suggests that risperidone might have a greater antidepressive than antimanic property [27]. Studies to test the antimanic properties of olanzapine are ongoing [28,29]. However, its mood-stabilizing properties seem to be limited [30]. Anecdotal records show an antimanic effect of quetiapine [31].

Gabapentin—another anticonvulsive medication just recently introduced into the treatment of bipolar disorder—has the advantage of no drug interactions in the cytochrome P450 system. The combination with lithium or other mood stabilizers, therefore, seems to be safe and has already shown moderate effectiveness [32].

Lamotrigine appears to be promising both as a mood stabilizer alone and in combination with lithium or valproate [33,34]. It is important to note that valproate increases the blood level of lamotrigine because it inhibits glucuronidation. This combination might be highly effective, but extreme caution is warranted. There is an increased risk of dermatological reactions like severe skin rash—even of Stevens–Johnson syndrome—and encephalopathy [35].

An even newer anticonvulsant drug thought to have antimanic potency is topiramate. More data on its properties as an add-on to mood stabilizers are still needed. It has a broad spectrum of activity, including GABAergic and antiglutamatergic mechanisms, as well as calcium channel antagonism. Topiramate is known to have adverse cognitive effects, such as decreased attention and word fluency in some patients, which might be a limiting factor. On the other hand, topiramate offers the potential to induce weight loss in obese subjects [36–38].

Mexiletine is a drug already well known in the treatment of epileptic patients. It seems to be somewhat promising in the treatment of manic episodes, but more trials are warranted [39]. There are some data on the usefulness of cholinesterase inhibitors like donepezil in the treatment of acute mania [40].

Calcium channel blockers may be hazardous in combination with lithium: multiple cases of toxicity, particularly neurotoxicity, have been reported. This side effect might be critical for elderly patients who may also suffer from bradycardia. The risk of neurotoxictiy seems to be somewhat higher with verapamil and diltiazem than in nifedipine. The stabilization of the sleep–wake cycle can be an important part of therapy. Disrupted sleep cycles, as in jet lag, can induce manic episodes in psychosocially stressful times or in the postpartum period. All avoidable disturbances should be cut down to a minimum [41,42].

IV. ACUTE TREATMENT OF BIPOLAR DEPRESSION

In any case of bipolar depression, the role of switch from severe depression to acute mania has to be taken into account. There seems to be a high risk with tricyclic antidepressants but also with new antidepressants such as mirtazepine. Bupropion seems to be the drug with the lowest risk of inducing mania.

A. Treatment of Bipolar I Depression

In psychotic depression, the combination of a mood stabilizer, an antidepressant, and an antipsychotic is the recommended treatment. ECT can be an alternative therapy. For severe depression without psychotic features, a mood stabilizer and an antidepressant are the treatment of choice. In milder depressive episodes, a mood stabilizer alone might be effective. Psychotherapy in addition to medication may also be effective in such patients.

B. Treatment of Bipolar II Depression

The treatment of bipolar II depression is very similar to the treatment of bipolar I depression. In milder cases, psychotherapy seems to be even more effective than in bipolar I depression. If there is only a minimal history of hypomania, mood stabilizers might be cautiously omitted; an antidepressant like an SSRI alone [43] or combined with psychotherapy could be considered the treatment of choice.

In depression with psychotic features, an antipsychotic should be added to mood-stabilizing and antidepressant medication. In some of these cases, electroconvulsive therapy can prove helpful but, on the other hand, it has the disadvantage of sometimes produc-

ing a relatively high relapse rate and there is little information about which medication would be most helpful for the maintenance phase in these patients.

C. Therapy-Refractory Depression in Bipolar Disorder

The antidepressant potential of all mood stabilizers is lower than their antimanic potential. In this context, refractoriness can be a great problem [44]. Lithium responsivity seems to decrease in certain illness patterns characterized by a depressive episode first, followed by a swing into a manic episode [45]. This suggests that lithium in these cases failed to improve the first depressive episode so that a compensatory overswing results in a second manic episode. In some cases, lithium discontinuation can lead to a failure to respond to lithium again as well as to other treatment approaches. One-third of the patients show an initially good response to lithium followed by a gradual loss of efficacy. Some clinical findings demonstrate that the addition of a second mood stabilizer leads to better results in these cases, better than introducing an antidepressant, but treatment is more often discontinued [46].

Lamotrigine has a high response rate in refractory depression, alone or in combination with previously ineffective mood stabilizers [47].

Antidepressants should be chosen carefully in both bipolar I and II depression, always keeping in mind their ability to switch depression into full-blown mania. Traditional unimodal antidepressants can precipitate mania in approximately one-third of bipolar patients [48] and cause cycle acceleration in one-fifth of this population. There is some suggestion that administering mood stabilizers as lithium, carbamazepine, or valproic acid at the same time may reduce the risk of switching into maniform states. (49)

Bupropion or serotonin reuptake inhibitors are the first-line choice for patients known to switch easily into mania or rapid cycling [50]. Bupropion is assumed to have mood-stabilizing properties of its own and is believed to be the antidepressant with the least tendency to induce manic switches. Furthermore, it is less likely than SSRIs to cause sexual dysfunction and is one of the few antidepressants associated with a weight loss rather than gain. Monoamine oxidase inhibitors and venlafaxine are supposed to be an alternative to these choices. Tricyclics should be avoided when clinicians are concerned about a possible manic switch. Patients who maintain high lithium serum levels may not need additional antidepressant medication at all [51]. As in mania, insomnia can be treated with an additional benzodiazepine. In psychotic syndromes or severe agitation, high- or medium-potency antipsychotics are the appropriate treatment.

If there is no adequate response to the first-line treatment, lithium should be added when not already part of the treatment. Psychotherapy should be considered in mild or moderate depression. If the patient develops a lithium-related hypothyroidism or elevated TSH, thyroid hormone should treat this. The treatment of a euthyroid patient could also be augmented with thyroid hormone, especially in cases of subtle rapid cycling. Physiological replacement dose of 75–11 μg/day may be useful especially in women. High-dose treatment of 150–400 μg/day is recommended for rapid cycling patients. Light therapy might be used in the seasonal pattern of bipolar disorder. Bright light (greater than 7500 lux) may be worth considering in patients with marked disruption of circadian rhythmicity and bipolar hypersomnia. Sleep deprivation may bring brief improvement, but could also precipitate hypomania or mania.

V. RAPID CYCLING

According to DSM-IV, whenever a patient has four or more episodes in 12 months (either manic, hypomanic, mixed, or major depressive), he is diagnosed as rapid cycling bipolar

disorder. There has to be a phase of remission between episodes of at least 2 months or episodes have to switch from one polarity to the opposite. The ultrarapid or even ultradian cycling is a course of illness neglected in DSM-IV; it means that a mood swing occurs within the same day or even the same hour. A great deal of research now focuses on these treatment-challenging courses of bipolar disorder.

Of bipolar patients, 13 to 20% suffer from rapid cycling [52–54]. The majority of the rapid cycling disorders have a late onset. Factors known to contribute to this course of illness are female gender, middle age, and hypothyroidism. Rapid cycling is approximately three times more common in women than in men [55]. The diagnosis of rapid cycling has implications for therapy. There is wide agreement that tricyclic antidepressants can induce this course, so they should be avoided in the treatment of depression in bipolar disorder. All antidepressants should be tapered more quickly than in unipolar depression. The agent believed to be the safest to treat depression in bipolar disorder is bupropion. Patients should be advised to record daily mood ratings—cycle acceleration can be hard to recognize in the beginning because of its subtle onset. Depression in rapid cycling is an extremely challenging condition for the clinician.

There also seem to be some nonpharmacological factors that can lead to cycle acceleration such as pregnancy, Graves' disease, or electroconvulsive therapy. Lithium in rapid cycling loses some of its usual power. Anticonvulsants seem to have a more beneficial effect. The first-line treatment for rapid cycling is valproate. The use of supraphysiological doses of thyroxine has been helpful in some patients with rapid cycling and lamotrigine proved to have some power in treating depression in patients with bipolar II disorder and a rapid cycling course [56].

VI. MEDICATION FOR CONTINUATION TREATMENT

The initial period lasting 4 to 6 months after an acute illness episode is the continuation period. The acute episode should have ended, but the patient may still have some symptoms and experience functional problems. The usual procedure is to continue mood stabilizer(s) while trying to taper other medications (antidepressants, antipsychotics, benzodiazepine). The dose of the mood stabilizer should be adjusted to balance maximum benefit against side effects.

During the continuation period, medication should be monitored weekly or every 2 weeks to prevent noncompliance. Antidepressants should be tapered sooner than in unipolar depression (e.g., sooner than 6–12 months).

VII. STRATEGIES FOR LONG-TERM MAINTENANCE

After the initial period has ended, the stabilized patient can still be vulnerable to new cycles of illness. To prevent this, a long-term or lifetime plan for prophylaxis must be considered. In bipolar I disorder, long-term or lifetime prophylaxis is the treatment of choice after two manic episodes, but should already be considered after one manic episode if it was severe or if there is a family history of bipolar disorder. Some experts advise long-time prophylaxis after one manic episode in patients with an onset before the age of 20.

In bipolar II disorder, prophylaxis can be considered after three hypomanic episodes, if severe or closely spaced or if there are antidepressant mood elevations, frequent depression, or a family history of bipolar I disorder. Long-term tolerability is highest in lithium

and valproate. Compliance with the prescribed medication should be an issue in each of the patient's visits. There should be a quick review of life events since the last visit. Topics that should also be addressed are substance abuse, sleep patterns, management of excessive stress, and suicidal ideations. Psychotherapeutic approaches like psychoeducation, cognitive-behavioral therapy, family and marital therapy intervention, and adjunctive therapies can be an integral part of therapy [57]. A visit for medication management is recommended every 2 to 3 months at minimum in a milder course and if the patient is stable. If some symptoms still persist or the patient still has some problems functioning, medication management should take place monthly.

In an especially severe course with ongoing care (e.g., as in rapid cycling),weekly medication management is recommended. Medication monitoring includes serum levels of lithium, valproate, carbamazepine, selected tricyclics every 3 to 6 months, thyroid function at least yearly, a general chemistry screen, and a physical exam if the patient's medical history is unknown, urine analysis if starting lithium and renal function tests every 6 to 12 months, and pregnancy test, if applicable.

There is a subgroup of patients who might not profit from prophylactic treatment. There is no consensus of how to identify this subgroup. When discontinuing a mood stabilizer this should always be done gradually over several weeks.

VIII. DEALING WITH COMMON SIDE EFFECTS OF MOOD STABILIZERS

Thirst and polyuria are side effects quite often seen in the beginning of lithium therapy. They can be very annoying and risk the patient's compliance. The reason for this effect is that vasopressin receptors and expression of water channels are diminished by lithium. Polyuria can be treated with amiloride, thiazides, or indomethacine. It is important to treat this symptom, since nocturia causes sleep disruption, which in return can result in mood disruption. Furthermore, the use of high-calorie beverages to treat polydipsia can cause weight gain. If mild renal insufficiency with elevated levels of creatinine is diagnosed, a different mood stabilizer should be added and lithium should be gradually tapered.

Cognitive complaints such as feeling dull or flat under lithium medication are not manageable with any add-ons. They are a reason to gradually switch to valproate or carbamazepine. Rigor or nystagmus is another effect on the CNS that can be induced by lithium medication. EEG can show abnormalities. Risk factors for these adverse effects are preexisting CNS structural disease, especially in elderly patients and with the combined therapy of lithium and neuroleptic drugs.

ECG abnormalities are due to changes in myocardial repolarization induced by lithium salts. Arrhythmia is seen very rarely within therapeutic ranges of lithium plasma levels. Dysfunction of the sinoatrial node and bradyarrhythmia are relative contraindications for lithium therapy.

Tremor caused by lithium therapy can be treated with β-blockers. Weight gain is seen in about one-third of patients treated with lithium. The average weight gain is 4 to 10 kg. Patients should check their weight regularly. Diets should be planned together with the clinician to avoid intoxication. Weight gain can be very challenging to the patient's compliance, especially in women. If the weight gain becomes unacceptable for the patient, another mood stabilizer should be considered for long-term therapy.

Sedation is another side effect that can occur under lithium therapy. If lithium induces diarrhea and nausea, then patients have to be observed closely. Dehydration can

lead to toxicity and therefore the clinician must be careful in balancing the fluid volume of lithium-treated patients. Milder nausea experienced early in treatment improves when lithium is administered with meals. Changing the time of day when dosing takes place can also bring some relief [58]. Diarrhea can be persistent, and antidiarrheal agents can be helpful.

Hypothyroidism is another adverse effect of lithium. Iodine uptake into the thyroid gland is decreased under lithium therapy. Formation and release of thyroxin are inhibited. TSH levels should be monitored regularly. If they are elevated, L-thyroxin should be added to the therapy.

Hyperparathyroidism and consequently hypocalcemia can be seen in nearly one-third of patients treated with lithium. Most of the patients do not have symptoms. Lithium has to be tapered if and when hypocalcemia becomes symptomatic.

The most common dermatological complaint of lithium users is about dry skin. Topical lotions are sufficient to treat this symptom. Psoriasis vulgaris can occur for the first time under lithium medication but can also be worsened if already preexisting. Acne is another effect of lithium on the skin. Any dermatosis induced by lithium should be treated in cooperation with a dermatologist. In most cases, it is sufficient to reduce lithium dosage; it is seldom the case that lithium has to be tapered due to dermatosis.

Lithium intoxication is seen in patients with decreased renal lithium excretion or with overdosage either accidentally or in a suicide attempt. Nearly all of these patients show central nervous system symptoms like tremor, ataxia, nystagmus, and a decrease of vigilance. Lithium's renal excretion can be reduced for several reasons. Gastroenteritis, renal insufficiency, dehydration, narcosis, delivery, and drugs like diuretics, NSAP, or some antibiotics are factors that can influence lithium excretion. Intoxication caused by decreased excretion of lithium can be avoided in most cases. The role of patient education should not be underestimated.

Valproate shares some side effects with lithium. Again, tremor can be treated with β-blockers. If sedation and weight gain (valproate is the anticonvulsant most often associated with weight gain) [59] become unbearable to the patient, another mood stabilizer should be considered for therapy. Diarrhea can be induced by valproate, but is less dangerous than under lithium medication. Nausea can be treated with H_2-blockers. Hair loss is preventable by adding on zinc and selenium supplements. Mild elevation of liver function parameters should be watched closely. Carbamazepine can have some alarming side effects: headache, nystagmus, ataxia, or sedation can be very disabling for the patient, as well as rash, which is rather common [60].

Leukopenia is the most dangerous side effect of carbamazepine, and should never be combined with clozapine, another drug that can induce a drop in leukocytes. Leukopenia is a reason to taper carbamazepine immediately. As with valproate, liver function parameters can be elevated.

Carbamazepine induces cytochrome P450 liver enzymes and shows drug–drug interactions very frequently. It can decrease plasma levels of other medications. Oxcarbazepine, a well-known drug in Europe, has the advantage of not requiring hematological or hepatic monitoring. It has recently become available in the United States.

Lamotrigine can lead to Stevens–Johnson syndrome. Rashes typically develop within the first 2 to 8 weeks of treatment. The risk can be minimized if the physician initiates therapy at the lowest possible dosage.

Psychiatrists prescribing anticonvulsants should be familiar with the skin eruptions that can be caused by them.

IX. SELECTING MOOD STABILIZERS FOR PATIENTS WITH MEDICAL COMPLICATIONS

Reduced liver function is a contraindication to use of anticonvulsants; in such cases, lithium is the preferred treatment option. In patients with CNS structural disease or renal disease, valproate or carbamazepine are recommended for treatment of bipolar disorder. Patients who have a comorbidity of alcohol or cocaine abuse should not be treated with carbamazepine. Valproate would be the best mood stabilizer for patients with heart failure.

X. SELECTING MOOD STABILIZERS FOR PATIENTS WITH AGE OVER 65

Valproate or lithium is the preferable treatment option for bipolar disorder in the elderly [61]. Carbamazepine often is not recommended because of its multiple drug interactions and because it can cause cardiac conduction disease. Some clinical trials suggest that lithium may be more efficacious than valproate in this special group of patients. For valproate, a "therapeutic window" (other than that in younger adults) was found, namely, 65 to 90 μg/mL. Generally, low rather than high doses should be used. Lithium dosage in the elderly requires adjustment because of changes in renal clearance and reduction of total body water [61]. If medication is added to the patient's current regimen, modest doses should be increased slowly. Drug interactions have to be considered carefully in the elderly population. Some widely used drugs that increase the blood level of lithium are thiazide diuretics, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and β-blockers.

Physically healthy elderly patients generally tolerate lithium well. The very old, physically ill, frail, or cognitively impaired may experience an increase in the frequency of side effects. The most prominent side effects in this special population are polyuria, gastrointestinal abnormalities, trauma, ataxia, and cognitive impairment. Polyuria may cause problems in patients with a comorbid prostatic hypertrophy. This combination can lead to urinary retention and promote urinary tract infections. Toxic blood lithium levels result in seizures and coma. Slow-release lithium tablets reduce the maximum blood level concentration.

The condition of mania is often misdiagnosed in the elderly. Confusion, irritability, paranoia, and impaired concentration are seen more commonly than in younger patients. Before starting treatment with lithium, neurological examinations should be administered. Patients with an onset of illness later than age 55 are not supposed to differ in their drug response from patients with an earlier onset.

XI. MANAGEMENT OF BIPOLAR DISORDER DURING PREGNANCY AND BREAST-FEEDING

Bipolar disorder occurs commonly in women during their childbearing years. The impact of untreated psychiatric illness on pregnant women and their offspring is often underestimated. Impulsive or self-injurious behavior and suicide, substance abuse, or inattention to prenatal care are only some of the risks. Discontinuing the mood stabilizer makes the risk of recurrence during pregnancy similar to the risk of recurrence at other times. The postpartum risk of recurrence is 3 times as high [63]. The risk of a subsequent relapse is greater if the number of prior affective episodes was high. Lithium, carbamazepine, and

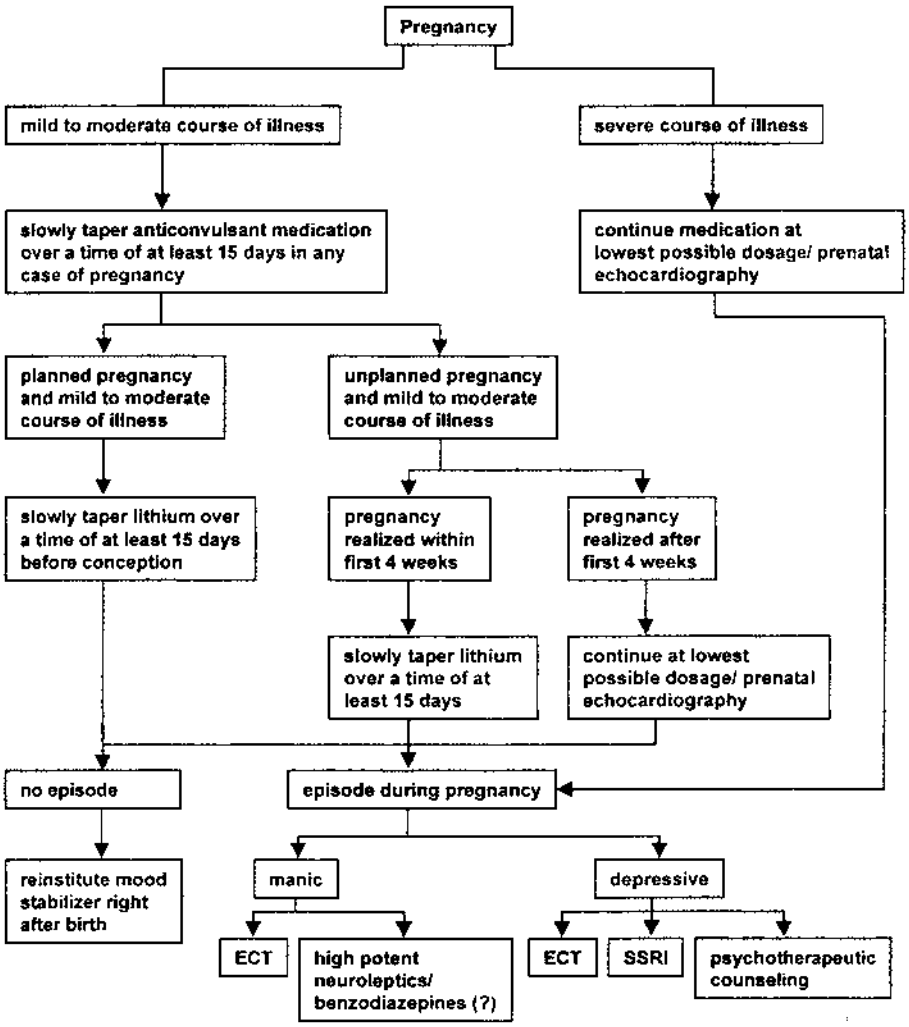

Figure 1 Management of patients with bipolar disorder during pregnancy—treatment options.

valproate all carry fetal teratogenic risk in early pregnancy and adverse effects in late pregnancy, labor, and delivery. Regardless of which medication is used, the treating clinician should document the contraceptive methods used and discuss the potential risks of intrauterine exposure to the different drugs in all female patients during their childbearing years.

Decisions should be made by the patient (and her partner) after being fully informed about all risks. Discontinuation might be considered in cases with a good previous course of illness (Fig. 1). Discontinuation of mood stabilizers should occur gradually over 15 to 30 days (i.e., not rapidly, if at all possible). This reduces the number of recurrences during pregnancy and the same applies to the patient subject to an unplanned pregnancy, as might often be the case. Medication should not be tapered abruptly but gradually over 2 weeks

(50% of all conceptions are inadvertent and most women learn that they are pregnant after a missed period and are already at 3 to 7 weeks gestation).

In the first trimester, neuroleptics, SSRIs, and ECT carry a lower teratogenic risk than lithium. There are very controversial findings about the teratogenic potency of lithium in the first trimester. Some studies suggest a connection between a cardiac malformation known as Ebstein's anomaly; others cannot confirm these findings (Ebstein's anomaly is characterized by right ventricular hypoplasia and congenital downward displacement of the tricuspid valve into the right ventricle) [64]. Ebstein's anomaly has a prevalence of 1:20,000 in the general population. Under lithium therapy, the risk increases 10 to 20 times or approximately 0.1 to 0.05%. A tricky decision is to be made by the clinician and the situation of each individual patient must be taken into account. If lithium therapy is also continued through the critical period of organogenesis (4 to 12 weeks after the last menstruation), the patient should be examined with prenatal high-resolution echocardiography around 16 to 18 weeks gestation [65]. The lowest effective dose of medication should be taken.

Newborn infants of lithium-treated mothers can develop a "floppy infant syndrome," as though under treatment with benzodiazepines or neuroleptics. When tapering lithium postpartum, reintroducing lithium prophylaxis right after delivery or during late pregnancy can reduce risk of recurrence. The doses have to be drastically reduced during delivery since the blood and fluid volume decreases immediately after the child is born and the risk of toxicity rises. When continuing anticonvulsives, folic acid supplements should be added to the regimen. It should be noted that anticonvulsants are believed to be highly teratogenic. An increased occurrence of spina bifida has been observed in the offspring of mothers treated with anticonvulsives. For carbamazepine, a so-called fetal carbamazepine syndrome, with dysmorphic features and mild mental retardation, is reported. The fetal valproate syndrome includes craniofacial, abdominal wall, cardiovascular, urogenital, and digital malformations. Therefore, if possible, usage of these drugs should be avoided during pregnancy.

There is no evidence of teratogenic potential of ECT—a therapy alternative to medication exposure. Risks to the fetus can be minimized by positioning the patient in the left lateral decubitus position, by monitoring the fetus and uterus, and by limiting the exposure to anticholinergic medications.

Antidepressants continued throughout delivery can cause symptoms of drug withdrawal in the newborn. These symptoms can include irritability, eye rolling, temperature instability, tachycardia, and seizures. All mood stabilizers are secreted through breast milk. Although there are some data suggesting that these medications do not pose an immediate risk to the newborn, breast-feeding is not recommended. However, there may be some women who insist on breast-feeding. Nursing infants' serum lithium concentrations have been reported to be 10 to 50% of the mothers' serum levels. The baby's hydration status should be monitored very carefully.

Valproate and carbamazepine are considered compatible with breast-feeding by some authors and the American Academy of Pediatrics (1994), although possible side effects include leukopenia and hepatitis. Liver function of the infant must be monitored.

XII. MOOD STABILIZERS AND SUICIDE RISK IN PATIENTS WITH BIPOLAR DISORDER

Bipolar patients are at great risk of committing suicide [66]. The mortality of bipolar patients is two to three times higher than that of the general population. (Bipolar disorder

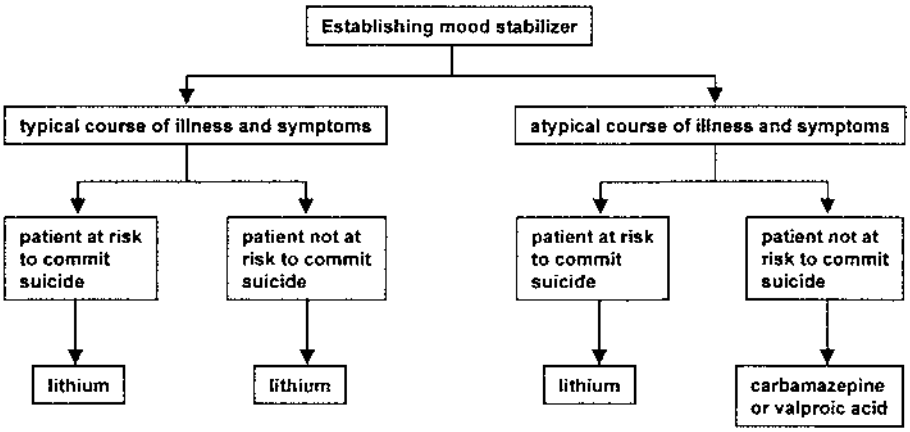

Figure 2 Bipolar disorder and suicide—treatment options.

is responsible for an estimated 30,000 deaths per year in the United States.) Of patients with bipolar disorder, 25 to 50% attempt suicide at least once! Only about 5% commit suicide by overdosing on their antidepressants—more women than men. So it does not seem to make sense to prescribe less toxic drugs—it might even be harmful if the drugs are less efficient [67].

Established risk factors for completed suicide in affective disorder are acute depression, mixed episodes, rapid cycling, substance abuse, aggression, and/or impulsivity. Young men in the early phase of their illness are the group at highest risk.

Mortality during lithium treatment is not significantly higher or only moderately higher than in the general population; after discontinuation of lithium, mortality again is significantly higher. The suicide rate in bipolar patients not treated with lithium is more than seven times higher than the suicide rate in bipolar patients treated with lithium [68]. This is strong evidence for the antisuicidal action of lithium—also for patients not showing satisfactory response to its episode-preventing effect! Similar observations have not been reported for other mood stabilizers, although anticonvulsants may have merit in the treatment of mixed states, rapid cycling, and comorbid substance abuse [69]. There is insufficient evidence that long-term antidepressant treatment diminishes suicide risk and this should be considered when choosing a mood stabilizer for long-time prophylaxis (Fig. 2), especially in patients with severe depression, suicidal thoughts, previous suicide attempts, or a combination of these.

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25

Development of New Treatment Options for Depression

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I. INTRODUCTION

Whereas antidepressants of the first generation were developed primarily on the basis of their chemical structure (e.g., tricyclic antidepressants), the newer generation of antidepressants has been developed strictly on the basis of their mechanism of action (e.g., selective serotonin or noradrenaline reuptake inhibitors). Although there was no evidence that the elderly antidepressants like imipramine are psychostimulants or a "happy pill," this notion is still often discussed in the media, over 40 years after the introduction of antidepressants for the treatment of depression. Figure 1 displays the development of antidepressive treatment modalities over the past 100 years, and the emerging treatment opportunities in the last 20 years are apparent.

When Kuhn [49,50] first described the antidepressant effect of imipramine, he based his assumption only on clinical observation. There were no standardized diagnostic criteria, no psychometric instruments, like the Hamilton Depression Rating Scale (HAM-D), and no computer to establish statistical significance levels and confidence intervals. Subsequent development resulted in a refined methodology, which is now the basis for internationally accepted drug development programs that make medications available in Europe and worldwide [24]. Whereas the methodology and the quality of the studies are now acceptable throughout different health authorities worldwide, pricing of the product is the limiting factor for the availability of medication in the individual countries.

One of the aims for developing new antidepressants was to have a better tolerability

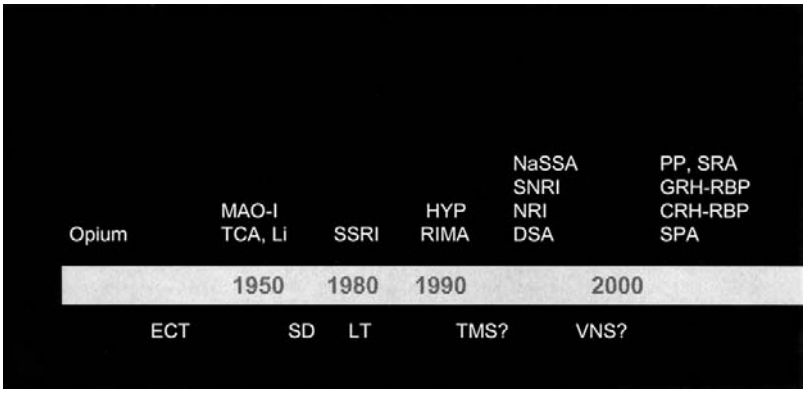

Figure 1 Different pharmacological and nonpharmacological, though biologically based, therapies in depression. ECT: electroconvulsive treatment; SD: sleep deprivation; LT: light therapy; rTMS: repeated transcranial magnetic stimulation; VNS: vagus nerve stimulation; MAO-I: inhibitors of monoamine oxidase; TCA: tricyclic antidepressants; SSRI: selective serotonin reuptake inhibitors; RIMA: reversible inhibitor of monoamine oxidase A; NaSSA: noradrenaline serotonin-specific antidepressant; SNRI: serotonin noradrenaline reuptake inhibitor; NRI: noradrenaline reuptake inhibitor; DSA: dual serotonergic antidepressant; SRA: sigma receptor antagonist; SPA: substance P antagonist; CRH-RBP: corticotropin-receptor-blocking peptide; GRH-RBP: glucocorticotropin-receptor-blocking peptide; PP: pentapeptides.

profile than the older antidepressants, especially regarding toxicity in overdose [18,32]. To achieve this goal, a more circumscribed mechanism of action was studied that was specifically targeted to eliminate anticholinergic (dry mouth, constipation, cognitive dysfunction), antihistaminergic (sedation, weight gain), as well as α_1-adrenolytic (orthostatic hypotension)-mediated side effects [61]. Particularly in the elderly, a beneficial side effect profile is important when lifelong antidepressant treatment is initiated, as it is for hypertension [78].

II. ANTIDEPRESSANTS OF THE FIRST GENERATION

Soon after antidepressants have been shown to be effective in open trials, randomized controlled trials (RCT) with the use of placebo were introduced in depression research. Morris and Beck [63] published the first comprehensive review of RCT. The authors noticed that there were many differences (e.g., diagnostic assessment of depression, control treatment, definition of response, and statistical analysis), which make it difficult to compare the individual studies. Interestingly, the first trial conducted in a setting of a general practice (GP) was published in 1970 [73]. This is of importance because more than 80% of prescriptions come from general practitioners.

Overall, the RCT indicated that tricyclic antidepressants (TCAs) as well as monoamine oxidase inhibitors (MAO-I) are significantly superior to placebo, but with a burdensome side effect profile. The largest long-term study of antidepressants has been performed with imipramine [30], which indicated that the dose that had been proven to be effective in the short-term treatment should also be used for continuation and prophylactic treatment

[31]. Furthermore, this study elegantly demonstrated the superiority of antidepressive medication over placebo and interpersonal psychotherapy.

The necessity of long-term treatment for depression soon underlined the importance of a low side effect profile for antidepressants, which was the impetus for the development of newer antidepressants.

III. NEWER ANTIDEPRESSANTS

Compared to the RCTs carried out with TCAs, newer antidepressants have been studied with a more refined methodology, because DSM-III and later DSM-IV diagnoses of major depression as well as standardized research instruments were more accepted [6]. These methodological standardizations facilitated the use of meta-analyses such as those by Loonen and Zwanikken [57], Bech and Cialdella [7], and Anderson and Tomenson [1].

A. Selective Serotonin Reuptake Inhibitors

To date, there are five selective serotonin reuptake inhibitors (SSRI) available worldwide: citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline [42,44]. They all have serotonin reuptake inhibition as the common mechanism of action but differ in pharmacokinetic properties. For instance, fluoxetine and its active metabolite have a half-life of 10 to 14 days, while other SSRIs are in the range of about 1 day. The metabolic pathway of P450 cytochromes also differentiates the individual SSRIs. The least interaction with other medications can be expected from citalopram and sertraline [12].

RCT indicated that the SSRIs are superior to placebo in their antidepressant efficacy and not different from TCAs, except possibly in severe depression, if clomipramine [20,21] or dual-acting antidepressants [58,60,83] are used for comparison.

Long-term studies with SSRIs support the notion that SSRIs are effective in preventing relapse (e.g., in the first 4–6 months after acute episode) and recurrence (6 months after remission has been achieved) [37,89].

Compared to TCAs, SSRIs have a more favorable side effect profile and it is of clinical importance that the side effects of SSRIs mitigate after 2 to 3 weeks, whereas those of TCAs persist [76].

In addition to the currently available SSRIs another SSRI, escitalopram, the S-enantiomer of citalopram, is now under clinical investigation and the first studies show a good tolerability and efficacy profile [62,68,85], which might exceed that of citalopram.

B. Selective Noradrenaline Reuptake Inhibitor

Recently, the selective noradrenaline reuptake inhibitor (SNRI) reboxetine has been introduced in Europe and will soon be available in the United States, which has a specific noradrenaline reuptake mechanism [22,46]. Besides antidepressant efficacy, this compound promises to specifically improve on social functioning compared to SSRIs [39,40]. One of the side effects of a specific noradrenaline reuptake inhibitor—urinary hesitancy in patients with prostate enlargement—has been shown to be treated effectively with tamsulosine [47].

C. Dual and Receptor-Specific Antidepressants

After the success of the SSRIs, researchers set out to develop compounds that exert both serotonin and norepinephrine reuptake blocking properties (= SNRIs). Antidepressants

with this mechanism of action have been termed dual-acting antidepressants. Venlafaxine [13,55], milnacipran [11,38], and duloxetine [72] fall into this category and, based on available data, it seems that low-dose (50 mg) venlafaxine is more a 5HT inhibitor than a noradrenaline reuptake inhibitor and vice versa for milnacipran. Both compounds (venlafaxine and milnacipran) have been shown to be significantly superior to placebo and as effective as TCAs [8,38]. However, compared with SSRIs, a significantly superior efficacy has been demonstrated in severely depressed patients [83]. The side effect profile of SNRIs is linked to their pharmacodynamic properties with nausea and agitation being the most frequent. Recently, another dual-acting antidepressant, duloxetine, was studied in a clinical development program in Japan and elsewhere [72,80].

Mirtazapine is a specific-acting antidepressant that enhances noradrenaline as well as serotonin neurotransmission via blockade of the presynaptic autoreceptor sites [45]. In addition, it exerts its antidepressant properties via blockade of postsynaptic $5HT_2$ and $5HT_3$ receptors. RCT demonstrated superior efficacy to placebo and comparable efficacy to TCAs, including clomipramine. Superior antidepressant properties have been demonstrated for fluoxetine. The side effect profile is superior compared to TCAs and different from SSRIs. The postsynaptic blockade of the $5HT_2$- and $5HT_3$-receptor site translates in clinical practice to typical SSRI side effects like nausea, agitation, headache and sexual dysfunction, which cannot be found with mirtazapine. However, weight gain is a limiting factor for some patients.

Nefazodone is another newly introduced compound with a combined serotonergic mechanism of action (5HT reuptake inhibition, $5HT_2$ blockade), which offers the benefit of not having sexual side effects [4]. However, the dosing regimen as well as some side effects (like lightheadedness) does not give this compound an overall advantage over SSRIs [26].

Whereas buspirone, a $5HT_{1A}$ agonist, was not used to a large extent in clinical practice, new compounds under investigation, such as gepirone [25,59], demonstrated clinically relevant efficacy in recent RCT. Flesinoxan and ipsapirone, on the other hand, also 5-HT_{1A}-activating compounds, have not been developed further since RCT did not show superiority over placebo.

Moclobemide, a reversible inhibitor of MAO-A, is marketed in Europe but not the United States [2,54]. This compound can be viewed as efficacious in mild-to-moderate, but not severe, depression. However, specific studies have not been carried out.

The mechanism of action of tianeptin is not as yet fully understood; however, clinical results reveal good efficacy, specifically if the symptomatology of the patients comprises both anxiety and depression [35,86]. Bupropion, a selective dopamine reuptake inhibitor, is marketed in the United States for depression and in Europe for smoking cessation. The availability for those different indications in different parts of the world stems more from marketing considerations than from data obtained in RCT.

D. St. John's Wort as an Antidepressant

Since ancient times, preparations of St. John's Wort ("Johanniskraut") have been used for antidepressant treatment properties [5,9,64]. In Germany, this treatment modality comprises up to 40% of the antidepressant market. Recently, it has been demonstrated for a few standardized formulations (e.g., LI-160, WS 5570) that the pharmacodynamic properties include serotonin, noradrenaline, and dopamine reuptake modulation together with

GABAergic effects [65]. Among the various constituents of St. John's wort extract, hyperforin seems to play a major role in exerting the antidepressant efficacy. This has been demonstrated not only in animal studies but also in placebo-controlled clinical trials [36,43,51,53]. There are a number of RCT with standardized hypericum extracts, with more to come. These studies indicate superiority over placebo and no difference to TCA. However, the quality of these trials is not always comparable to synthetic antidepressants with regard to sample size, dosage of reference compound, etc. [81]. The ongoing trials, specifically placebo-controlled continuation and maintenance trials, will answer these questions in the near future. A recent placebo-controlled study performed by Volz et al. [84] indicated that it might be worthwhile to further investigate hypericum extracts for somatoform disorder. Compared to synthetic antidepressants, hypericum extracts exhibit significantly fewer side effects and most likely this is one of the reasons for its high prescription rates [41].

Table 1 Available Antidepressants and Their Pharmacodynamic Properties

	NA	5-HT	DA	Ach	H
Modern antidepressants					
SSRI: selective serotonin reuptake inhibitors					
Citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline	−	+++	−	−	−
NaSSA: noradrenaline and serotonin-specific antidepressant	++	++	−	−	+
Mirtazapine					
SNRI: (selective) serotonin and noradrenaline reuptake inhibitors					
Milnacipran, venlafaxine, duloxetine	+++	+++	−	−	−
(Selective) noradrenaline reuptake inhibitors					
Reboxetine	+++	−	−	−	−
Serotonin-reuptake enhancer					
Tianeptin		??			
Serotonin $5HT_{1A}$-agonists					
Buspirone, Gepirone		++			
Dopamine reuptake inhibitor					
Bupropion			+++		
DSA: dual serotonin antidepressant					
Nefazodone	−	++	−	−	−
Older antidepressants					
Tricyclics:					
e.g., Amitriptyline, clomipramine, desipramine, imipramine	+++	++(+)	−	+++	+
Tetracyclics					
Maprotiline	+++	−	−	+++	++
Others:					
Mianserine	++	−	−	+	++
Trazodone	−	+	−	+	+
MAO inhibitors	+++	+++	++		
MAO-A inhibitors (reversible):					
Moclobemide	++	++	−	−	−

DA: dopamine; NA: noradrenaline, Ach: acetylcholine, 5HT: serotonin, H: histamine.

IV. NOVEL ANTIDEPRESSANTS

Efforts at developing new antidepressants are currently pursuing drugs with novel mechanisms of action. In particular, a number of strategies have focused on agents that block receptors for brain peptides. These approaches have been built upon theories that alterations in the activity of specific brain peptides could play a major role in stress responses or in the development of symptoms of anxiety or depression.

The most advanced of these pharmacological approaches is the use of substance P antagonists. Substance P is a neurokinin peptide with wide distribution in the brain [71]. It was identified in the early 1930s by von Euler and its amino acid sequence was identified some 40 years later [87]. Its potential role as a neurotransmitter in the brain was demonstrated in 1975. In the late 1980s, a neurokinin receptor (NK_1) that preferred substance P was cloned and early antagonists, which were large molecules and had less than optimal availability, were developed. Recently, a number of companies have developed relatively small molecules with better availability and these are under active clinical investigation for their potential efficacy in depression. One compound—MK-869—has been tested in major depression and has been reported to be effective [48].

Initially, substance P antagonists were tested as pain modulators, but studies were remarkable in their consistent lack of efficacy for this indication. The mapping of substance P in mammalian brain pointed to high concentrations in areas (e.g., the amygdala) thought to be involved in stress modulation, anxiety, or depression and this led investigators to explore the possible use of NK_1 antagonists in animal models of stress. Kramer and colleagues have described elegantly that MK-869—an NK_1 antagonist— can exert significant suppression effects on separation-induced vocalizations in an animal model of stress. In addition, NK_1 antagonists have been reported to have potent antiemetic effects.

MK-869 has been tested in a multicenter trial in which 300 mg/day of the agent were compared with paroxetine (20 mg/day) and placebo in 213 patients [48]. Both drugs separated from placebo in reducing total scores on the Hamilton Depression Rating Scale (HDRS) and the Hamilton Anxiety Rating Scale (HARS) and there was a suggestion—although not statistically significant—of possible superiority of MK-869 over paroxetine on anxiety measures at the conclusion of the study. MK-869 did not appear to produce more rapid effects.

The drug's side effect profile appeared relatively benign. Principal side effects appeared to be irritability, nausea, and fatigue. The drug appeared to produce significantly less sexual dysfunction than did the SSRIs.

Subsequently, the manufacturer of MK-869 has decided to suspend development of this particular compound in favor of much more potent second-generation antagonists that can be prescribed at lower doses. In addition, a number of pharmaceutical companies with small-molecule antagonists in their portfolios have also started investigation of their compounds in major depression. This approach is innovative and opens new avenues for treatment.

Recently, netamiftide, a novel pentapeptide, has been studied by Feighner et al. [27–29] and found to be effective in severe treatment-resistant depression. Interestingly, efficacy was achieved by five or ten doses which continued for the remaining month of the initial phase.

Another recent approach has been to develop antagonists for corticotropin-releasing hormone (CRH). This peptide, found in the hypothalamus and hippocampus, has been reported to play an initiating role in the hypothalamic–pituitary–adrenal (HPA) axis re-

sponse to stress. The peptide stimulates the pituitary to release adrenocorticotropin hormone (ACTH) which, in turn, stimulates the adrenal gland to secrete cortisol. Cortisol feeds back at the levels of the brain and pituitary to inhibit further stimulation of CRH and ACTH and ultimately to decrease its own release. Potential antagonism of the stress-induced increases in HPA axis activity underlie early attempts to develop CRH antagonists as antidepressants. However, more recent studies have concentrated on CRH outside the HPA axis (e.g., in the amygdala) which is not responsive to negative feedback by cortisol. CRH outside the HPA axis may play an important role in general stress responsivity and the pathogenesis of anxiety and depressive disorders. A glucocorticoid receptor antagonist is currently under investigation [66,75].

Early prototypes (e.g., a helical CRH) were potent antagonists but, again, their large structures led to poor bioavailability or permeability of the blood–brain barrier. Studies in rats required intracerebral administration. More recently, a number of antagonists have been developed and these are generally smaller and enjoy greater permeability into the brain. Several prototypes have passed phase I screening for safety and are now headed for early dose finding phase II efficacy studies. The CRH antagonist R121919 had been studied in an open clinical trial by Zobel et al. [91] and reported to be effective in a lower, compared to a higher, dosage. The authors compared the results of this open trial with a historical open trial with paroxetine and concluded similar efficacy. However, due to unforeseen side effects on the liver, development had been stopped.

A third approach has been to develop drugs that act as antagonists of the sigma receptor [79]. The sigma receptor was thought for many years to be linked to opioid systems, but over time data have emerged that indicate that this receptor has less to do with opioids than was originally thought. More recently, a number of studies have pointed to some sigma antagonists as exerting effects on other systems such as glutamate, GABA-B acetylcholine, and neuropeptides. Sigma antagonists may increase release of norepinephrine from presynaptic terminals and are effective in several animal models used to determine possible antidepressant properties. Still, the exact mechanisms of action vis-à-vis relief of depression are unclear.

Sigma antagonists were originally studied in schizophrenia, with relatively limited efficacy on positive symptoms. Recently, one such compound, igmesine, has begun to be

Table 2 Novel Treatment Approaches

Proposed mechanism	Substance	Clinical results
Substance P antagonists (NK_1 antagonist)	MK-869	Equal efficacy to paroxetine, better side effect profile
Corticotropin-releasing hormone (CRH) antagonists	—	Open trial exerts antidepressant efficacy. Development stopped due to side effects
Glucocorticoid-receptor antagonist	—	Not yet available
Sigma-receptor antagonist	Igmesine	Not yet available
Blockage of melatonin receptors	Agomelatin	Not yet available
Pentapeptide	Netamiftide	Open trial positive for severe treatment-resistant depression, even after 5–10 doses
$5HT_{2C}$ inverse agonist	Deramciclane	Positive studies in generalized anxiety disorders available

Table 3 Current and Possible Future Mood Stabilizers

Substance	Dosage mg/day[a]	Blood level
Lithium carbonate	400–800	0,6–0,8 mVal/L
Carbamazepine	400–1200	17–42 μmol/L
Oxcarbamazepine	600–1500	10–50 μg/mL
Valproic acid, Na-Valproate	750–1500	50–120 μg/mL
Lamotrigine	200–400	1–10 μg/mL
Topiramate	100–200	3–15 μmol/L
Atypical antipsychotic	TBD	—

[a]Initial dosage lower.
TBD: to be determined, likely as an add-on strategy to mood stabilizers.

studied in major depression. Results are not yet available. Still, sigma antagonists represent a novel approach to antidepressant therapy [79]. Recently, deramciclane, a specific-acting serotonergic antidepressant, has come under investigation [52,69]. The other novel compound studied in animal experiments is agomelatin [88].

V. EMERGING OPPORTUNITIES WITH MOOD STABILIZERS

Whereas for a long time only lithium was used for long-term treatment of bipolar (BP) disorder, now there are valproic acid, carbamazepine, and, as an add-on therapy, clonazepam available with mood stabilizing properties [23,33,77]. The side effect profile and toxicity of lithium are not the only reasons why further developments were necessary [3,34]. Based on the kindling hypothesis of Post et al. [74], it is thought that BP patients in a later stage of their illness respond more positively to anticonvulsants than to lithium. Within the group of mood stabilizers, there is a transatlantic gap (e.g., in the United States valproic acid and in Europe carbamazepine are predominantly used as monotherapy or as combination therapy with lithium). The U.S. approach is backed up by RCT [10], whereas open data obtained in Europe speak for carbamazepine. There is some indication that carbamazepine has a favorable profile in schizoaffective disorders [82], whereas valproic acid is beneficial specifically in rapid cycling [17]. Both compounds have limiting side effects, such as weight gain or changes in plasma levels of concomitant medication (carbamazepine lowers and valproic acid increases levels of concomitant medication).

Further candidates for treatment of bipolar disorder under current investigation are lamotrigine [15,90] and topiramate [56,70]. So far, topiramate is promising because it is not burdened with the side effect of weight gain. Lamotrigine recently demonstrated efficacy in the treatment of acute depression within BP [14]. The studies for gabapentin, however, did not show a significant mood-stabilizing property. It emerged that this compound is more helpful for the anxiety component of the BP spectrum. Although oxcarbamazepin is significantly less burdened with enzyme-inducing properties than carbamazepine, there are no studies in BP disorder available in the literature. Our clinical experience, however, indicates that oxcarbamazepine is as effective as carbamazepine.

The role of atypical antipsychotics for treatment of not only the acute phase of mania but also mood-stabilizing properties is now under current investigation [15,77].

VI. CONCLUSION

The introduction of TCAs in the 1950s was the first revolution of antidepressant therapy followed by the SSRIs in the late 1980s. The relative lack of side effects of the SSRIs and their efficacy in disorders other than major depression (including anxiety disorders and eating disorders) made them widely prescribed and accepted medications [42]. The SSRIs fostered a mechanism-based approach to antidepressant research that will hopefully lead to antidepressants with more distinct mechanisms of action (e.g., substance P antagonists) [48]. More effective and better tolerated antidepressant medications improve patient's quality of life, foster greater acceptance of the disease in our society, and ultimately provide wider benefits for patients [19]. This is important because depression ranks highest among the identified diseases in the loss of daily adjusted life years (DALYs), a measure that indicates the loss of an individual's productivity due to the disease from which they suffer [67].

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The Depressed Patient: From Nonresponse to Complete Remission

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I. INTRODUCTION

Major depressive disorder is a highly prevalent and disabling psychiatric disorder. Data from the National Comorbidity Survey showed a 4.9% prevalence of current major depression and a 17.1% prevalence of lifetime major depression [l]. When compared with chronic medical illnesses, depression results in equal or exceeding decrements in functioning and well-being [2]. Major depression not only afflicts the individual but also has an important societal impact. Due to its direct and indirect costs, major depression places a heavy burden on the community. Different therapies of major depressive disorder have been developed in an attempt to reduce this impact on a person's life and society. The effectiveness of antidepressant treatment is said to be very high and, according to the Agency for Health Care Policy and Research guidelines, depression can almost always be treated successfully with medication, psychotherapy, or a combination of both [3,4]. This therapeutic optimism is somewhat conflicting with everyday clinical practice where major depression often does not resolve completely and recurs frequently. One of the main reasons for this discrepancy may be found in the different definitions of "successful treatment." Indeed, treatment outcome is not a uniform concept and different definitions are applied according to the parties doing the defining (e.g., pharmaceutical industry, society, and health-care providers, physicians, and patients). This chapter will take a closer look at treatment outcome in major depression and will attempt to disentangle the many overlapping and often vaguely defined descriptions of (un)successful treatment for depression.

II. OUTCOME DEFINITIONS IN MAJOR DEPRESSION

Many definitions exist and are used inconsistently (e.g., nonresponse, partial response, response, partial remission, remission with residual symptoms, asymptomatic remission, and recovery). These inconsistencies make research findings difficult to interpret and hinder comparisons across studies. Frank et al. [5] proposed an internally consistent, empirically defined conceptual scheme for the terms episode, response and partial remission, full remission, recovery, relapse, and recurrence (Table 1). It was stated that such a coherent scheme "would aid in the design, conduct and analysis of investigations aimed at understanding cause and pathogenesis, as well as those focused on the evaluation of short- and long-term effects of treatment." Besides the conceptual scheme, Frank et al. also put forward some operational criteria based on the Schedule for Affective Disorders and Schizophrenia [6], the 17-item Hamilton Rating Scale for Depression (HDRS) [7], or the 21-item Beck Depression Inventory (BDI) [8] (Table 2). A remarkable finding in these definitions is that different duration criteria were suggested for the different measures (e.g., an episode defined by the 17-item HDRS implies a HDRS score >15 for longer than 2 weeks, while an episode defined by the 21-item BDI requires a BDI score >15 for longer than 4 weeks). This might also lead to different duration criteria in the definition of episode, full remission, and recovery.

However, besides the categorical outcome criteria proposed by Frank et al. [5], many continuous outcome definitions of depressive disorder are currently employed: endpoint severity (absolute score on a chosen depression rating scale), depression improvement (absolute change in score from baseline to endpoint), and percent improvement (percent change from baseline to endpoint). Some discrepancies found in the literature covering predictors of outcome can be attributed to whether a categorical or continuous outcome definition was used as illustrated by Tedlow et al. [9]. In the study by Tedlow et al., 248 patients with DSM-IIIR diagnosis of major depression were treated with fluoxetine 20

Table 1 Definitions of Terms Designating Change Points in Major Depressive Disorder

An episode is a period, lasting longer than D days, during which the patient is consistently within the fully symptomatic range on a sufficient number of symptoms to meet syndromal criteria.
A response is the point at which a partial remission begins.
A partial remission is a period during which an improvement of sufficient magnitude is observed that the individual is no longer fully symptomatic (i.e., no longer meets syndromal criteria for the disorder) but continues to evidence more than minimal symptoms. Treatment is not a requirement of the definition; partial remission can be spontaneous.
A full remission is a relatively brief ($>$E days but $<$F days) period during which an improvement of sufficient magnitude is observed that the individual is asymptomatic (i.e., no longer meets syndromal criteria for the disorder and has no more than minimal symptoms). Treatment is not a requirement of the definition; partial remission can be spontaneous.
A remission that lasts for F days or longer is a recovery. Recovery can be spontaneous and can last for an indefinite period.
A relapse is a return of symptoms satisfying the full syndrome criteria for an episode that occurs during the period of (partial) remission, but before recovery as defined above.
A recurrence is the appearance of a new episode of major depressive disorder and, thus, can occur only during a recovery.

Source: Ref. 5.

Table 2 Operational Criteria for Terms Designating Change Points in Major Depressive Disorder

Schedule for affective disorders and schizophrenia	
Clinical ranges	
Asymptomatic	2 symptoms present
Fully symptomatic	5 symptoms present
Durations	
Episode	4-week symptomatic
Full remission	2-week to <8-week asymptomatic
Recovery	8-week asymptomatic
17-item Hamilton rating scale for depression	
Clinical ranges	
Asymptomatic	score of 7
Fully symptomatic	score of 15
Durations	
Episode	2-week fully symptomatic
Full remission	2-week to <6-month asymptomatic
Recovery	6-month asymptomatic
21-item Beck depression inventory	
Clinical ranges	
Asymptomatic	score of 8
Fully symptomatic	score of 15
Durations	
Episode	4-week fully symptomatic
Full remission	3-week to <4-month asymptomatic
Recovery	4-month asymptomatic

Source: Ref. 5.

mg/day for 8 weeks. Patients were evaluated both pre- and post-treatment with the 17-item HRDS, the Clinical Global Impression Scales for Severity (CGI-S) and Improvement (CGI-I) [10]. The authors investigated the relationship between baseline severity of depression and anxiety, and different definitions of treatment outcome. Both continuous and categorical outcome definitions were used. Continuous outcome definitions were endpoint severity (endpoint HDRS score), depression improvement (absolute change in HDRS from baseline to endpoint), global improvement (CGI-I score at endpoint), and percent depression improvement (percent change in HDRS score from baselin!e to endpoint). Categorical definitions were HDRS score >10, 50% reduction in HDRS; HDRS score >10 and 50% reduction in HDRS; HDRS score >7, 75% reduction in HDRS; HDRS score >7, 75% reduction in HDRS CGI-S = 1. The absolute change in depression score (depression improvement) showed no relationship with baseline severity of depression or anxiety. However, when using percent change in the HDRS score as an outcome variable, treatment proved to be modestly better for subjects with lesser baseline anxiety or depression. This

relationship became more robust when categorical definitions of outcome were applied, with lesser baseline symptoms predicting a better response.

III. RESPONSE

In the definition of Frank et al. [5], a response is thought of as the point at which partial remission begins. Unlike other outcome definitions like remission and recovery, response requires treatment. It is often applied in clinical trials to evaluate efficacy of an antidepressant in the early phase of treatment. The most commonly used criterion of response is a 50% reduction or more from a baseline score on a depression rating scale like the HDRS, the BDI, or Montgomery and Åsberg Depression Rating Scale [11]. These different scales show a different item coverage and a different perspective of observer (self-rating versus observer-rating) and so may yield different rates of response when used in a common study sample. Epidemiological data and clinical trials have shown that the standard of a 50% improvement fails to reflect the reality of depression outcome and does not cover outcomes like partial remission and residual symptoms. Indeed, a 50% reduction in the HDRS score of a severely depressed patient may classify him as a responder but still leaves him with prominent depressive symptoms.

Different definitions or subclassifications of response may concern the magnitude and the pattern of improvement (timing and course), and were recently summarized by Trivedi and Baker [12]: response, minimal response, nonresponse, early and late response, sustained response (Table 3). These latter outcome definitions (minimal response, early and late response, sustained response) are important in determining the onset of action of antidepressants and may differentiate between true drug response and placebo response.

For many years, investigators have tried to measure the speed of onset of antidepressant drugs. In timing this onset of action, response must be an early, significant reduction in depressive symptoms and must be linked to a subsequent, clinically significant treatment outcome (i.e., a sustained response) [13]. Different definitions for early response (e.g., an absolute change or percent improvement in HDRS score) are used and may affect the results of studies, with stricter response criteria resulting in a later onset of action [14–18] (Table 4). In an elegantly designed study by Nierenberg et al. [16], the time until onset of antidepressant response was assessed in 182 outpatients with DSM-IIIR major depression who had a sustained response to fluoxetine (8 weeks of treatment with 20 mg/day of fluoxetine). Onset of action was defined as a 30% decrease in score on the 17-

Table 3 Classification of Response According to Symptom Improvement and Timing of Improvement

Response: 50% improvement in symptom severity from baseline on the HDRS.
Minimal response: a 25 to 49% improvement in symptom severity from baseline on the HDRS.
Nonresponse: <25% reduction improvement in symptom severity from baseline on the HDRS
Early response: 50% improvement in symptom severity from baseline on the HDRS, before week 4.
Late response: 50% improvement in symptom severity from baseline on the HDRS, after week 4.
Sustained response: two consecutive weeks of improvement in symptoms that meet the response criterion.

Table 4 Different Criteria for Onset of Action

20% reduction in 17-item HDRS score.	Ref. 15
30% reduction in 17-item HDRS score.	Ref. 16
Decrease of 4 points in the MADRS score.	Ref. 17
CGI score of either 2 (much improved) or 1 (very much improved)	Ref. 18

item HDRS, which persisted and led to a 50% decrease by week 8. The authors found that at weeks 2, 4, and 6, the probabilities of having on onset of action were 56%, 25%, and 9%, respectively. The mean time to onset of action was 3.8 weeks. When response was defined as a 50% decrease in HDRS score, the probabilities of having a response at weeks 2, 4, and 6 became 18%, 37%, and 26%. The mean time to response became 4.9 weeks.

However, besides the magnitude and timing of response, research has also focused on the temporal pattern of this response. Quitkin et al. [18] developed a method of describing the longitudinal pattern of response to antidepressants. Patterns of response were based on weekly ratings of clinical status (CGI-I). Improvement was defined as a CGI score of either 2 (much improved) or 1 (very much improved). Delayed improvement was judged as being first observed after 2 weeks of treatment. Improvement was said to be persistent if it was not followed by a relapse in any succeeding week. In a sample of 150 nonmelancholic depressed patients (treated in a double-blinded study during 6 weeks with phenelzine, imipramine, or placebo), true drug effect was characterized by a 2-week delay of onset and persistence of improvement. These findings were further elaborated by Stewart et al. [19] using pattern analysis to predict differential relapse of remitted patients with major depression during 1 year of treatment with fluoxetine or placebo. The efficacy of fluoxetine during the continuation phase (the period from the end of the short-term treatment until 6 months) and maintenance phase (beyond 6 months) seemed to be limited to patients with an initial true drug response (delayed and persistent improvement).

IV. PARTIAL REMISSION

Partial remission is defined as a period during which an improvement of sufficient magnitude is observed that the individual is no longer fully symptomatic (i.e., no longer meets syndromal criteria for the disorder), but continues to evidence more than minimal symptoms. Operational criteria are a 17-item HDRS score ranging from 8 to 14, or a 21-item BDI ranging from 9 to 14 [5]. About 50 to 70% of patients in clinical trials will respond ($>$50% improvement) to antidepressants and only 25 to 35% will experience full remission [20]. Thus, many patients will only achieve partial remission (i.e., the patient is no longer fully symptomatic but continues to evidence residual symptoms).

Studies by Paykel et al. [21] and Van Londen et al. [22] have highlighted some remarkable findings regarding the prevalence, nature, and consequences of the residual symptoms in partial remission. First, residual symptoms in partially remitted patients are highly prevalent. Second, they resemble the typical depressive symptomatology. Third, residual symptoms in partial remission are associated with a higher risk of relapse.

In a study by Paykel et al. [21], patients with primary unipolar major depression (Research Diagnostic Criteria) were followed every 3 months to remission and thereafter. Remission was defined as no longer meeting the inclusion criterion of major depression

Table 5 HDRS Distribution in Patients No Longer Reaching Criteria for Major Depression

17-item HDRS score	%
0–4	47
5–7	22
8–10	7
11–12	12
13–15	5
16–18	8

Source: Ref. 21.

during two consecutive months. In the remitted patients, residual symptoms were regarded as present when the 17-item HDRS score was >8. Residual symptoms were found in 32% of the remitted sample; the HDRS score ranged from 8 to 18 (Table 5). The symptoms present were those typical of depression: at least 47% of subjects showed ratings at the level of moderate or more on the items of depressed mood, work, and interests, psychic anxiety, and genital symptoms. Mean scores on all individual items of the HDRS could differentiate significantly between subjects with or without residual symptoms, except for some items typical of severe depression (late insomnia, retardation, agitation, hypochondriasis, weight loss, and loss of insight). Residual symptoms were predictive of relapse. Overall, 76% of subjects with residual symptoms relapsed over follow-up, as opposed to 25% of those without residual symptoms. Analogous results were obtained by Van Londen et al. (22) in a follow-up study of patients with DSM-III-R major depression. After 9 months, 49% of patients had reached full remission and 45% were in partial remission. Full remission was defined as at least two consecutive months with symptoms below the threshold for depression and without residual symptoms. The absence off residual symptoms was conceptualized by a score of <2 per symptom on the MADRS. Partial remission was defined as at least two consecutive months, with symptoms below the threshold for depression but with residual symptoms. For subjects with partial remission, the sum score had to be 10 on the MADRS, and only one symptom was allowed to have a score equal to 3; any further symptom score had to be <3. Subjects with only partial remission were found to have a significantly greater risk of relapse in the subsequent 12 months than patients in full remission. Patients with partial remission relapsed particularly in the first 4 months, while fully remitted patients had recurrence for the most part after 12 months of remission.

V. FULL REMISSION AND RECOVERY

Full remission is a relatively brief period during which an improvement of sufficient magnitude is observed that the individual is asymptomatic (i.e., no longer meets the syndromal criteria for the disorder and has no more than minimal symptoms). When this asymptomatic state is maintained during a longer period, full remission becomes recovery [5]. The definition of remission and recovery implies a common criterion of severity (asymptomatic or no more than minimal symptoms) and a different criterion of duration. Being asymp-

tomatic is often translated into a 17-item HDRS >7 or a BDI >8. More controversy exists about the different criteria of duration. In the original proposition by Frank et al. [5], full remission was defined as a period >2 weeks and <6 months and recovery >6 months. However, in the subsequently mentioned research articles other duration criteria are used or are not specified at all.

Full remission is considered to be the gold standard in antidepressant therapy. However, having a closer look at these fully remitted patients may reveal that they are not as asymptomatic as they are supposed to be. Residual symptoms seem to persist, not only in the depressive realm but also in the area of social and cognitive functioning.

In a study by Nierenberg et al. [23] 215 outpatients with DSM-III-R major depression were treated with a fixed dose of fluoxetine (20 mg/day) for 8 weeks. Of these 215 patients, 108 (50%) reached full response (17-item HDRS score >7). In these full responders, the authors then assessed the presence of subthreshold or threshold depressive symptoms, according to the Structured Clinical Interview for DSM-III-R, Patient Edition (SCID-P). Only a minority (18%) of those with a final 17-item HDRS score >7 were found to be totally free of SCID-P major depressive symptoms, while the majority were not as asympomatic as they were thought to be (Table 6). The three most common symptoms were sleep disturbances, fatigue, and diminished interest or pleasure. Fava et al. [24] looked for residual symptoms in 49 outpatients with primary major depressive disorder (Research Diagnostic Criteria), who were successfully treated with antidepressant drugs and had reached full remission [5]. Residual symptoms were assessed with a modified version of Paykel's Clinical Interview for Depression [25]. Only 6 (12%) of the 49 patients screened did not present with residual symptoms. The most frequently reported symptoms were generalized anxiety, somatic anxiety, and irritability.

The importance of residual depressive symptomatology in fully remitted/recovered patients is pointed out by a prospective study by Judd et al. [26] in which patients with unipolar major depressive disorder were followed naturalistically for 10 years or longer. Recovered patients were divided into two groups: asymptomatic recovery and recovery with subthreshold depressive symptoms (SSD). Residual SSD compared to asymptomatic recovery patients relapsed >3 times faster to their next major depressive episode (me-

Table 6 Frequency of Threshold and Subthreshold Depressive Symtoms (as defined in the SCID-P) in Fully Remitted Patients

Number of symptoms	%
1	18
2	26
3	23
4	19
5	10
6	2
7	3

Source: Ref. 23.

dian = 68 vs. 231 weeks). The risk for early relapse associated with residual SSD recovery was so strong that it appeared to override the effect of the history of prior major depressive episodes.

However, residual symptoms in fully remitted patients extend beyond the core depressive symptomatology. Research has gathered substantial evidence for long-lasting changes in social functioning and cognition, even after succesful treatment (i.e., full remission) of major depressive disorder. Some of these research findings will be discussed further.

As early as 1973, Paykel and Weissman [27] demonstrated the persistence of impaired social functioning in women after symptomatic improvement. Although improvements in social functioning occurred over an 8-month follow-up period, residual impairment remained. In the study of Mintz et al. [28], analogous conclusions were made. By means of a comprehensive review of the scientific literature, the authors investigated the effects of antidepressants and psychotherapy on work impairment in depressed patients. The trajectory of outcome for work appeared to be slower than that observed for symptoms. In fully remitted patients, work outcome became better and better as the duration of treatment increased, reaching a maximum at about 4 to 6 months. More recently, Furukawa et al. [29] studied the time course of symptomatic change and social functioning in patients recovering from major depression. The study included 95 patients with DSM-IV unipolar major depressive disorder, who had received no antidepressant or antipsychotic medication in the preceding 3 months. Symptomatic change was measured with the 17-item HDRS, while social functioning was assessed by the Global Assessment Scale (GAS) [30] and the Social Adjustment Scale-Self Report (SAS-SR) [31]. A patient was considered as remitted when he/she scored >7 on the 17-item HDRS, and recovered when he/she scored >7 for two consecutive months or more. Within 2 years of follow-up, 74 (78%) reached remission and 53 (56%) reached recovery. Twenty (21%) of the recovered patients demonstrated sustained recovery over 6 months. In remitted patients, GAS ratings, although greatly improved from baseline, still were not in the normal range. GAS ratings only reached the normal range in recovered patients and still improved if recovery was sustained. Similar trends were observed for the SAS-SR.

Besides impaired social functioning, other aspects of depression, such as dysfunctional attitudes and depressive cognitions, may persist after the resolution of depressive symptomatology. An example of these cognitive residual symptoms was given by Power et al. [32]. Using a subscale version of the Dysfunctional Attitude Scale [33] in fully remitted depressed patients (BDI >6), the authors found a dependency subscale score that remained elevated. In a study by Fava et al. [34], the relationship between dysfunctional attitudes and depression severity was examined during 8 weeks of treatment with fluoxetine in 115 outpatients with DSM-III-R major depressive disorder. Dysfunctional attitudes were assessed by the Dysfunctional Attitude Scale (DAS) [35] and the Cognitions Questionnaire (CQ) [36]. Depression severity was measured by the 17-item HDRS and the 21-item BDI. The DAS measures cognitive distortions, fixed negative values, and perfectionistic attitudes, while the CQ is directed toward negative attitudes and perceived uncontrollability as a response to hypothetical situations. Treatment with fluoxetine was associated with a significant decrease in dysfunctional attitudes as measured by the DAS and the CQ, linearly associated with the decrease in depression severity. However, the mean DAS and CQ scores after treatment did not approach normal values. The importance of residual cognitive components is perhaps best shown indirectly by the positive effect

of cognitive behavioral therapy in the prevention of recurrent depression as evidenced in the studies by Fava et al. [37] and Paykel et al. [38].

VI. CATEGORICAL OR CONTINUOUS DEFINITIONS OF OUTCOME VERSUS THE FLUCTUATING TIME COURSE OF THE DEPRESSIVE ILLNESS

The aforementioned outcome definitions are subject to temporal changes. During an extended period of time, a depressed patient will move from one criterion to another, in both directions. This dynamic course of symptoms was convincingly proved by Judd et al. [39] in a prospective 12-year study of subsyndromal and syndromal depressive symptoms in unipolar major depressive disorder. The authors conducted weekly analyses of depressive symptom severity in a large cohort of major depressed patients evaluated prospectively for up to 12 years. Depressive symptoms were divided into four levels of severity: (1) depressive symptoms at the threshold for major depression (MDD); (2) depressive symptoms at the threshold for minor depression or dysthymic disorder (MinD); (3) subsyndromal or subthreshold depressive symptoms (SSDs); and (4) no depressive symptoms. During follow-up, patients were symptomatically ill 59% of the time (Table 7). The vast majority of patients spent their follow-up weeks at 3 or 4 different levels of depressive symptom severity, and they changed symptom levels almost twice a year. Depressive symptoms at the level of SSDs, MinD, and MDD were associated with a stepwise increment in psychosocial disability [40].

VII. SUMMARY

About 50 to 70% of patients in clinical trials will respond (>50% improvement) to antidepressants, but only 25 to 35% will experience full remission. About 80 to 90% of fully remitted patients will experience some further core depressive symptomatology. Residual symptoms found in fully and partially remitted patients are an important risk factor for subsequent relapse or recurrence.

Treatment outcome is not a unitary concept and covers different domains of pathology (e.g., core depressive symptomatology, social, and cognitive dysfunctioning). Although these different dimensions of pathology are well correlated, all showing a gradual decrement in the course of recovery from major depression, social and cognitive functioning seem to exhibit a more protracted trajectory. A consequence of this may be that different definitions of outcome should be applied according to the phase in the process of

Table 7 Percentage of Weeks Spent at 4 Different Levels of Depressive Symptom Severity in Patient with MDD During a 12-Year Follow-Up

No depressive symptoms	42%
Subsyndromal depressive symptoms	17%
Depressive symptoms at the MinD level	27%
Depressive symptoms at the MDD level	15%

Source: Ref. 39.

recovery (e.g., outcome criteria based on depressive symptomatology from the early stage of treatment to full remission, followed by measurements of social and cognitive functioning). This differential assessment of treatment outcome may then be linked to differential therapeutic interventions—the so-called concept of staging and sequential therapy [41,42]. A failure to attain fully symptomatic remission in the early phase of treatment should invite the modification of the antidepressant treatment (augmentation, combination, change) while the persistence of cognitive/social dysfunctioning after symptomatic resolution could be targeted by cognitive behavioral psychotherapy or interpersonal psychotherapy.

An often neglected part of the outcome definitions in major depressive disorder is the time criterion. In many research studies, outcome criteria like response, remission, and residual symptoms are used without specifying the specific time point in the course of treatment, and are thereby difficult to interpret. However, in clinical practice, outcome must be measured at specific time points (critical decision points), after which the treatment strategy should be maintained or changed.

Recovery from major depression is a complex phenomenon, as reflected in the many obscure definitions designed to describe its course. This obscurity involves both the content and the time point of definitions and may hamper current and future depression research. Although stated by Frank et al. [5] two decades ago, there is still a need for a coherent scheme, that "would aid in the design, conduct, and analysis of investigations aimed at understanding cause and pathogenesis, as well as those focused on the evaluation of short- and long-term effects of treatment."

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Pharmacogenetics of Mood Disorders: Is There a Future?

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I. INTRODUCTION

Pharmacogenetics is the study of genetically based, inter-individual variability in response to drugs and susceptibility to drug-induced adverse effects. Almost a century ago, it was recognized that differences among individuals in their response to ingested substances might have a genetic basis [1,2]. In the 1950s, the role of genetic susceptibility in adverse drug reactions was described by Motulsky [3]. Vogel [4] introduced the term pharmacogenetics. The major role of genetic polymorphism of the cytochrome P450 system in differences in drug metabolism was first described in the 1970s [5] and has been extensively studied since then.

Since pharmacogenetics has been around for some time, why has it only now become the focus of the great interest that has been expressed in a number of influential papers in major scientific journals (e.g., Ref. 6)? The answer to this question lies in the recent delivery of the draft sequence of the human genome, culminating more than a decade of intensive government-sponsored and privately funded research. In the current, so-called "post genomic" era, the next major task is to define and catalogue the totality of genetic variation within the human genome sequence. This variation, it is anticipated, will serve as the major tool in mapping genes for complex traits [7,8]. Prominent among such traits are most of the major psychiatric disorders and also pharmacogenetic characteristics that

have a polygenic basis. A number of government-sponsored, private, and academic consortia are devoting enormous resources to identifying the most common of the genetic variants, single nucleotide polymorphisms (SNPs). These are variations in a single base pair of DNA sequence and they are estimated to occur every 1000 bases. The information that is being gathered is deposited in publicly accessible databases. Since pharmacogenetics seeks to correlate variation in gene structure (genotype) with variations in response to drugs and susceptibility to adverse effects, this is an invaluable resource. The ultimate, end product of these efforts are diagnostic tests available to the clinician that will be used to guide the choice of treatment. Pharmacogenetic information will also be of immense value in streamlining clinical trials of new drugs by stratifying patients according to genetic predictors of response and adverse effects. This will greatly reduce the sample sizes needed and thereby the time lag and expense of bringing new therapeutic agents to the clinic. Although the chip technology needed for tests of this type is well advanced, it is still prohibitively expensive. However, by the time large-scale pharmacogenetic testing is practically feasible, this obstacle will be overcome.

An important field allied to pharmacogenetics is pharmacogenomics. While the starting point of pharmacogenetics is patient-oriented research, the human genome sequence is the starting point of pharmacogenomics. Putative drug targets derived from the human genome sequence (to a great extent "in silica," i.e., at the computer level) are screened for their potential in developing therapeutic agents. This process differs markedly from conventional approaches in which compounds are screened for their potential to act on known targets. Targets can also be identified by means of a variety of techniques that are used to screen relevant tissues for messenger RNAs that are expressed either in relation to the target disease or as a consequence of drug effects. The emerging field of proteomics is an important element of these efforts. While pharmacogenetics and pharmacogenomics are closely related and the terms are often used interchangeably, the different starting points and agendas of the two fields suggest that it is useful to maintain the distinction.

What promise does pharmacogenetics hold for the treatment of mood disorders and what are the problems and pitfalls on the way to its realization? These issues will be considered in this chapter. Initially, we will review basic concepts in the pharmacogenetics of mood disorders and outline the clinical and genetic rationale. Then, we will review the role of genetic influences on the pharmacokinetics of antidepressant drugs. We will next consider pharmacodynamic factors on the background of data on the role of serotonin transporter gene variation in predicting antidepressant response. The section thereafter will deal with studies on the pharmacogenetics of mood-stabilizing drugs, particularly lithium. Finally, we will consider pivotal issues in the design and execution of research studies in the pharmacogenetics of mood disorders, primarily from the perspective of clinical research. For a more extensive discussion of the role of pharmacogenetics in psychopharmacology, the reader is referred to a forthcoming volume devoted to this topic [9].

II. RATIONALE FOR THE PHARMACOGENETICS OF MOOD DISORDERS

A. Clinical Rationale

From their experience in the clinic and in the context of clinical trials, psychiatrists are fully aware of the very substantial variability in the response of patients with the same

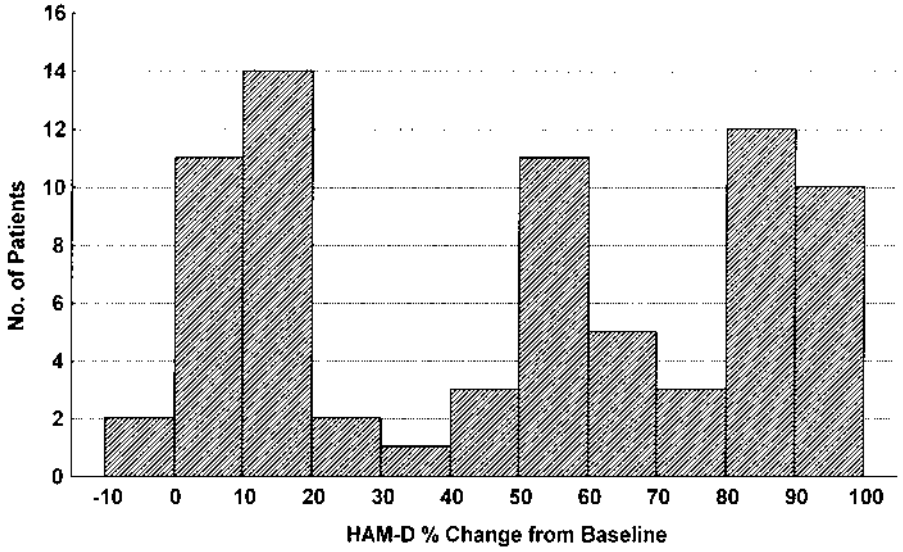

Figure 1 Distribution of 21-item Hamilton Depression Scale (HAM-D) change scores (percent change from baseline) in patients with unipolar, nonpsychotic major depression treated with SSRI 20 mg for 4 weeks (Agid and Lerer, unpublished data).

diagnosis to the same drug. Figure 1 shows data from a prospective, algorithm-based study conducted in our clinic (Agid and Lerer, unpublished data). In this study, 90 patients with unipolar, nonpsychotic major depression (DSM-IV) who had not received antidepressant medication in the current episode of illness were treated according to a standard treatment algorithm. The initial step of the algorithm was treatment with a specific serotonin reuptake inhibitor (SSRI)—fluoxetine in most cases—at a dose of 20 mg/day for 4 weeks. Subsequent steps are not relevant to the present discussion. Among the 74 patients who completed the full 4 weeks of SSRI, 20 mg/day (last observation carried forward data were similar but are not presented here), 44 (59.5%) were responders, as defined by a Clinical Global Impression (CGI) rating of at least "much improved" at week 4. This rate of response is similar to that observed in clinical trials of fluoxetine and paroxetine. The striking point made by Figure 1 is the tremendous variability of response.

We analyzed the demographic and clinical data of the sample in order to identify possible predictors of response. Nonresponders to fluoxetine had less schooling ($p = 0.03$), fewer adverse life events ($p = 0.01$), were more likely to have been previously hospitalized ($p = 0.04$), had higher HAM-D scores at baseline ($p = 0.0003$), and rated themselves as more depressed on a 10-cm visual analog scale ($p = 0.0002$). HAM-D items that significantly differentiated responders from nonresponders were late insomnia ($p = 0.008$), psychic anxiety ($p = 0.0007$), somatic symptoms and appetite ($p = 0.04$), and diurnal variation ($p = 0.04$).

A number of the items that differentiated responders and nonresponders are consistent with previous reports. Overall they seem to point to a more severe clinical picture in the nonresponders that is less situational. While interesting, these findings as well as others in the literature cannot serve as a basis for reliable, a priori discrimination of potential

responders and nonresponders to SSRI treatment. It is this niche that pharmacogenetics seeks to fill.

B. Genetic Rationale

A putative role for pharmacogenetics in predicting response to antidepressant and other mood-stabilizing drugs requires consideration of the role of genetic factors in such effects. Are there sufficient data upon which to base such an assumption? In fact, there are few clinical studies that explore a role for genetic factors in response to antidepressant drugs. Pare et al. [10] examined the records of 170 patients who had been treated with antidepressant drugs [imipramine or monoamine oxidase inhibitors (MAOI)], mostly in the context of controlled trials. Seven cases were identified in which a first-degree relative had also been treated with a drug from one of these two classes; in some cases there were more than one first-degree relative, giving 12 pairs in all. Treatment effects were concordant in all proband-relative pairs (response in 5 and nonresponse in 7). The authors also observed a tendency for patients to respond (or not to respond) to a drug of the same class on repeated administrations (13 of 17 patients consistent for MAOI and 7 of 9 consistent for imipramine), although it was not always certain that different episodes of illness were being considered in each case. Subsequently, Pare [11] reported even higher levels of consistency of response within the same patient for MAO inhibitors (two different drugs in this class): 9 of 16 concordant for response and 7 of 16 concordant for nonresponse; none discordant for response. For two different drugs of the tricyclic antidepressant (TCA) group, there was only one discordant response out of 12 cases. Conversely, of 65 cases in which the same patient was administered a TCA and MAOI, there were only 35 concordant responses out of 65 trials in the same patient. Similar findings were reported by Dally and Rhode [12]. Pare and Mack [11] reanalyzed familial response data from the Pare et al. [10] study and showed that concordance of response to antidepressants between relative pairs was in the context of an antidepressant from the *same* group (MAOI or TCA) and not from different groups. In a new series of patients, Pare and Mack [11] again observed concordance of response to antidepressants among first-degree relative pairs (10 out of 12 pairs), which was again most evident for drugs of the same class. Concordance of response to imipramine among relative pairs was also observed by Angst [13,14] in patients with "endogenous depression." Another report of interest is that of O'Reilly et al. [15], who observed that 4 of 8 members of a single-, two-generation family who met DSM-IIIR criteria for major depression, did not respond to TCA treatment but did respond to the MAOI, tranylcypromine. In the absence of family and twin studies, which are the classic methods for demonstrating the genetic basis of a complex trait (such studies are very difficult to conduct in a pharmacogenetic context), the above findings are of considerable interest and suggest that genetic factors may indeed play a role in response to antidepressants. Data on genetic factors in response to lithium are discussed later in this chapter.

Differences between individuals in their response to drugs can be attributed to two major sets of factors. Pharmacokinetic factors encompass the processes that influence bioavailability of a drug (i.e., the concentration of the drug that is available at its site of action). Pharmacodynamic factors are differences in the targets upon which the drug acts. A simple view is that pharmacokinetics refers to the effect of the individual upon the drug and pharmacodynamics to the effect of the drug on the individual [16]. Both sets of factors are under a variable degree of genetic control. Both influence the response of the individual to a given drug and may interact within the same individual and with the environment

[17]. The aim of pharmacogenetics is to identify these factors, establish their genetic basis, and incorporate the information in diagnostic procedures that will enhance clinical practice by a more effective match between patient and drug.

Classical genetics of human disease deals with monogenic disorders in which a single mutation in a single gene is causatively related to the phenotype. The genetics of complex illnesses such as diabetes, hypertension, coronary artery disease, and most of the major psychiatric disorders do not fit this simple paradigm. The same distinction is applicable to pharmacogenetics. Genetic factors that underlie pharmacokinetic effects and influence drug bioavailability (such as the effect of CYP2D6 polymorphism on the metabolism of a variety of psychotropic and other drugs, which is inherited as an autosomal recessive trait) may be monogenic [17]. For the most part, however, pharmacogenetic traits are likely to be polygenic and multifactorial. A polygenic trait is one that is influenced by a number of different genes, each of which contributes a portion of the effect and may do so additively as well as interactively (epistasis). The term multifactorial indicates that both genetic and environmental factors contribute substantially and variably to the phenotype.

Traditionally, pharmacogenetic studies seek an association between a specific gene and the response or adverse effect phenotype under study. This is a classic candidate gene approach that is based on an a priori hypothesis regarding the role of the protein coded by the gene in the particular phenotype. The genetic variation that is most frequently studied in pharmacogenetic studies is the single nucleotide polymorphisms [18]. A polymorphism is a genetic variation that occurs with a frequency of 1% or more in the population. SNPs are differences between individuals in a single base of the genomic sequence. SNPs occur throughout the human genome at a density of approximately 1 per 1000 bases of DNA. SNPs may be classified by their location, occurring in exons (coding regions) or introns (noncoding regions) of genes and in regulatory regions such as the promoter. SNPs in coding regions need not necessarily influence the structure of the protein and are termed synonymous. SNPs that do alter the structure of the proteins need not have functional consequences and are termed conservative or nonfunctional. On the other hand, SNPs located in intronic regions can have an impact on the coded protein by influencing splicing [19]. SNPs in regulatory regions can have major effects on the expression of the gene. Major efforts are now in progress by public and industry-based consortia to generate SNP databases that will be an invaluable resource for pharmacogenetics in the very near future. It should be noted that an SNP or other genetic variation might be statistically associated with a phenotype without having a direct effect. This phenomenon is due to linkage disequilibrium (LD) and it results from the fact that the variant examined is close enough to the true predisposing variant that it does not undergo recombination during meiosis and is inherited with it. The marker may be at a different site in the gene itself or may be located outside the gene.

III. GENETIC VARIATION IN DRUG-METABOLIZING ENZYMES

The dose of a drug that is ingested by a patient is only indirectly related to its concentration at the effector site(s) where the drug acts (bioavailability). Numerous other factors determine this concentration. They include the absorption, transport, metabolism, and elimination of the drug and its metabolites. Age, gender, and disease may all influence the efficiency of each step of the process and thus the bioavailability of the drug.

Drug metabolism is generally divided into two stages termed phase I and phase II reactions. Phase I reactions include oxidation, reduction, and hydrolysis, introducing a

polar group into the molecule. In phase II, an endogenous hydrophilic substance is conjugated with a polar group in the molecule generating water-soluble compounds. Oxidation is an important mechanism in phase I and it is primarily catalyzed by the cytochrome P450 enzymes that are principally located in the liver endoplasmic reticulum. Cytochrome P450 (CYP) enzymes are subdivided into families designated by a number (CYP2), subfamilies designated by a letter (CYP2D), and individual enzymes designated by a number (CYP2D6). CYP enzymes, particularly CYP2C9, CYP2C19, and CYP2D6 are polymorphic. CYP2D6 polymorphism results in there being poor (PM), extensive (EM), and ultrarapid (UM) metabolism of the test drug, debrisoquine, as well as a number of antidepressant and other psychotropic drugs that are metabolized by CYP2D6. Poor metabolism is inherited as an autosomal recessive trait and is found in approximately 7% of Caucasians. Amplification of functional CYP2D6 genes accounts for ultrarapid metabolism. Poor metabolizers will have abnormally high blood levels of the drug, even when administered at regular doses, and are more susceptible to adverse effects. Ultrarapid metabolizers will be less likely to benefit from the therapeutic action of the drug. Variability of plasma levels within the EM group may be accounted for by differences between heterozygotes for the defective allele and homozygotes for the wild type. Among the psychotropic drugs that are metabolized by CYP2D6 are the tricyclic antidepressants, nortriptyline, amitryptyline, clomipramine, desipramine, and imipramine; the SSRIs, fluoxetine and paroxetine; and also maprotiline and mianserin [16,17,20].

Pharmacokinetic variability between individuals may be evaluated at 3 levels, each of which carries advantages and disadvantages [20]. Plasma levels of the drug may be monitored (therapeutic drug monitoring), the phenotype may be established by administration of a test drug (debrisoquine, sparteine, or dextromethorphane in the case of CYP2D6), or the genotype may be examined directly. Genotype is not influenced by extraneous factors and can serve as an important role in screening for potential nonresponders to standard drug doses (UM) or susceptibility to adverse effects (PM). Tricyclic antidepressants are good candidates for pretreatment genotyping because of their narrow therapeutic index and the reasonably well-established correlation between plasma levels and clinical effects. For SSRIs, the utility is less clear since a clear correlation between plasma levels and therapeutic effectiveness has not been established. Moreover, standard drug doses are used and these produce plasma levels within the therapeutic range. These conclusions may need to be reevaluated in the context of possible interactions between drug bioavailability and relevant variations in the target proteins of the drug. In such a case, more subtle differences could be important even though they do not have a major influence in and of themselves.

Antidepressants are also metabolized by other CYP450 enzymes such as CYP2C19, CYP3A4, and CYP1A2. A detailed consideration of genetic variability in these enzymes and its clinical relevance is beyond the scope of this chapter [16,17,20]. Each enzyme has the potential to impact significantly on individual variability in responsiveness to antidepressant drugs and susceptibility to adverse effects. With the advent of DNA chip technology, it will be possible to implement large-scale studies and to gather the type of data that are needed to draw conclusions and plan strategy for pharmacogenetic testing at the clinical level. There may be a higher concentration of patients with genetically based abnormalities of drug metabolism in hospitals and tertiary care facilities than in the community. This could be due to the fact that patients in such centers are more likely to be nonresponders or to have suffered adverse effects.

IV. PHARMACODYNAMIC FACTORS IN ANTIDEPRESSANT RESPONSE

The serotonin transporter (5HTT) is located on the presynaptic neuron of serotonergic nerve terminals and is the site of action of SSRIs and other antidepressants that block the uptake of serotonin. Therefore, it is an important potential candidate for pharmacogenetic studies of antidepressants. The 5HTT is encoded by a single gene, SLC6A4 on chromosome 17, and includes 14 exons spanning approximately 35 kb. There are two common polymorphisms in SLC6A4. One is in the promoter region and is termed 5HTTLPR (5HT transporter-linked polymorphic region). It consists of a 44-base pair insertion or deletion. The long [l] variant has been reported to generate more gene transcription than the shorter variant(s) [21]. The other polymorphism in SLC6A4 consists of a variable number of tandem repeats (VNTR) in the second intron. It has three alleles (STin2*9, Stin2*10, Stin2*12) represented by different numbers of repeats. 5HTTLPR was reported to be associated with personality traits [21] and has been extensively studied as a candidate gene for mood disorders with variable results [22]. Association of the intron 2 VNTR polymorphism with mood disorders is also variable [23].

An association between the serotonin transporter gene and response to antidepressants was first reported by Smeraldi et al. [24] (Table 1). One-hundred-two patients with DSM-IV major depression and psychotic features received fluvoxamine + pindolol or fluvoxamine + placebo in a 6-week double-blind controlled study. Response to the study drugs was compared among the patients grouped according to 5HTTLPR genotype. It was found that patients carrying either one or two copies of the long (l) allele had a significantly better response to fluvoxamine (+ placebo) than patients homozygous for the short (s) allele. When the same comparison was conducted for patients receiving fluvoxamine + pindolol, there was no difference between the genotypes. It was speculated that differences in gene transcription between the genotypes expressed in the number of transporters might be associated with different degrees of negative feedback on 5HT release (mediated via $5HT_{1A}$ somatodendritic receptors in the raphe nuclei). Pindolol blocks $5HT_{1A}$ receptors and would thus abolish these differences.

Subsequent studies (Table 1) have provided a fair amount of support for the initial observation of Smeraldi et al. [24]. In a further report from the same group, Zanardi et al. [25] studied patients treated with paroxetine for 6 weeks. Genotypes including the I allele of 5HTTLPR were associated with better and also faster response to paroxetine. Patients with l-l genotypes had better response than patients with l-s genotypes and patients with l-s genotypes had better responses than those with s-s genotypes. Pollock et al. [26] studied patients with late-life depression treated with paroxetine or nortriptyline. Onset of antidepressant effect was faster in patients homozygous for the l variant of 5HTTLPR than in l-s heterozygotes or s-s homozygotes. There were no differences among the genotypes in response to nortriptyline. The difference between paroxetine and nortriptyline is of great interest because of the noradrenergic mechanism of nortriptyline and tends to support a pharmacogenetic hypothesis linked to the principal site of action of SSRIs.

Substantial complexity is introduced by the findings of Kim et al. [27] who studied Korean patients with major depression treated with fluoxetine or paroxetine and genotyped both 5HTTLPR and the VNTR in intron 2 of the gene. They too found an association of 5HTTLPR with treatment response but with the short variant. 5HTTLPR s-s homozygotes were more frequently represented among good responders than l-s or l-l genotypes. Kim

Table 1 Pharmacogenetics of Antidepressant Response: Studies on the Role of the Serotonin Transporter Gene

Ref.	Subjects	Design	Polymorphism	Finding
24	102 patients (Northern Italian) DSM-IV Major Depression Psychotic Features	Double-blind, 6 weeks Fluvoxamine 300 mg/d + pindolol 2.5 mg/d vs. fluvoxamine 300 mg/d + placebo	5HTTLPR (Promoter)	*ll* homozygotes and *ls* heterozygotes—better response to fluvoxamine than *ss* homozygotes With addition of pindolol—no difference between the genotypes
28	68 patients (Northern Italian) DSM-IV bipolar I, depressive episode Nonpsychotic	Total sleep deprivation, one night	5HTTLPR (Promoter)	Better response in 5HTTLPR *ll* homozygotes
27	120 patients (Korean) DSM-IIIR Major Depression	Prospective, random assignment 6 weeks Fluoxetine 20–50 mg/d or paroxetine 20–60 mg/d	5HTTLPR (Promoter) VNTR (Intron 2)	*ss* 5HTTLPR homozygotes—better response than *ll* or *ls* genotypes *ll* intron 2 homozygotes—better response than other genotypes
26	57 patients completing trial (Ethnicity not specified) DSM-IV Major Depression Nonpsychotic, nonbipolar	Double-blind, 12 weeks Paroxetine 20–30 mg/d vs. nortriptyline from 25 mg/d (per blood level, 50–150 ng/mL	5HTTLPR (Promoter)	Faster response to paroxetine in *ll* homozygotes than *ls* heterozygotes or *ss* homozygotes No difference among genotypes in response to nortriptyline
25	60 patients (Northern Italian) DSM-IV Major Depression Nonpsychotic	Open with single-blind placebo run-in, 4 weeks Paroxetine 40 mg/d	5HTTLPR (Promoter)	Presence of *l* copy of 5HTTLPR associated with better and faster response to paroxetine *ll* genotypes, better response than *ls*; *ls* genotypes, better than *ss*
29	63 patients (Ethnicity not specified) DSM-IV bipolar I or II	Retrospective Patients with history of antidepressant-induced mania (IM+) compared to patients with no history (IM−)	5HTTLPR (Promoter) VNTR (Intron 2)	Excess frequency of 5HTTLR *s* allele and *ss* homozygotes in IM+ group No difference in VNTR allele or genotype frequency

et al. [27] also found an association with the intron 2 VNTR. There was a significant excess of patients homozygous for the 12-repeat variant among the drug-responsive patients. It should be noted that the allele distribution of the 5HTT polymorphisms studied was very different among the Korean subjects than among European populations. This was true of both the intron 2 VNTR and 5HTTLPR. For example, homozygosity for the short variant of 5HTTLPR was 19% in the patients studied by Benedetti et al. [28], but was 56.7% in the Korean subjects of Kim et al. [27]. As discussed in an earlier section of this chapter, frequency of alleles varies greatly among populations. It is possible that association be demonstrated with a polymorphic locus in two populations but that different alleles be implicated in the respective populations. This would suggest that the locus itself is not implicated but is in linkage disequilibrium with another implicated locus close by.

Two other studies of the 5HT transporter gene should be noted (Table 1). Benedetti et al. [28] studied the relationship of 5HTTLPR to the antidepressant effect of total sleep deprivation in patients with bipolar depression. Patients homozygous for the l variant were found to manifest the strongest effect. Recently, Mundo et al. [29] examined the relationship of the 5HT transporter gene to induction of mania by antidepressants in patients with bipolar disorder. While the intron 2 VNTR did not show an effect, the authors observed a higher rate of homozygosity for the short variant of 5HTTLPR in patients with a history of antidepressant-induced mania.

Overall, the findings relating to 5HT transporter gene and antidepressant effects provide an intriguing insight into the potential and also the complexities that characterize the pharmacogenetics of mood disorders. A great deal of further work will be needed involving large patient samples carefully stratified for intervening variables such as ethnicity before it will be possible to draw definitive conclusions and to proceed to the stage of clinical application.

V. PHARMACOGENETICS OF MOOD-STABILIZING DRUGS

Beginning in the 1970s, a series of studies has examined the relationship between response to prophylactic lithium administration and family history of bipolar disorder [30]. The overall trend of these studies is to find that a positive family history of bipolar disorder is associated with lithium response or that rates of bipolar disorder are higher among the relatives of lithium-responsive patients than among the relatives of lithium nonresponders [31–33]. There are some notable exceptions, however [34]. An excess of mood disorders among the relatives of lithium-responsive patients can be interpreted in two ways. Lithium responsiveness may characterize a subtype of bipolar disorder that has higher familial loading. The alternative possibility is that lithium responsiveness is a familial, pharmacogenetic trait. It is difficult to separate these possibilities. If the first possibility is correct, then "lithium-responsive" families could be of particular importance in the search for bipolar disorder genes by linkage analysis. The second possibility can also be the focus of linkage analysis by setting lithium responsiveness rather than bipolar disorder as the phenotype for which linkage is sought. Early results from work of this type are discussed below.

Compared to studies on the prevalence of bipolar disorder in the relatives of lithium responders and nonresponders, there are far fewer family studies in which the pharmacogenetic trait of lithium response has been used as the phenotype. There is a report that children of bipolar lithium responders have a response concordant with that of their parents

[35]. Similarly, Grof et al. [36] reported a significantly higher response rate to lithium in the relatives of bipolar probands who were lithium responders than in a comparison group.

The putative mode of inheritance of lithium-responsive bipolar disorder has been studied by segregation analysis. Smeraldi et al. [32] found support for a major gene effect in the families of responders. Alda et al. [37,38] found evidence for an autosomal recessive mode of inheritance. These analyses could serve as a backdrop for linkage studies, but there are not enough data to allow the mode of inheritance to be definitively specified.

In spite of the fact that mode of inheritance has not been established, linkage studies of bipolar disorder, using lithium responsiveness to refine the phenotype, have been performed. In one study that used both association and linkage approaches, a polymorphism in the phospholipase C gamma-1 genes was examined. In a case control sample, modest association with lithium-responsive bipolar disorder was observed (OR = 1.88). A linkage study in a family sample also showed some support (lod = 1.45; $p = 0.04$) [39]. Turecki and colleagues [40] have completed a whole genome scan of 21 families (247 subjects, 108 affected) in which regions on chromosome 6, 7, 15, 21, and 22 yielded lod scores suggestive of linkage. In the chromosome 15q14 region, a lod score of 3.46 ($p = 0.000014$) was obtained with the marker ACTC and on chromosome 7q11.2 and a lod score of 2.62 ($p = 0.0001$) with the marker D7S1816. Using responsiveness to lithium as the phenotype, the highest lod score was 1.53 ($p = 0.003$) for the marker D7S1816. This group has also conducted association studies comparing lithium-responsive patients and controls. Besides the modestly positive association for phospholipase C gamma-1 noted above, negative results were obtained for several candidate genes including MAO-A, CRN, proenkephalin, and genes related to GABA function [30].

There are also a number of association studies directly comparing responders and nonresponders to lithium in a case control design. Negative results were obtained by Serretti et al. for the dopamine D3 receptor gene [41], GABA–21 subunit and dopamine D2 and D4 receptors [42], tryptophan hydroxylase [43], and the serotonin 2A, 2C, and 1A genes [44]. Steen et al. [45] found association of a polymorphism in the inositol phosphate 1-phosphatase gene with lithium responsiveness in Norwegian but not Israeli patients. Del Zompo et al. [46] examined the serotonin transporter gene and found a trend toward association of lithium nonresponsiveness with the 1 allele.

Overall, no clear trend has emerged as yet from association studies of candidate genes with lithium responsiveness and most results have been negative. However, the family data that tend to support a genetically distinct lithium-responsive subtype of bipolar disorder and the genome scan results reported by Turecki et al. [40] are promising. Taken together, they suggest that pharmacogenetic stratification could be a key to localizing genes that predispose to a pharmacogenetically distinct variant of bipolar disorder. The potential implications for understanding the pathogenesis of the illness and for individually tailoring treatment are substantial.

VI. STUDY DESIGN FOR THE PHARMACOGENETICS OF MOOD DISORDERS

Most current pharmacogenetic studies in mood and other psychiatric disorders are "opportunistic" in the sense that they are not designed for the purpose of asking questions specifically relevant to pharmacogenetics and do not take into account special design considerations. They make use of previously studied clinical samples for which drug response or adverse effect data are available, taking blood for DNA and genotyping, or blood sampling

for DNA is added on to a clinical trial without modifying the protocol in any way. Such approaches have yielded some important findings and can have an important place at the early stages of research. Eventually, purpose-built pharmacogenetic studies will be needed. This section considers pivotal issues in their design.

A. Which Design to Use

The most frequently used experimental context for determining pharmacogenetic influences on drug response is a comparison of responders and nonresponders to the drug or of individuals who develop adverse effects and those who do not. This appealingly simple case-control design should be readily applicable in psychopharmacology. Data from such studies are amenable to analysis by either of two approaches: the first is a categorical approach in which patients are grouped according to the phenotype (responder or nonresponder, develops adverse effect or does not) and the frequency of the genotype of interest in these groups is compared. The second approach utilizes the response variable (or adverse effect measure) in a continuous fashion and compares scores at a single time point or over a period of treatment in patients grouped according to genotype. There are already numerous examples of the application of these approaches to psychopharmacology.

Case-control designs are very susceptible to the effects of population stratification (i.e., differences in allele frequencies between populations can lead to spurious results if patients from different population groups are represented in different proportions in the case and control groups). Family-based association studies employ a proband and both parents and use the untransmitted alleles of the parents as a virtual control group. Data can be analyzed by the haplotype relative risk (HRR) method or by the transmission disequilibrium test (TDT) [47]. While this approach addresses the problem of population stratification efficiently, since parents and probands are by definition from the same population group, recruitment of samples is more difficult and it is not applicable to older patients whose parents may not be available. Family-based designs are more applicable in children and adolescents, such as in pharmacogenetic studies of attention deficit hyperactivity disorder. Other approaches implemented in the search for disease-predisposing loci, such as affected and discordant sibling pair designs, are unlikely to be of value to pharmacogenetics because of recruitment difficulties because such designs can only be implemented retrospectively and because it is highly unlikely that siblings will be matched for exposure to the drug of interest. Therefore, it is clear that case-control designs will remain the cornerstone of pharmacogenetic research in mood and other disorders.

B. Is a Placebo Control Group Needed?

Random assignment, placebo-controlled, double-blind designs are the "gold standard" for clinical trials research in medicine. In studies where a pharmacogenetic component is "added on," placebo controls will be available because they are part of the basic design of the study. Are placebo controls needed for "purpose-built" pharmacogenetic studies? In clinical trials of drug efficacy, the placebo group controls for nonspecific effects and allows a superior effect of the active drug to be demonstrated with a satisfactory level of statistical confidence. In a pharmacogenetic study, control for efficacy is not needed, unless it is a trial of a new drug. Therefore, the placebo group is controlling for an association of the gene or genes being tested with response to treatment at a nonspecific level (i.e., it allows it to be established whether the gene variation is associated with response to the active drug or is associated with response regardless of the nature of the treatment). Pla-

cebo control substantially increases the complexity and expense of a study. Also, placebo control groups for studies that are pharmacogenetically oriented and not efficacy oriented raise ethical questions and are likely to encounter significant difficulties with Internal Review Boards, particularly in countries where approval for efficacy-oriented placebo-controlled studies is already difficult to obtain. However, it must be acknowledged that the exclusion of nonspecific effects will reduce false positives and enhance the likelihood of replication. Ultimately, investigators will need to carefully balance cost-benefit considerations. Placebo run-in phases could be a partial answer since they reduce the placebo response rate in the trial itself and ethically are less problematic.

C. Sample Size

Large samples are required for pharmacogenetic studies because of the inherent nature of the research design, which seeks to associate response to a drug with a genetic variant, such as SNP. In the case of a bi-allelic SNP, the frequency of the rarer allele in the population dictates the minimum sample size. This can be calculated by power analysis in which the minimum difference in the frequency of the allele of interest between responders and nonresponders that the study seeks to detect is entered. The rarer the allele in the population, the larger the sample that will be needed. Also, the more alleles the variant has, the larger the sample size that will be required.

Another important aspect must be considered. Consider a hypothetical trial in which 200 patients are randomly assigned to an antidepressant drug or to a placebo. It is widely accepted that placebo response rates in antidepressant trials are 40% or more. If the rate of response to the active drug is 60%, it must be assumed that 40% of these responders are patients who would have responded to placebo. Therefore, only 36 of the 60 responders to active drug are true drug responders. These patients are the true targets of our pharmacogenetic study. The fact that a study is placebo-controlled does not resolve this problem. It can only be effectively addressed by studying a sufficiently large sample.

D. Further Considerations in the Design of Studies

1. Demographic Variables

Since the basis of interindividual variability in drug response is multifactorial, the impact of demographic variables such as age and gender cannot be ignored. A particular genetic variant may not be functionally relevant without the addition of other factors that also influence the phenotype. An example is the influence of certain 5HT-receptor variants on predisposition to neuroleptic-induced tardive dyskinesia, which was demonstrated in a sample of older patients but was not observed in a sample of patients who were two decades younger [48].

2. Population Effects

It is well known that the frequency of genetic polymorphisms can differ markedly among ethnic groups. An example is given in Figure 2 from a multicenter European study in which the frequency of a $5HT_{2C}$ receptor ser9gly polymorphism was studied in mood disorders [49]. The difference in frequency of the gly9 allele among the different countries that participated in the study is striking, from more than double ($>20\%$) in Greek normal control subjects as compared to Bulgarian and Croatian normal controls. These data clearly indicate how the unequal inclusion of individuals of different ethnic backgrounds in groups

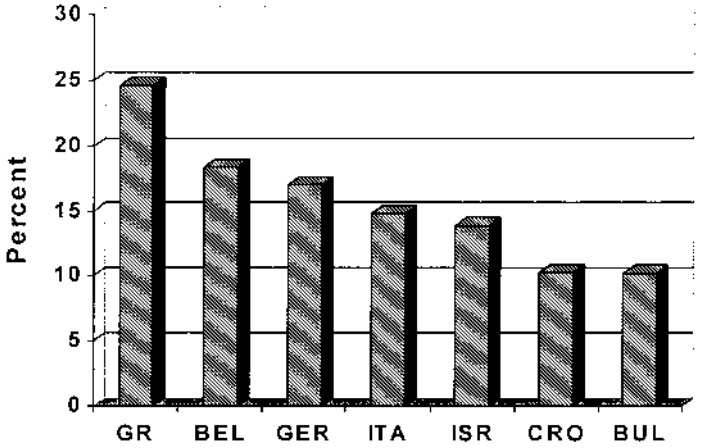

Figure 2 Frequency of $5HT_{2C}$ ser allele in European populations. (GR = Greece; BEL = Belgium; GER = Germany; ITA = Italy; ISR = Israel; CRO = Croatia; BUL = Bulgaria.)

of subjects being compared can lead to spurious results that reflect ethnic stratification. Methods have been proposed to address these issues [49,50]. Nevertheless, it is essential that studies be designed to take them into account prospectively.

3. Definition and Evaluation of the Phenotype

Drug response to psychotropic drugs is a phenotype that is very difficult to operationalize. There is an extensive literature that debates how to define a "responder" to an antidepressant drug in the context of a clinical trial. Definitions may be based on percent improvement on a particular rating scale or by using a specific score on that scale as a threshold. There are conventions that are fairly well accepted for clinical trials of psychopharmacological agents. It remains to be established whether these conventions can be readily transferred to pharmacogenetic studies.

4. Interaction Among Multiple Loci

Traditionally, pharmacogenetic studies have focused on single genes and on single polymorphisms within these genes. It is becoming clear that this approach is too limited to adequately address the complexity of the situation. Single SNPs may not show an association with treatment effects or with disease susceptibility while combinations do. These may be within a single gene, as demonstrated for the effect of complex haplotypes of SNPs in the coding region and the promoter of the β_2-adrenergic receptor gene on the bronchodilator response to asthma therapy [51]. Interactions (epistasis) may be demonstrated between SNPs in different genes as recently observed in a study of genetic susceptibility to sporadic breast cancer [52]. In the context of pharmacogenetics, Segman et al. [53] have observed an interaction between a polymorphism in the cytochrome P17 gene and the dopamine D3 receptor gene that is associated with more neuroleptic-related abnormal involuntary movements in patients who carry both mutant genotypes. These considerations will need to be given particular emphasis in the design of pharmacogenetic studies in the future because they have a major impact upon the size of the samples that need to be collected.

VII. CONCLUSIONS

Pharmacogenetics is the study of genetically determined, interindividual differences in therapeutic response to drugs and susceptibility to adverse effects. The principal objective of pharmacogenetics is to identify and categorize the genetic factors that underlie these differences and to apply these observations in the clinic. Pharmacogenetics addresses a core issue in pharmacotherapeutics, the individualization of drug treatment to the specific patient, and promises to provide the tools for making rational clinical decisions that are based on the patient's genetic profile. This will be a major advance in therapeutics that will have enormous impact on patient care and also important pharmacoeconomic implications. Furthermore, the complex and lengthy process of new drug development could be considerably shortened. While there is certainly a future for the pharmacogenetics of mood disorders, translating the promise into reality is likely to take substantially longer than anticipated. It will require considerable investment of resources in the design and execution of appropriate clinical studies as well as the development of novel and considerably more efficient approaches to data analysis.

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Theories of the Etiology of Anxiety

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I. INTRODUCTION

Anxiety is a multidimensional concept that includes cognitive, affective, physical, and behavioral elements. Cognitions such as intense worry and uncertainty are coupled with feelings of apprehension and fear. Physiological reactions based on the autonomic nervous system, including increased heart rate, sweating, tremor, vascular, and gastrointestinal changes, are frequently accompanied by behavioral changes such as escape, avoidance, or, in extreme cases, paralysis (freezing). This constellation of reactions is normal and adaptive, but in some there are exaggerations to the point where the individual's psychosocial well being is threatened. In these cases, an anxiety disorder has emerged and these are among the most common of all psychiatric disturbances [1]. In this chapter, we begin with a historical account of theories of anxiety formation followed by an examination of the major contemporary understanding of the etiology of anxiety disorders. As there is a high degree of clinical overlap and comorbidity among the anxiety disorders as defined in modern classificatory systems such as DSM-IV [2], we will take a unitary view, mentioning specific disorders when indicated.

II. HISTORICAL OVERVIEW

Given the primal nature of anxiety as an emotional phenomenon, identifiable throughout the animal kingdom, it is surprising that ancient medical texts did not devote more attention to its description. Hippocrates (460–370 B.C.) appears to have overlooked anxiety when

he proposed his theory of emotions, giving predominance to the four bodily humors of black bile (sad, loveless, "melancholic"), yellow bile (angry, irritable, "choleric"), phlegm (rigid, passive, "phlegmatic"), and blood (lively, optimistic, "sanguine"). One possibility is that anxious phenomena were considered more the domain of the philosophers. Marcus Tullius Cicero, the Roman orator, politician, and philosopher (106–43 B.C.), made a clear distinction between "state" and "trait" anxiety and hinted at a learning theory of anxiety and conditioned fear in his book *Tusculaum Disputationum*.

William Cullen (1769), the Edinburgh physician, introduced the term "neurosis," which he believed was a disorder of the nervous system in the absence of fever. The term included disorders such as hysteria, hypochondriasis, melancholia, and asthenia. Despite this, discussions of anxiety remained predominantly philosophical when Soren Kierkegaard (1813–1855) began exploring the problem of "human dread." This remained so with the writings of the existential philosophers that followed (see existential theories). During the 19th century, increased scientific attention was paid to anxiety with detailed chapters on "angst" in both German and French psychiatric textbooks. A surge of interest in anxiety followed Charles Darwin's "The Expression of Emotions in Man and Animals" [3], in which he presents evidence for an adaptive value of anxiety and its importance in evolution. Darwin also highlighted the importance of early bonding experiences that were later developed by Freud and Bowlby. One of the first biological theories of anxiety emerged from the separate writings of the Danish physician Carl Lange (1834–1900) and the American psychologist William James (1842–1910). James and Lange proposed that different emotions arise as a result of peripheral sensory feedback. The James–Lange theory implied that behavioral components of anxiety were automatic and that emotional and cognitive responses were secondary to autonomic manifestations of anxiety in the periphery. Argument about central versus peripheral origins of anxiety began and to some extent continue today. The most provocative and detailed modern theories of anxiety began, however, with the work of Sigmund Freud (1856–1939).

III. PSYCHODYNAMIC THEORIES OF ANXIETY

Anxiety lies at the very heart of Freud's view of neurosis. There is no single psychodynamic theory of anxiety but a series of views held by Freud which, when traced, reflect the evolution of his thought on the human psychic apparatus. Freud's interest began following a visit to the Salpetriere in the 1880s that introduced him to the study of hysteria. Freud was aware of the peripheral model known as the James–Lange theory and the opposing central theories based on neurocircuits involving the medulla oblongata—and rejected them [4]. Instead, he embarked on a search for the central psychological mechanisms of anxiety that changed throughout his life, and even at the age of 70 he was producing revisions to his earlier theories of anxiety [5]. Freud contrasted realistic anxiety with neurotic anxiety. Realistic anxiety was seen as the rational and understandable reaction to danger and had adaptive advantage in Darwinian terms [4]. The understanding of neurotic anxiety, on the other hand, required an exploration of the inner workings of the mind. It is impossible here to summarize Freud's prolific writings on the topic of anxiety and emphasis will be given to some of the major advances made by Freud and developed subsequently by the neo-Freudians.

In Freud's first major paper on anxiety, he argued for a separation of anxiety neurosis from neurasthenia, splitting the neuroses into "actual neurosis" (anxiety neurosis) and the "psychoneuroses," which included hysteria and obsessive-compulsive neurosis [6]. In his

analysis of patients with anxiety neurosis, Freud did not find the psychological conflicts that characterized the psychoneuroses. Instead, he found common sexual practices such as continence and coitus interruptus, which were believed to result in accumulated libido undischarged as sexual energy in orgasm. Libidinal energy was transformed directly into the somatic symptoms of anxiety. Freud was aware of potential criticism of this theory on the basis that those who are by nature anxious and apprehensive may be more inclined to practice restraint in sexual matters and other aspects of their life. He counter-argued that women, being passively dependent on the sexual behavior of men, be used as an example. Passionate women, being more inclined to sexual behavior and satisfaction than "anesthetic" women, are more certain to react to manifestations of male anxiety (impotence, coitus interruptus) [4].

Understanding the origins of anxiety in the psychoneuroses depended on Freud's developing idea of the unconscious and the psychic apparatus. Freud's discovery of childhood sexuality and the Oedipus complex led to the topographical model of the mind, which he later altered to include the superego and so conceived the structural model. The structural model consists of a tripartite psyche consisting of the ego, the id, and the superego. The id is seen as being the source of the instinctual drives that energize it. The ego consists of a number of related functions that control and divert id drives, permitting their gratification within the limits of superego control. The superego, formed following the resolution of the Oedipus complex, is the repository of ideals and social conscience that guides the ego's execution of id driving forces [7].

In later reformulations, Freud conceived of anxiety not emerging merely as a mechanical overflow of affect, but as a signal to the ego. In realistic anxiety, anxiety could be seen adaptively as a warning of danger and the signal for taking flight. In the case of neurotic anxiety, the ego generates anxiety as a signal to flight from libidinal demands (i.e., treating the internal danger as if it were external). Defensive maneuvers, such as fight/flight required in the case of external anxiety, also hold true for neurotic anxiety where the anxiety is transformed into symptoms (predominantly repression and allied defenses) which results in the anxiety becoming bound [4]. Anxiety, therefore, can be seen as involving all components of the psychic apparatus whether as undischarged libidinal energy or as an affect generated by the ego in reaction to internal danger and a signal to mobilize internal defenses where there is a conflict between id drives and superego control.

IV. DEVELOPMENTAL THEORIES OF ANXIETY

Freud initiated the exploration of anxiety in a developmental context. The prototypical experience of anxiety comes with the act of birth and the interruption of internal respiration. While this may remind us of Klein's later theory of suffocation in panic disorder, Freud believed this first experience of anxiety has been thoroughly incorporated into us through evolution. Two aspects were of particular relevance for Freud; first, the experience of anxiety was toxic and second, this first state of anxiety arose out of separation from the mother. In later childhood, these feelings are revisited at times of separation or when confronted with strangers. The disappointment and longing for the mother are transformed into anxiety. Infantile anxiety was closely related to neurotic anxiety, as it is derived from unemployed libido and replaces the missing love object by an external object or situation [4]. In later years, such "free-floating" anxiety could readily attach to any suitable idea [4] that relates to modern conceptualizations of generalized anxiety, or to objects and situations as in the phobias.

The next major developmental phase of relevance to the neuroses is the Oedipus complex. In both sexes, there is a striving for the parent of the opposite sex. In males, rivalrous feelings toward the father come to a head with murderous fantasies, guilt, and fear of retaliation by the father in the form of castration. The castration anxiety is resolved following internalization of the father as the superego. In the female, the child renounces her wishes toward her father upon realization of her mother's disapproval in order to maintain the bond with her mother [7]. The Oedipal stage occurs around the ages of 3 to 5. In Eriksson's reformulation, this is the developmental phase of "initiative versus guilt." The child who inadequately resolves the conflict between initiative and guilt is hypothesized by Eriksson to be at risk of developing phobic disorders [8].

Bowlby, drawing on ethological and psychodynamic sources, suggests that early attachment experiences may be important in the genesis of anxiety and other disorders. A key function of parenting is to establish a secure base from which the child can explore the world. Appropriately secure attachments are of central importance in providing the secure base [9]. Anxiety has an adaptive function in promoting attachment and has evolved because of its contribution to species survival. Of the three principal patterns of attachment described by Ainsworth and colleagues [10], Bowlby has focused on the pattern of "anxious attachment" as being of particular relevance to the anxiety disorders. Children with this type of attachment are prone to uncertainty when their parent is unavailable and tend to be clinging and anxious in their exploration of the world. Anxious attachment is promoted by inconsistent availability of the parent, separations, and threats of abandonment by the parent as a means of control [9].

Bowlby proposed that separation anxiety in children and agoraphobia in adults are best understood through the earlier development of anxious attachments. Uncontrolled studies in agoraphobic patients have suggested parental overprotection and lack of affection are common [11,12]. Parker [13], using the Parental Bonding Instrument (PBI), found agoraphobic and social phobic patients were more likely to report low-care–high-protection or "affectionless control." Maternal overprotection has been confirmed in some other [14,15] but not all studies [16,17]. Studies investigating Bowlby's suggestion that threatened or actual early parental separation and loss predispose to the development of anxiety in adulthood are contradictory. Early studies [18–20] did not find increased rates of parental loss and separation while a controlled study reported a positive correlation [14].

Correlational studies suggesting links between early-life experiences and later anxiety disorders may reflect a causal relationship but several alternative explanations need consideration. First, self-report measures of early parenting may reflect response bias secondary to mood or personality disturbance. Second, family styles of overprotection and low care may be secondary to the child's anxiety disorder or premorbid anxious personality. Third, a genetic predisposition to anxiety may affect both child and parent, resulting in altered parenting styles of overprotection [21].

V. LIFE EVENTS AND THE DEVELOPMENT OF ANXIETY

Systematic enquiry into the relationship between events rated as stressful and subsequent illness began with the work of Hinkle and Wolff [22] who found clusters of illness most frequently appeared following an individual's perception of difficulty in adapting to life situations. The development of the Social Readjustment Rating Scale [23] and subsequent

techniques of measuring stressful life events has kindled interest in the role such events may play in the causation and timing of mental illness. Most of this research is retrospective and has concentrated on discrete temporal events usually relating to the 12-month period prior to the onset of symptoms. While there are many methodological difficulties in conducting such research [24], there appears to be a relationship between anxiety symptoms or disorders and stressful life events.

A number of studies have evaluated the relationship between stressful life events (as measured by either questionnaire or interview) and self-report anxiety symptoms in nonpsychiatrically ill populations. Lauer [25] administered the Social Readjustment Scale to a large group of English and American university students and found a significant association between anxiety scores and self-reported life changes over an unstated period. This relationship was confirmed in most subsequent studies involving students, general practice attenders, and normal controls [26,27]. On the other hand, Flannery [28], in a study of 97 adults attending evening classes, found a relationship between anxiety and stressful life events in the women but not in the men studied.

Studies assessing the relationship between life events and the onset of anxiety disorder have demonstrated strong associations. Barrett [29] assessed 202 volunteers with a Research Diagnostic Criteria diagnosis of anxiety or depressive disorder for life events in the 6 months prior to the onset of the disorder. Both groups reported more events than a reference group and anxiety patients rated financial difficulties and arguments as particularly distressing. In a classic study of 164 young women attending a general practice in London and interviewed by a psychiatrist using the Present State Examination, Finlay-Jones and Brown [30] reported on the type of life event associated with depression, anxiety, and mixed anxiety/depression. Life events for the 12 months prior to onset of disorder were rated using the Bedford College Interview Schedule and compared with 80 women with no psychiatric diagnosis and 39 women who had an onset of depression or anxiety more than 12 months earlier. Loss events were significantly associated with depression, danger events with anxiety, and women with mixed anxiety/depression were more likely to report both severe loss and danger.

Of the anxiety disorders, the relationship between life events and agoraphobia has been the most studied. Roth [31] first noted the high incidence of threatening life events before the onset of agoraphobia with panic attacks in 135 patients. Since that time, a number of studies have noted the association between life events and the onset of agoraphobia [18,20,32,33]. Interpersonal difficulties are the most commonly reported precipitating event occurring in up to 76% of agoraphobics [34]. Events viewed as uncontrollable, undesirable, or lowering of self-esteem were found to precede the onset of panic attacks in patients with panic disorder [35].

VI. PERSONALITY AND ANXIETY DISORDERS

Personality is defined as the enduring pattern of "perceiving, relating to, and thinking about the environment and oneself" [36]. Implicit in this definition is that the repertoire of cognitions, emotions, and behaviors has stability over time and across a range of social and personal contexts. Personality description is usually made according to dimensional characteristics or with respect to specific core features grouped together to define a category (e.g. DSM-IV). Traditionally, the relationship between personality and major mental illness has been viewed in two opposing ways. First, personality dimensions may essen-

tially reflect a subclinical form of the major mood disturbance. The second view is exemplified by the psychoanalytic tradition where the major illness is seen as developing out of personality traits that make an individual vulnerable. In the former view, the form of the illness is seen in continuity with premorbid character traits, whereas in the latter, the symptomatic state is distinct from the form of the personality. Although limited, the literature suggests there is a linkage between some personality dimensions or categories and clinical anxiety states; however, most studies have identified personality factors in retrospect following the diagnosis of a major anxiety disorder. This methodology limits the findings for a number of reasons: (1) there is no accurate determination of the personality predating the illness; (2) personality traits may be exacerbated by the illness; and (3) the illness itself may distort the individual's view of the premorbid personality [37].

Studies assessing dimensional personality characteristics in anxious patients have generally suggested there is high neuroticism and low extroversion [38–40]. Kerr et al. [38] used the Maudsley Personality Scale to investigate personality characteristics of 57 patients with multiple phobias, generalized anxiety, and probable panic attacks. High scores of neuroticism in anxious patients were found to normalize in the well state, whereas scores on the introversion scale remained low relative to controls on follow up. High neuroticism/low extroversion was confirmed by Hoehn-Saric [39] in patients with generalized anxiety and panic attacks. Extroversion was found to be significantly lower in those with generalized anxiety compared with panic patients. Using factor analytic techniques, Cloninger has determined a personality model based upon three genetically independent factors with predictable responses to novel, aversive, and appetitive stimuli [41]. Cloninger suggests that individuals with high scores of "harm avoidance" and low scores on "novelty seeking" dimensions are susceptible to cognitive anxiety while the opposite configuration is associated with somatic anxiety. Allgulander and coworkers [42] demonstrated marked reductions in harm avoidance in patients with generalized anxiety disorder following treatment with paroxetine, suggesting some of these personality dimensions may be state dependent.

In comparison with depressed patients, anxious patients reported more premorbid personality traits of social anxiety, hypersensitivity, dependence, immaturity, hysteria, and anergia [43]. In contrast, Murray and Blackburn [44] found similar premorbid personality characteristics in depressed and anxious groups. Using the Cattel 16 Personality Factor Questionnaire, the anxious patients reported abnormalities of emotional immaturity, shyness, worry, seclusiveness, tension, and introversion that remained on recovery. In one of the few prospective studies in the area, Nystrom and Lindegard [45] tested 3019 males recruited while registering their car. Six years later, 114 of these were identified through registers of public psychiatric units of whom 34 had an anxiety disorder and 37 unipolar depression. Premorbid psychasthenia and subclinical anxiety traits, as measured by the Sjobring Personality Scale, were found to have a strong relationship with anxiety states. Psychasthenia and subclinical depressive traits were also found in depressed individuals.

Studies using categorical diagnoses have found elevated rates of personality disturbance in anxious patients. Koenigsberg and colleagues [46] found high rates of DSM-III Personality Disorder in a study of 2462 patients with highest rates in panic disorder (50%) and phobia (48%). Over 90% of these patients had either dependent, avoidant, borderline, or histrionic personality. Mavissakalian and Hanann [40] found 27% of 60 agoraphobic patients had a DSM-III Axis 2 disorder, again the most common being avoidant, dependent, or histrionic. Reich and colleagues [47] confirmed the predominance of Cluster C

disorders and, in particular, Dependent Personality Disorder in 88 patients with panic disorder.

VII. COGNITIVE AND BEHAVIORAL THEORIES OF ANXIETY

Cognitive-behavior therapy (CBT) is a popular and effective treatment for most anxiety disorders. As with psychoanalysis, no single theory pertains to the cognitive-behavioral understanding of anxiety, but a group of theories related to thought and behavior abnormalities frequently work in concert to initiate and maintain anxiety. The understanding of these theories drives the therapy in CBT where there is a strong emphasis on education of the patient.

In 1920, Watson and Rayner translated Pavlov's classic conditioning experiments in dogs to create a conditioned phobic response to rats in little Albert [48]. It was suggested that fear may be learned as a conditioned response. Several criticisms have emerged from this work. First, while Watson's student Mary Cover Jones [49] was able to replicate this phenomenon, others could not [50,51]. Second, conditioning theory demands single-trial conditioning, which is very rare in laboratory settings [52]. Third, establishing a conditioned response in the laboratory demands finely turned timing of conditioned and unconditioned stimuli, which are very unlikely to occur in real life situations [53]. The importance of Watson and Rayner's work is that it led to extensive research into the behavioral understanding of anxiety and the technique of systematic desensitization, an effective treatment for phobic avoidance, pioneered by Wolpe [54].

More recently, cognitive theories of anxiety have complemented behavioral understanding leading to a more complete model of the etiology of anxiety. Notions of "free-floating anxiety" in the absence of clear danger have been challenged by cognitive theorists who emphasize frequently reported thoughts and images of danger in the anxious patient. Beck and colleagues [55] have argued that anxious individuals systematically overestimate the threat of a given situation, galvanizing a set of inherited responses originally designed to protect us from harm. These changes include autonomic hyperarousal, inhibition of ongoing behavior, and vigilance for possible sources of danger [56]. Peripheral manifestations of autonomic arousal are then misinterpreted by the patient as a sign of serious physical or mental derangement leading to further arousal. In this model, anxiety is maintained by peripheral mechanisms originally hypothesized in the James–Lange theory of anxiety. Fearful cognitions in anxiety include medical emergency (e.g., heart attack), mental disorder (e.g., "going crazy"), or social catastrophe (e.g., public disgrace) [55]. Beck has emphasized the role of dysfunctional thinking in anxious patients, which contributes to the misattributions of peripheral stimuli. Cognitive distortions in the anxiety disorders include "catastrophization" (dwelling on the worst possible outcome), selective abstraction (selecting negative rather than neutral or positive aspects of a situation), dichotomous (polarized or "black and white") thinking, and hypervigilant scanning [55].

The cognitive and behavioral abnormalities discussed above can work alone or in concert in the anxiety disorders. In generalized anxiety disorder, Beck and coworkers [55] suggest there is a reactivation of developmental fears (dysfunctional assumptions) regarding the person's acceptability to others, competence, responsibility, and self control. In the cognitive model of panic disorder, Clark [57] emphasizes the catastrophic misinterpretation of bodily sensations as threat of impending physical or mental disaster. The initial stimuli can be external (e.g., the situation of a previous panic attack) or internal

(thoughts, images, or bodily sensations). A vicious cycle of amplification of anxiety ensues fueled by apprehensive scanning and somatic misinterpretations. Once initiated, panic disorder is maintained by a tendency to become hypervigilant for bodily cues of danger and avoidance of situations known to initiate symptoms. Avoidance is a major factor in the maintenance of the phobias and may contribute to the development of anticipatory anxiety, apprehension, and dread [58].

VIII. EXISTENTIAL ANXIETY

The exploration of human anxiety is not the exclusive domain of psychology. Philosophers from early times have sought to understand the origins of anxiety with the existential philosophers making the most recent contributions. "Existentialism" is a term given to a disparate group of philosophers dissatisfied with the traditional thinking of their time. While existential themes can be seen in the writings of Dostoevsky, the term "existentialism" originates from the Danish philosopher Soren Kierkegaard following exploration of modes of existence in "The Point of View," published posthumously in 1859 [59]. It was, however, Kierkegaard's concepts of human dread and freedom that became central in the thinking of later existentialists—Jaspers, Heidegger, and Sartre. While the various existential thinkers differed widely in their views, a number of themes have emerged from their work which directly address the origins of anxiety in humans. Yalom [60] identifies four ultimate concerns for the individual: death, freedom, isolation, and meaninglessness.

Death is an inescapable aspect of existence and it is hypothesized that we live with the ultimate terror of death. Choron [61] distinguishes three types of death fear: (1) what comes after death; (2) the event of dying; and (3) ceasing to be. It is the third of these fears that Kierkegaard emphasized as a dread of nothingness [62]. The existential anxiety is the tension that emerges between the knowledge of the inevitability of death and the wish to continue to be. One way to combat the fear of nothingness is to transfer it to something so that we can mount a self-protective defense. As a result, primal death anxiety is rarely encountered in clinical practice and is only seen when conventional defenses are stripped away (e.g., in analysis or in dreams). Yalom [60] suggests a number of defenses are particularly used to reduce death anxiety including denial, suicidal thoughts as a control over the helplessness of death, compulsive working (e.g., "workaholic"), personal beliefs of inviolability (narcissism), aggressive drives for power and control, and rescue fantasies (e.g., by God), etc.

While freedom is generally viewed as a positive concept, for the existentialist it is inextricably linked with dread. In its existential sense, "freedom" refers to the absence of a well-structured universe. In the absence of inherent design, the individual is entirely responsible for their world in terms of choices and actions. The individual strives for order in a disordered world. For Heidegger and Sartre, the individual is free to constitute the world however they wish but bares full responsibility for doing so—they are the authors of their world and the responsibility is terrifying. As with death, there is no escaping this freedom. To avoid the responsibility of freedom is to live "inauthentically" [63] or in "bad faith" [64]. Yalom [60] suggests a number of responsibility-avoiding defenses are commonly used to reduce the anxiety associated with freedom including: denial of responsibility, displacement of responsibility, compulsive behaviors, etc.

The third ultimate concern is isolation. Yalom [60] defines three types of isolation: (1) interpersonal isolation results when factors such as distance, personality style, etc., prevent social gratification: (2) intrapersonal isolation results when unconscious mecha-

nisms isolate one part of the self as in the defense of isolation of affect; and (3) existential isolation. Existential isolation refers to an unbridgeable gap between individuals based on the knowledge that each of us enters into existence alone and must leave alone. No matter how close we may get to another, one must ultimately undergo the journey of death alone. Isolation is then related to death and freedom in that the individual is alone in coming into existence, being responsible for their own existence, and leaving existence. Most of the defensive operations used to reduce the sense of isolation involve interpersonal relationships and include searching for love, avoidance of being alone, fusion with another, or with a group, etc.

The final existential concern identified by Yalom [60] is meaninglessness. Existential meaninglessness is related to freedom and responsibility. If the universe lacks structure and meaning, then the individual needs to construct meaning in a personal sense. Anxiety can result if insufficient purpose and meaning is achieved, or if the individual fears that this may be the appraisal on their death. Meaning can be achieved through altruism, creativity, and dedication to a cause, etc.

From a therapeutic viewpoint, there are many similarities with the psychodynamic therapies. Anxious concerns are understood to be defended against by classic defense mechanisms and are seen in a developmental context. The process of therapy is essentially aimed at gaining insight and effecting change on the basis of these insights. The major differences are related to the understanding of the origins of anxiety as outlined above and how to combat them. Armed with an understanding of their inner conflicts, patients may work through the grief related to the realization of fundamental life concerns and make choices aiming for a more "authentic" existence.

IX. NEUROANATOMICAL SUBSTRATES OF ANXIETY

Identification of key neural circuits involved in the genesis of anxiety has relied heavily on studies in animals, primarily rodents [65,66]. Experimental models of anxiety in animals have relied on inhibition of behavioral tendencies in certain tasks [67]. Frequently such tasks depend on learned behaviors that are probably less reliable for understanding the neural basis of fear than are spontaneous fearful behaviors. Furthermore, many animal models are markedly dependent on responses to clinically effective pharmacological agents for validity [68]. Benzodiazepines have been the most widely employed agents in animal testing and the extent to which such models can be generalized to other classes of agents is still open to question. Nevertheless, external signs of fear in animals are well recognized. Both electrical stimulation of the brain and agents acting on specific neurotransmitters have provided valuable insights into the neuroanatomical substrates of fear and anxiety.

As noted by Charney and Bremner [69], during the development of an anxiety state, afferent inputs are relayed from sensory organs through the dorsal thalamus to cortical brain regions. Secondary cortical association areas then process the information. Particular brain regions have been identified as involved in fear and anxiety including the amygdala, hippocampus, entorhinal cortex, orbitofrontal cortex, and the cingulate. Processing of information within these regions is believed to account for the experience of anxiety by individuals, while modulation of neurotransmitter inputs to neural circuits formed between regions can account for the anxiolytic effect of various therapeutic drugs. A number of hypotheses involving these brain regions have been advanced to account for fear and anxiety in humans and other species.

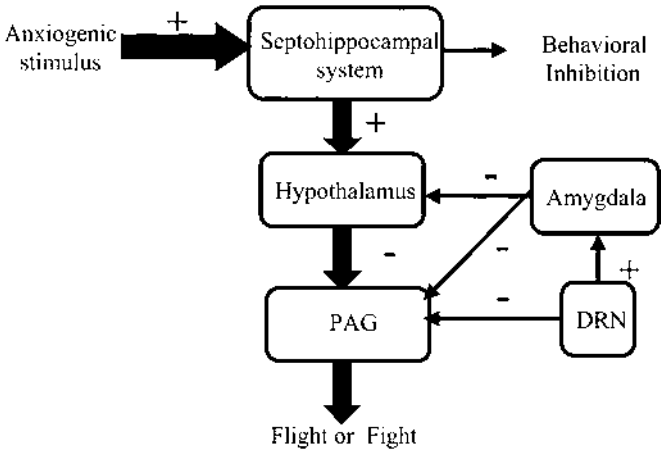

Figure 1 Representation of Gray's septohippocampal scheme for anxiety. Inputs to the septo-hippocampal system activate the behavioral inhibition system and increase anxiety. Increased neurotransmitter activity from both serotonergic pathways (the raphe nuclei) and noradrenergic pathways (from the locus coeruleus) are proposed to increase anxiety. Anxiolytic drugs are proposed to have their therapeutic effects by decreasing the firing of the raphe nuclei, the locus coeruleus, or both.

A. Septohippocampal System

One hypothesis proposes that the septum and hippocampus form a behavioral inhibition system (Fig. 1) that serves as a substrate upon which anxiolytic drugs exert their effects [70]. Gray also included the Papez circuit as part of the system, although subsequent work has suggested that this pathway may have little involvement in emotion [71]. The septohippocampal system is posited to be part of a neural network that acts as a comparator of actual and anticipated stimuli. When a mismatch is detected, the behavioral inhibition system is activated. The system either inhibits ongoing behavior or increases vigilance, depending on the nature of the inputs. Since septohippocampal lesions do not alter the behavioral effects of anxiolytic agents in animals, key inputs to the system must be affected by drug administration. Both serotonergic inputs from raphe nuclei and noradrenergic inputs from the locus coeruleus have been implicated. It has been proposed that septohippocampal activity is enhanced by increased activity of both monoamines and that anxiolytics exerted their effects by reducing monoaminergic activity [70]. An additional circuit comprising the amygdala, hypothalamus, and the periaqueductal gray (PAG) region of the central gray has been proposed to mediate flight-or-fight responses.

B. Amygdala

More recently, the role of the amygdala in fear responses has been described [71,72]. Both the lateral and basolateral nuclei of the amygdala receive processed sensory information. Both of these nuclei project to the central nucleus of the amygdala, which in turn projects to both the hypothalamus and the brainstem. These latter areas directly mediate the signs of fear and anxiety. The amygdala, because of its relevant projections (see Fig. 2), may represent a central fear system [73]. For example, direct projections from the central nucleus of the amygdala to the lateral hypothalamus probably activate sympathetic

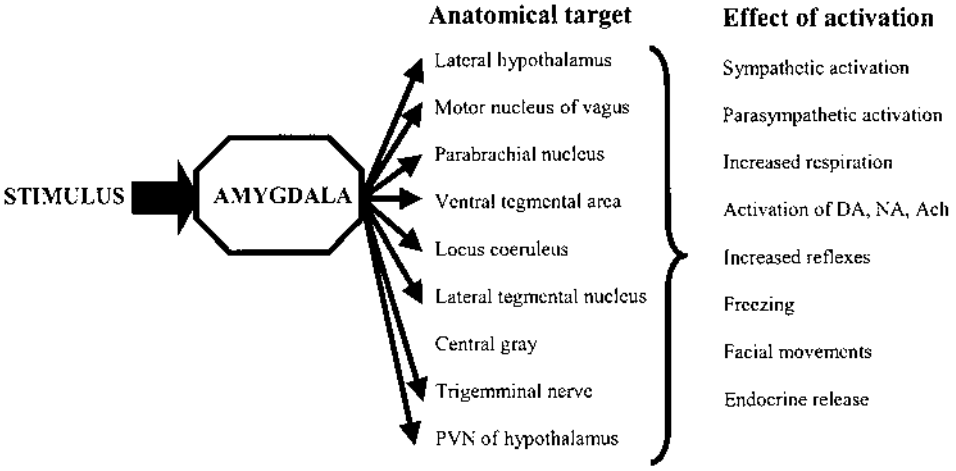

Figure 2 Connections of the amygdala to other anatomical regions and the consequences of activation. The amygdala appears to play a key role in assessing threatening stimuli and initiating response to those stimuli. Through activation of the various anatomical targets the autonomic and endocrine responses to threat are activated. (Modified from Ref. 69.)

responses during fear and anxiety [74]. Similarly, projections to the dorsal motor nucleus, vagus, and ventrolateral medulla most likely mediate changes in heart rate and blood pressure. Furthermore, respiratory effects may be influenced through projections of central amygdala to the parabrachial nucleus. Neuroendocrine responses to fear and anxiety are likely mediated by projections of central amygdala to the paraventricular nucleus of the hypothalamus or to the bed nucleus of the stria terminalis. Other projections and their symptom consequences are summarized in Figure 2.

Further evidence for the role of the amygdala in anxiety states comes from clinical observations of behavioral and autonomic changes, resembling fear, following electrical activation of the amygdala after temporal lobe seizures [75]. Similarly, direct electrical stimulation of the amygdala in humans results in subjective feelings of anxiety or fear, as well as autonomic signs usually associated with fear [73]. Furthermore, in rats, chemical stimulation of this brain region with drugs such as the GABA antagonist bicuculline, produced elevated blood pressure and heart rate [76]. Together these observations suggest that activation of the amygdala during fear may explain all of the fear/anxiety symptoms experienced. Conversely, if the amygdala is critical for the interpretation and manifestation of anxiety states, then lesions to the area should be anxiolytic. Indeed, in monkeys, bilateral removal of the temporal lobes including the amygdala, hippocampus, and surrounding cortical areas resulted in so-called "psychic blindness" [77]. This is best described as a lack of fear of usually feared animate and inanimate objects (e.g., snakes). While patients with lesions to the amygdala rarely exhibit a full Kluver-Bucy syndrome, they do exhibit blunted emotional reactivity [73]. Similarly, in the rat lesions of the amygdala result in a blockade of innate fears (e.g., increasing the number of contacts with a sedated cat) [78].

The amygdala represents one brain area that is critically involved in mediating the specific signs of fear and anxiety. Inputs to the amygdala from other critical brain areas

are also proposed to play a role in mediating anxiety and fear responses. In particular, inputs from the dorsal raphe nucleus (DRN) utilizing serotonin as a neurotransmitter are thought to augment avoidance of aversive stimuli [79]. A further serotonergic pathway arising in the DRN, which inhibits the PAG, is proposed to underlie the flight-or-fight response [79]. Furthermore, noradrenergic neurons arising in nucleus locus coeruleus have long been associated with fear responses in animals [80].

Clearly, the neural circuits underlying anxiety are complex and rely upon the interaction of several different systems. In turn, these systems communicate with one another through the agency of chemical neurotransmitters. Of the various transmitters, most attention has been directed toward noradrenaline, serotonin, and γ-aminobutyric acid (GABA). Other systems, dopamine, opiates, and neuropeptides have also been implicated in mediating anxiety responses.

X. NEUROCHEMICAL BASIS OF ANXIETY

A. Noradrenergic System

Cannon first proposed a role for noradrenaline in fear responses, while elevations of adrenaline and noradrenaline in the plasma of animals and humans in response to fear have been demonstrated [81]. Overactivity of the nucleus locus coeruleus and the ascending noradrenergic systems has been implicated in anxiety [82]. The locus coeruleus is the major noradrenergic-containing nucleus of the brain and is regulated by feedback onto α_2 autoreceptors. Electrical stimulation, lesioning studies, and pharmacological manipulation of the locus coeruleus have been extensively studied in stump-tailed monkeys [80]. Stimulation of the locus coeruleus produces an alerting effect, similar to that observed after threatening confrontations, leading to the notion that behavior was related to fear and anxiety [83]. The responses observed were specific to stimulation of the locus, as stimulating adjacent areas did not produce the behaviors. Similar responses to electrical stimulation could be elicited by the α_2 antagonists piperoxane and yohimbine administered intravenously. On the other hand, clonidine at a dose of 1 μg/kg infused intravenously was able to reduce the behavioral effects resulting from locus coeruleus stimulation. The same dose of clonidine reversed the effects of yohimbine and piperoxane [84]. Such actions would be predicted from the known effects of these agents on α_2 autoreceptors. Propranolol, a β-blocker, has also been shown to decrease behavioral responses and partially block the effects of stimulating the locus. Agents as diverse as diazepam and morphine also block electrical stimulation and the effects of piperoxane and yohimbine [80]. Diazepam probably exerts its effects by promoting the inhibitory effects of GABA, thereby decreasing the firing rate of locus coeruleus neurons and noradrenaline turnover. Morphine binds to inhibitory opiate receptors on the locus coeruleus [84].

Evidence for a similar role in humans has been obtained by electrical stimulation of the locus coeruleus, which has been reported to result in feelings of fear and imminent death [85]. Other evidence for a catecholamine hypothesis of anxiety is to be found in the clinical effects of drugs known to influence adrenergic function. The effects of the β-adrenergic agonist isoproterenol have been extensively studied. Frohlich and coworkers [86] identified a group of patients they described as having a "hyperdynamic β-adrenergic circulatory state." This was characterized by high left ventricular ejection rate, occasional systolic hypertension, systolic ejection murmur, and bounding arterial pulse. Such patients, but not normal controls, experienced increased cardiac awareness, increased pulse rate,

and anxiety attacks during infusion of the β-agonist isoproteronol. Acute anxiety attacks following isoproteronol infusion have been described in a small group of patients suffering from episodic anxiety [87]. Intravenous administration of propranolol (2 to 5 mg in 2 to 3 min) was able to block the chemically induced anxiety. Later workers have shown that panic attacks could be induced in patients susceptible to such attacks by intravenous infusions of isoproteronol [88,89]. The mechanism of the effect is not clear but probably does not involve a direct effect of isoproteronol on the locus coeruleus, since the drug does not cross the blood–brain barrier. More likely, the effect is indirect, activating the locus coeruleus "alarm signal" by cues from the peripheral cardiac effects of isoproteronol (i.e., the James–Lange peripheral model of anxiety). On the other hand, the α_2 antagonist yohimbine has been shown to cross the blood–brain barrier and is reportedly anxiogenic in humans [90,91]. In panic disorder patients, about 80% had an anxiety response to yohimbine, which was subsequently blocked by 8 to 12 weeks of treatment with a benzodiazepine anxiolytic [92]. The anxiogenic effects of yohimbine are probably mediated by a direct action on presynaptic α_2 adrenoreceptors located on the locus coeruleus [93].

B. Serotonergic System

Much of the evidence for a role of serotonin in anxiety states relies on data from animal studies. It is well recognized that the main cell bodies of serotonin-containing neurons arise in the brainstem and innervate virtually all cortical areas. Both the dorsal and median raphe nuclei contain cell bodies that give rise to ascending serotonergic pathways. Within this broad anatomical framework there are subtle functional connections of neurons [94]. Coupled with a plethora of serotonin receptor subtypes, it is clear that the serotonergic system provides multiple anxiogenic/anxiolytic targets.

Animal studies suggest that inhibition of serotonergic neurotransmission is anxiolytic, while an increase is anxiogenic. Administration of the serotonin synthesis inhibitor, p-choloro-phenylalanine (PCPA), has been shown to produce an anxiolytic profile in several animal behavioral models [95,96]. These effects could be reversed by treatment with the serotonin precursor, 5-hydroxytryptophan [97], as well as by $5HT_{1A}$ agonists such as 8-OH-DPAT [98]. Destruction of serotonin neurons with toxic agents such as 5,7-dihydroxytryptamine (5,7-DHT) in localized regions leads to diminished anxiety responses. For example, File and coworkers [99] showed that DHT lesioning of the dorsal raphe but not the median raphe resulted in anxiety reduction. On the other hand, more recent investigations suggest that both electrolytic and neurotoxic lesions of the median raphe nucleus are anxiolytic [100]. This may reflect the involvement of the different nuclei in different aspects of the behavioral tests employed. Furthermore, it has been proposed that the dorsal raphe is important for cognitive processes, while the median raphe modulates fear and anticipatory anxiety [101].

Studies with both serotonin agonists and antagonists, in general, produce anxiogenic and anxiolytic responses, respectively [102]. Responses to individual agents depend on the nature of the receptor subtype involved and its anatomical location (pre- or postsynaptic). Activation of specific serotonin receptors and the responses elicited in particular anxiety disorders are described in detail elsewhere in this volume.

Clearly the role of serotonin in the etiology of anxiety disorders is a complex one. Simple models based on increases or decreases of absolute levels of serotonin are inadequate. Neural circuits involving various serotonin-receptor subtypes and interactions with other neurotransmitters offer more sophisticated explanations of the etiology of anxiety.

Furthermore, these models, even though they themselves are probably inadequate, are of heuristic value providing specific testable hypotheses and further iterations of the model.

C. GABA–Benzodiazepine System

The efficacy of benzodiazepines and other drugs acting at GABA receptors in treating anxiety have argued for a role of GABA and its receptor systems in the etiology of anxiety. Manipulation of GABA levels or the use of GABA agonists does not produce a reliable anxiolytic effect in animals [103,104]. An important role for GABA was clearly implicated when it was demonstrated that benzodiazepines and other drugs with anxiolytic properties (barbiturates, ethanol) potentiate and prolong the inhibitory effects of GABA at central neurons. Subsequently, it was shown that benzodiazepines and GABA-A receptors form part of the same macromolecular complex (see Fig. 3). Although they bind to different sites on the complex, they are functionally coupled through modulation of the chloride ion channel. Benzodiazepines facilitate GABA transmission by positive allosteric modulation [105]. The GABA-A receptors are pentameric membrane-bound proteins distinguished by their subunit composition. In mammalian brain, seven different classes of subunits, most with multiple isoforms, have been identified: α_1–α_6, β_1–β_3, γ_1–γ_3, δ, ε, θ [106]. The majority of GABA-A receptors are composed of α, β and γ subunits [107]. The specific combination of subunits and isoforms varies across the central nervous system and accounts for the diversity of effects observed by drugs acting at GABA-A receptors [107]. Combinations of α and γ subunits are necessary to bind benzodiazepines; specifically the N-terminal extracellular domain of certain α subunits is critical. Only the α_1, α_2, α_3, or α_5 subunits bind the benzodiazepines [106]. Gene knockout strategies have been employed to examine the effects of alterations of GABA-A receptors on the physiological and pharmacological

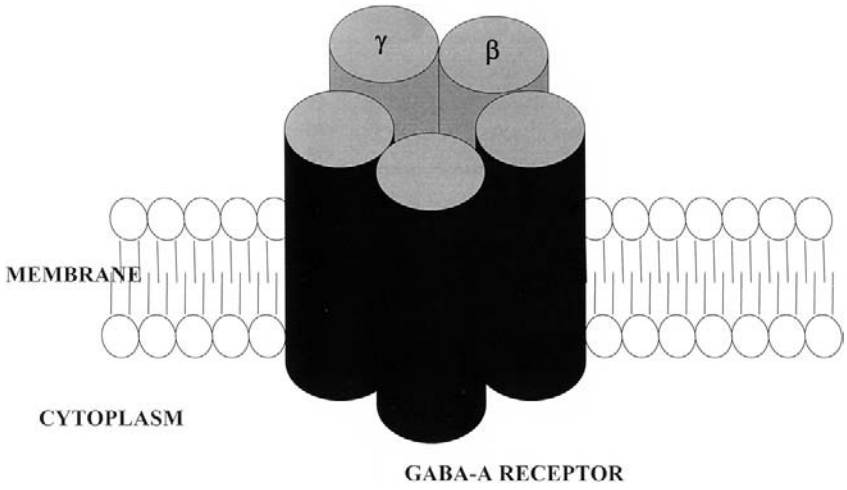

Figure 3 The GABA-A receptor. These receptors form a ligand-gated ion channel for the chloride ion. Binding of GABA to its site facilitates the influx of chloride ions and inhibits the firing of that neuron. Multiple allosteric modulatory sites, including one for benzodiazepines, are present on the GABA complex. The receptor consists of five peptide subunits, which in turn have four membrane-spanning domains. The benzodiazepine binding site is present on the extracellular domain of the α-subunit (see Fig. 4).

responses of mutant mice. This strategy suggested that the α6 subunit is associated with marked anxiety responses to natural and learned aversive stimuli; the β3 subunit is associated with motor impairment, epileptic seizures and hyperresponsiveness to sensory stimuli. Notwithstanding the methodological limitations of the knockout paradigm, these observations provide interesting clues as to the possible role of GABA-A receptor subtypes in the etiology of anxiety [106].

An alternate strategy has been to examine the role of so-called knockin point mutations [106]. The N-terminus of the extracellular domain of the α subunits, which bind benzodiazepines, have a histidine (His) residue at position 101 (see Fig. 4). GABA-A receptors composed of α4 and α6 subunits, which do not bind benzodiazepines, have an arginine (Arg) at this residue. A point mutation that converts histidine to arginine at position 101 causes benzodiazepine-sensitive receptors to become insensitive. The His to Arg point mutation has been introduced into the germ line of mice in the genes that encode α_1, α_2, and α_3 subunits and the behavioral effects examined. In mice with the mutation in the α1 subunit, diazepam administration failed to produce sedation and amnestic effects [108]. Similarly, the sedative and anticonvulsant effects of zolpidem, an agent with high affinity for α1-containing GABA-A receptors, was abolished in these mice [109]. By contrast, mice with the mutation in the α_2 subunit showed resistance to the antianxiety effects of diazepam, but not to its sedative, anticonvulsant, or muscle-relaxant effects [110]. Selective His to Arg mutations of the α3 subunit did not appear to have any of the resistance apparent in the other two strains [110].

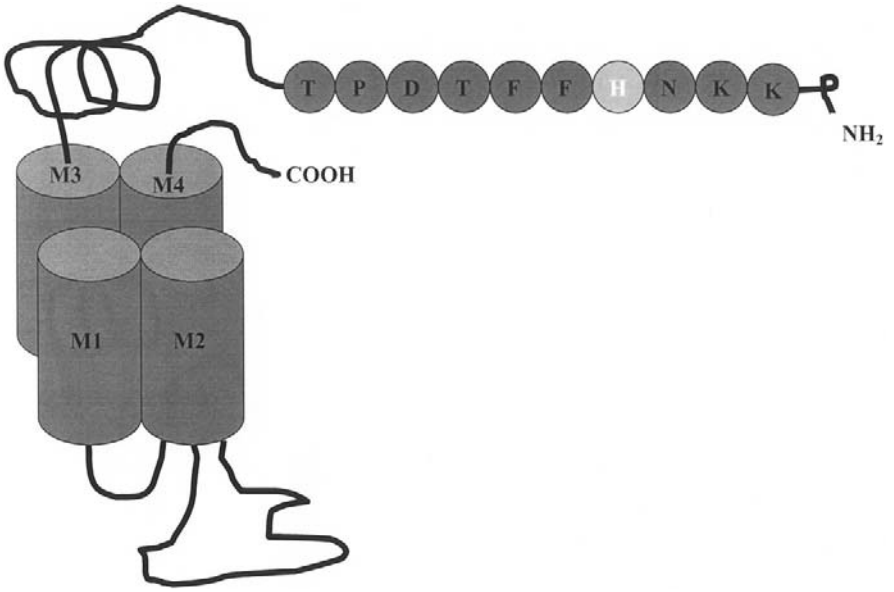

Figure 4 The α-subunit of the GABA-A receptor containing the benzodiazepine-binding domain. A conserved histidine residue at position 101 of the binding domain is indicated. Point mutations that convert the histidine to arginine result in loss of binding of the classical benzodiazepines, such as diazepam. The four transmembrane-spanning domains of the α subunits are represented as the cylinders (α-helical protein structures) M1, M2, M3, and M4.

These findings clearly implicate GABA-A receptor subtypes in the mechanism of action of benzodiazepines and hold promise for the development of selective antianxiety agents based on specific neuronal receptor targets. Furthermore, developments at the molecular level suggest that a refinement of the understanding of the neurobiology and genetics of anxiety disorders may follow.

A unique feature of the GABA-A benzodiazepine receptor system is its bidirectional agonism [102]. Conventional benzodiazepines act as agonists at the receptor while both antagonists and inverse agonists have also been identified. Compounds acting as inverse agonists at the receptor, such as the β-carboline FG7142, are anxiogenic in humans [111]. Observations such as these led to the notion that endogenous compounds may play a role in the genesis of anxiety states. Several compounds have been proposed. Tribulin, a low-molecular-weight compound found in normal human urine [112], has been shown to be increased in some anxiety disorders and in lactate-induced panic attacks [113]. Diazepam-binding inhibitor (DBI), an 86-amino-acid polypeptide, and one of its fragments, octadecaneuropeptide (ODN), have also been proposed as inverse agonists [114]. The demonstration that the benzodiazepine antagonist, flumazenil, was-anxiogenic in panic patients suggests that an endogenous agonist may occupy the receptor site [115]. Endogenous neurosteroids, including allopregnanolone (AP), allotetrahydrodeoxycorticosterone (THDOC), and 3-α-hydroxy-5-α-dihydroprogesterone (3-α-OH-DHP), produce modulatory effects by binding to steroid receptor sites on the GABA-A receptor complex, as shown in Figure 3 [116]. The metabolite of pregnanolone (AP) can exert a positive allosteric effect on GABA at the GABA-A receptor [117], while THDOC has been shown to reduce anxiety-related behaviors following i.c.v injection in the rat [118]. The role of such compounds in anxiety states in humans is attracting further interest both from the point of view of etiology and therapeutics.

An interesting observation has been the demonstration of the presence of the diazepam metabolite, N-desmethyldiazepam, in postmortem brain tissue obtained before the introduction of benzodiazepines into widespread clinical use [119]. It appears that such compounds may arise from dietary sources such as potatoes and other plants, and have been shown to contain this metabolite [105]. Perhaps the endogenous ligand for the benzodiazepine–GABA-A receptor is a benzodiazepine derivative itself. To date, there are no studies of the levels of N-desmethyldiazepam in patients with anxiety disorders.

XI. GENETIC ETIOLOGY

Several approaches to the study of the genetic etiology of anxiety disorders have been undertaken. These approaches are examined in more detail elsewhere in this volume in relation to specific anxiety disorders. Many studies have attempted to determine the extent to which anxiety disorders are familial and to estimate their heritability. Detailed reviews of the genetic epidemiology of anxiety states [120] and their molecular genetics [121] have appeared. Recently a meta-analysis of the genetic epidemiology of anxiety disorders have been published [122]. This study provides a concise summary of findings to date. The meta-analysis supported the notion that anxiety disorders aggregate in families, the greatest evidence being for panic disorder. The major source of familial risk of an anxiety disorder was shown to be genetic. This was supported by large twin studies in panic disorder, generalized anxiety disorder, and phobias. However, the estimated heritabilities across the disorders was a relatively modest 30 to 40%. The largest proportion of the

variance in the liability to an anxiety disorder is explained by environmental factors peculiar to an individual.

Notwithstanding their weaknesses, twin and family studies suggest that molecular strategies could be employed to identify specific genes associated with particular anxiety disorders. To date, this approach has not been widely employed [121]. As noted above, genes coding for the subunits of the GABA-A might be profitably explored. A proline-to-serine point mutation at residue 385 of the $\alpha 6$ subunit has been identified, which mediates sensitivity to benzodiazepines [123]. While this finding may be more relevant to alcoholism, it does illustrate the utility of the approach. A study of 8 polymorphic subunits of the GABA-A receptor in panic disorder showed no evidence of linkage [124]. Studies of the GABA-A subunit genes in other anxiety disorders may be more fruitful.

Given the putative involvement of many neurotransmitters in the etiology of anxiety disorders and their numerous receptor targets there is no doubt that further studies for candidate genes in anxiety disorders will evolve. This approach coupled with linkage studies and animal models (such as knockout and knockin strains as described above) have the potential to identify important genes for the etiology of anxiety disorders. It is unlikely that a single gene will completely explain the etiology of any one anxiety disorder. However, genetic techniques may uncover previously unheralded therapeutic targets.

XII. CONCLUSION

Freud [6] remarked that anxiety was

> . . . a riddle whose solution would be bound to throw a flood of light on our whole mental existence.

Advances in the understanding of the function of the brain in recent years has seen a renewed focus on the nature and treatment of anxiety, as well as an interest in the classification of anxiety states. The latter continues to be a subject of debate between the advocates of a General Neurotic Syndrome [125] and those advocating separate disorders [126]. This has not been a barrier to intensive research on the neurobiology and neurocircuitry associated with anxiety. The neurobiological approach proceeds from the premise that underlying a psychological dysfunction is a biological one. Knowledge of the relationship between functional processes in the brain and symptoms of anxiety is still evolving. This evolution has been accompanied by a greater understanding of the neural circuits involved in anxiety states. The amygdala now appears to be an important mediator of some facets of the conditioned fear response in animals and perhaps in humans, while the septo-hippocampal system and its connections to the PAG region also plays a role in mediating the anxiety response. Continued research in animal models of anxiety states as well as neuroimaging studies in humans should provide a greater understanding of these aspects of anxiety, as well as provide new neural targets for therapeutics. Genetic studies suggest a familial pattern of inheritance for anxiety disorders, but specific genes for individual disorders have not been identified. Family and twin studies suggest that environment makes a significant contribution to the etiology of anxiety states. This suggests that the psychological approach to the understanding of the etiology of anxiety, far from being in abeyance, can offer many insights. Both psychological and social approaches to anxiety simply represent another perspective on the problem. Understanding anxiety based on the neurobiological, neuropsychological, and psychological point of view promises a greater depth than in the past. Coupled with knowledge of the mechanism of action of effective

treatments, further progress toward the etiology of the disorders should be forthcoming. These endeavors are beginning to unravel the mystery that is anxiety.

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29

Animal Models of Anxiety and Anxiolytic Drug Action

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I. INTRODUCTION

A. Animal Models and Anxiolytic Drugs

Animal models of anxiety attempt to represent some aspect of the etiology, symptomatology, or treatment of human anxiety disorders in order to facilitate their scientific study [146,154,220]. Within this context, animal models of anxiolytic drug action can be viewed as treatment models relevant to the pharmacological control of human anxiety. A major purpose of these models is to identify novel anxiolytic compounds and to study the mechanisms whereby these compounds produce their anxiolytic effects (for reviews, see Refs. 10,37,45,64–66,79,132,137,192,202,209,218–221).

B. Correlation, Isomorphism, and Homology

Validation of behavioral animal models of psychotherapeutic drug action has typically proceeded along three lines: correlation, isomorphism, and homology [221]. A correlational model (or "screening test") is selectively sensitive to target therapeutic agents (e.g., diazepam), so that target compounds can be distinguished from nontarget compounds. An isomorphic model implies that the animal's "anxiety" response is in some way similar to a human "anxiety" response (e.g., avoidance), and a homologous model implies that the cause of an "anxiety" response in the animal is sufficient to cause an anxiety response in humans (e.g., the threat of an aversive stimulus). Isomorphism and homology are important criteria because *some* functional similarity between the animal model and human anxiety is a logical requirement if the model is used to study the mechanisms of a drug's *anxiolytic*

action [221]. Whether these "anxiety" reactions in animals express a subjective state that is similar to that experienced by anxious humans, or whether these reactions lie on the same continuum as "abnormal" human anxiety conditions, are difficult but important questions (for an insightful discussion of this issue, see Ref. 133). For animal models of anxiety, however, one simplifying assumption is that aversive stimulation, real or anticipated, can produce anxiety-like behaviors in both humans and in infrahumans, and these should be diminished by antianxiety agents [220].

Historically, the correlational (or pharmacological) validation of animal models of anxiety has been a preeminent concern, and even today is initiated on the basis of their relative sensitivity to a few, standard, clinically proven compounds, such as the 1,4-benzodiazepines (e.g., diazepam). However, the triazolobenzodiazepines (e.g., alprazolam) are closely related to standard 1,4-benzodiazepines and produce anxiolytic as well as antidepressant effects in humans [57,117,190], as do $5HT_{1A}$-type agents such as buspirone [12,86,101,105,113,187,188] and classical antidepressant drugs such as imipramine [2,127,189,233]. The relative sensitivity of animal models to various classes of clinically proven anxiolytic drugs is an important issue and will be the focus of the present review.

II. ANIMAL MODELS OF ANXIETY

For convenience, the models reviewed in the following sections are grouped into two categories. The first category involves animals' reactions to nonpainful stressors (e.g., exposure to a novel test chamber). The second category involves animals' reactions to painful stressors (e.g., exposure to electric foot-shock). The models will be evaluated in terms of their relative sensitivity to "classic" anxiolytic agents (e.g., benzodiazepines), $5HT_{1A}$ compounds (e.g., buspirone), and antidepressants (e.g., imipramine).

Comparable sensitivity to the effects of benzodiazepines, $5HT_{1A}$ anxiolytics, and antidepressants could be taken as support for the validity of a test as a *general* model of anxiolytic drug action, because all three of these drug classes are effective in the treatment of human anxiety disorders (see above). On the other hand, a model sensitive to only one drug class (e.g., benzodiazepines) is "class-specific," but may nevertheless be important (e.g., in the preclinical testing of benzodiazepine-type anxiolytics, and for studying the mechanisms whereby these drugs produce their effects) [79,149]. Thus, while general models of anxiolytic drug action are of particular interest, class-specific models should not be discounted (see Ref. 97 for a review of the pharmacology of benzodiazepine-type anxiolytics, and their actions at the $GABA_A$-receptor subtype; 178 for a review of the pharmacology of buspirone-type anxiolytics and their interactions at the $5HT_{1A}$-receptor subtype; and Ref. 78 for a review of the pharmacology of antidepressant drugs and their actions on monoamine neurotransmission).

A. Models Based on Reactions to Nonpainful Stressors

1. Light–Dark Exploration

In the light–dark exploration test, rodents (typically mice) normally avoid the brightly lit side of a two-compartment chamber, preferring instead to explore the dimly lit side. Anxiety reduction is indicated by increased transitions between the two compartments, and/or increased exploration of the brighter compartment, whereas nonspecific effects are indicated by changes in general locomotor behavior [11,36–39]. Early studies showed that a

variety of benzodiazepine anxiolytics, including diazepam, clonazepam, flurazepam, and chlordiazepoxide, produced dose-dependent increases in the number of light/dark transitions in mice, whereas an antipsychotic (chlorpromazine) did not [36,38]. The relative potency of benzodiazepine anxiolytics in increasing "exploratory" transitions was consistent with their relative potency in the clinical treatment of human anxiety [36]. Furthermore, a nonspecific stimulatory effect of anxiolytics on general activity did not explain these increases in light–dark transitions [38].

Later studies showed that standard anxiolytics also increase exploratory activity in the lit section of light–dark test chambers, or conversely, decrease exploration in the dark chamber, while a number of nonanxiolytic agents do not produce this pattern of results [29,31–33,126]. This suggests that the light intensity of the brightly lit compartment is aversive, suppressing exploration, and that this suppression is selectively antagonized by standard anxiolytics [88,210].

Like traditional benzodiazepines, the mixed anxiolytic-antidepressant drug alprazolam also increased side transitions and time spent on the bright side of a light–dark apparatus [102,103,210]. Standard antidepressant drugs, such as the tricyclic (TCA) imipramine, the monoamine oxidase inhibitor (MAOI) moclobemide, and the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, paroxetine, and citralopram, have also yielded anxiolytic-like effects in some cases [13,47,48,104,198]. However, in other cases, null or even anxiogenic effects have been found [89,130,200,210,242]. In contrast, $5HT_{1A}$ compounds such as buspirone, ipsapirone, and indorenate have had fairly uniform, anxiolytic effects in the light–dark exploration test [20,31,33,58,126,170,199]. Only in a few cases, however, has the magnitude of the effects of $5HT_{1A}$ agents been comparable to that of benzodiazepines.

In summary, the light–dark exploration test is reliably sensitive to the anxiolytic effects of benzodiazepines across a wide range of doses [37]. Although the effective dose ranges tend to be narrower for $5HT_{1A}$ agents, the test is sensitive to these compounds as well, particularly buspirone and ipsapirone [198,210,242,243]. Finally, the effects of antidepressant drugs on light–dark exploration are inconsistent, but clear anxiolytic effects have been registered in some studies.

2. Social Interaction

In this test, pairs of rats are placed in an open arena, and the time they spend in active social interaction (e.g., sniffing, grooming) is measured. Social interaction is suppressed when animals are tested under bright lights or in an unfamiliar test environment, relative to low light/familiar conditions, and this suppression is the index of anxiety [68]. As a control for nonspecific changes in locomotor activity, line crossings are also measured (for reviews, see Refs. 64–66). Several benzodiazepine anxiolytics, including chlordiazepoxide, diazepam, and flurazepam, all antagonized the suppression of social interaction under unfamiliar, high light conditions, without producing concomitant changes in motor activity. Other compounds, such as neuroleptics and CNS stimulants, did not produce selective anxiolytic effects on social interaction [67,68,70–73].

Using a modified procedure in which novelty of the partner is the primary anxiogenic stimulus, Gardner and Guy [83] and Guy and Gardner [95] found increases in social interaction after administration of a number of different benzodiazepines (e.g., diazepam, oxazepam, loprazolam, nitrazepam) at doses that did not significantly affect ambulation. Social interaction in this modified test was not selectively affected by other centrally active agents

(e.g., phentolamine, metergoline, propranolol, amphetamine). These results also suggest that social interaction under conditions of novelty is very sensitive to the effects of benzodiazepine-type anxiolytics.

Antidepressant drugs, however, have inconsistent effects on social interaction. File and colleagues [74,116] reported that the mixed anxiolytic-antidepressant drug alprazolam did not produce anxiolytic effects on social interaction, whereas Gardner and Guy [83] reported that alprazolam had anxiolytic effects in their version of the test. Similarly, Pellow and File [176] found that the TCA imipramine was not anxiolytic after either acute or chronic administration, whereas Popik and Vetulani [185] found that chronic imipramine was anxiolytic. The MAOI antidepressant phenelzine produced an *anxiogenic* profile in the social interaction test even after chronic administration [116], whereas mianserin, a tetracyclic antidepressant, produced a significant anxiolytic profile [124]. SSRIs such as paroxetine and sertraline can have significant anxiolytic effects on social interaction after chronic administration [54,123,135].

Although there are exceptions [64], $5HT_{1A}$ agents such as buspirone generally have reliable, benzodiazepine-like profiles in the social interaction test [30,42,53,95,98,179].

In summary, the social interaction test, like the light–dark exploration test, is reasonably sensitive to both benzodiazepine and $5HT_{1A}$-type compounds; the effects of antidepressants are inconsistent. These tests may be analogous to human anxiety to the extent that avoidance of novel social or environmental stimuli is common in both rodents and humans.

3. Elevated Plus Maze

In the elevated plus maze, rodents normally avoid the two open arms of the maze, and restrict most of their activity to the two closed arms. Open-arm avoidance appears to be driven by an aversion to open spaces, leading to thigmotaxic behavior [229]. An antianxiety effect is indicated by an increase in the proportion of activity in the open arms of the maze (i.e., an increase in the percentage of time spent in the open arms and in the percentage of entries into the open arms). Changes in total entries and/or changes in the number of closed-arm entries indicate nonspecific drug effects on locomotor activity. (For reviews of procedures and methods, see Refs. 109, 173). Benzodiazepine anxiolytics (e.g., chlordiazepoxide, diazepam) increase the proportion of activity in the open arms, whereas nonanxiolytic agents (e.g., amphetamine, caffeine) generally do not [3,100,114,115,138, 174,176].

Mixed anxiolytic-antidepressant compounds such as alprazolam also have reliable anxiolytic effects in the elevated plus maze [74,90,116,118,186]. However, the effects of standard antidepressant drugs in the plus maze have been inconsistent. On the one hand, both acute and chronic administration of TCAs (imipramine, amitriptyline) failed to produce anxiolytic profiles in the plus maze [23,69,138,142,174]. SSRIs such as fluoxetine have been reported to be *anxiogenic* [99,128,184,211,212], *anxiolytic* [16,89,92,131], or *ineffective* [99,136,194]. These inconsistent effects have been found after both *acute* [92,211] and *chronic* [131,212] drug administration.

On the other hand, the tetracyclic antidepressant mianserin produced significant anxiolytic effects after chronic administration [191], and MAOIs such as phenelzine and befloxatone produced anxiolytic effects whether given acutely [17,171] or chronically [116].

The effect of $5HT_{1A}$-type compounds (e.g., buspirone, ipsapirone, gepirone) in the elevated plus maze are also mixed. There are reports of clear anxiolytic effects [18,40,53,

87,91,98,106,151,182,214], and reports of no clear anxiolytic effects [24,40,41,175,177, 211], even after chronic drug administrations [156,157,193].

Although chronic regimens with buspirone or ipsapirone did not produce anxiolytic effects in the plus maze [156,239], there is some evidence that these negative findings may have been related to dose. Soderpalm et al. [215] found that 5 weeks of buspirone (10 mg/kg b.i.d.) significantly increased open-arm activity whereas the same regimen at lower doses (2.5 or 5.0 mg/kg) was without effect. A number of other studies support the hypothesis that high doses of $5HT_{1A}$ compounds may be necessary for their anxiolytic effects to emerge after chronic treatment in the elevated plus maze [22,143,158,211].

In summary, the elevated plus maze is clearly sensitive to benzodiazepine-type anxiolytics. However, the effects of antidepressant drugs (both chronic and acute) are mixed, as are the effects of $5HT_{1A}$ compounds. There is some evidence that high doses of chronically administered $5HT_{1A}$ compounds may be necessary to detect their anxiolytic effects in the elevated plus-maze.

4. Separation-Induced Ultrasonic Distress Vocalization

Rat pups separated from their mother and littermates emit high-frequency (30–50 kHz) distress calls which, in turn, elicit retrieval behavior from the mother [165]. Distress calls are infrequent just after birth, increase up to maximal levels at about 10 days of age, and then decrease to minimal levels at about 17 days of age. The eliciting stimulus (separation), under certain conditions (e.g., dependency), would seem capable of producing fear or anxiety in humans. An anxiolytic drug effect in this response system is indicated by a significant reduction in high-frequency vocalization in the absence of behavioral sedation [56,80,81,112].

Separation-induced ultrasonic vocalizations are suppressed by benzodiazepine anxiolytics (e.g., diazepam, chlordiazepoxide) at doses that do not disrupt behavior in general, while other agents (e.g., amphetamine, haloperidol, naloxone, clonidine) do not selectively suppress vocalization [8,75,80,81,167]. A possible false positive was metergoline, which inhibited ultrasound at doses that were not behaviorally impairing, as did morphine [19].

Benton and Nastiti [8] found that ultrasonic calling in mouse pups was also inhibited by the $5HT_{1A}$-type anxiolytic ipsapirone, and they provided data that this inhibition was not related to drug-induced decreases in body temperature. Kehne et al. [122] and Nastiti et al. [160] found that buspirone, ipsapirone, and gepirone suppressed ultrasonic vocalization in preweanling rats at doses that did not disrupt motor coordination on an inclined screen test. The selective $5HT_{1A}$ agonists flesinoxan and 8-OH-DPAT also inhibited ultrasonic vocalization, although some degree of hypothermia and sedation was associated with these effects [168], particularly at higher doses [75].

Alprazolam has potent suppressive effects on ultrasonic calling in separated pups [153]. Separation-induced vocalization in pups is also reliably reduced by a number of antidepressant compounds such as clomipramine, fluvoxamine, fluoxetine, and zimelidine [168,169], all of which block the reuptake of 5HT to varying degrees. In contrast, antidepressants that are more selective for blockade of NE reuptake (e.g., desipramine and maprotiline) actually *increase* distress vocalizations [237]. Although it seems possible that the inhibition of ultrasonic vocalizations by antidepressants depends specifically on their ability to inhibit 5HT reuptake, Olivier et al. [168] reported that the 5HT reuptake stimulant, tianeptine, also blocked ultrasonic vocalizations. In any case, the results thus far suggest that separation-induced vocalizations in young rodents are sensitive to the anxiolytic effects of both benzodiazepine and serotonergic drugs.

B. Models Based on Reactions to Painful Stressors

1. Shock-Probe Burying

Rats shocked once from an electrified probe that is attached to the wall of a test chamber characteristically spray bedding material toward or over the probe, with rapid, alternating movements of the forepaws (i.e., "burying behavior") [181], while avoiding further contact with the shock probe. A reduction in the duration of probe burying, in the absence of a decrease in general activity, is used as the primary index of anxiety reduction [230]. An increase in the number of probe contacts is sometimes used as a second index of anxiety reduction [224]. (For reviews of methods and procedures, see Refs. 221, 228.)

Low doses of standard anxiolytic agents (e.g., chlordiazepoxide, diazepam) reduce the amount that rats bury the shock probe without suppressing general activity [7,9,195,221–225,227,230,231]. Chlordiazepoxide also antagonizes the shock-induced elevations in plasma corticosterone and adrenaline that occur during the burying test [49]. The effects of anxiolytic compounds on probe burying can be distinguished from those of several nonanxiolytic agents [7,195,225,230–232], although the drug-class specificity of the test is sensitive to procedural variations [15,35,163,224,230]. The suppression of burying behavior by benzodiazepine anxiolytics does not appear to be secondary to analgesia [223] or associative deficits [9].

An early study [227] found that buspirone, like chlordiazepoxide, decreased the amount of probe burying and concurrently increased the number of contact-induced probe shocks rats received. These bidirectional, anxiolytic drug effects increased as a function of drug dose and were independent of changes in general activity [227]. Higher doses of buspirone (64 mg/kg), which suppress general activity, do not produce selective effects on either probe avoidance [150] or probe burying [35].

Later studies [129] reported that ipsapirone also reduced burying behavior in the absence of effects on general activity. Similar results have been found in an extensive series of experiments by Lopez-Rubalcava et al. [141] and by Fernandez-Guasti and colleagues [59,61–63,139,140]. These researchers found dose-dependent decreases in the burying behavior of both male and female rats after the administration of buspirone, ipsapirone, indorenate, and the selective $5HT_{1A}$-receptor agonist 8-OH-DPAT. Flesinoxan, another selective $5HT_{1A}$-receptor agonist, was anxiolytic in the shock-probe burying test after either acute [94] or chronic [93] administration.

Little work has been conducted examining the effects of antidepressants in the shock-probe burying test, and the results thus far are equivocal. One study [7] showed that chronic treatments with imipramine, desipramine, and pargyline did not produce significant effects on burying behavior, whereas two other studies [60,144] showed that chronic desipramine reliably suppressed burying behavior. The mixed SSRI/$5HT_{1A}$-agonist EMD 68843 produced a selective, dose-dependent reduction in burying behavior after acute administration [226]. Whether the latter result was due to serotonin reuptake inhibition, $5HT_{1A}$-receptor activation, or some combination of both is not known, but future studies should clarify this interesting finding.

Broekkamp et al. [15] and Njung'e and Handley [163,164] described a variant of the burying test in which mice are individually placed in a test chamber with glass marbles evenly distributed across the surface of the bedding material. The number of marbles "buried" is used as the index of anxiety. Various anxiolytics reduced marble "burying," including diazepam, chlordiazepoxide, flunitrazepam, clonazepam, and meprobamate. However, false positives were found for scopolamine and atropine. Although $5HT_{1A}$-type compounds (e.g., buspirone) suppressed marble-burying behavior, the effect only occurred

at doses that also reduced general activity [164] and disappeared after chronic treatment [111].

The effects of cyclic antidepressants on mouse marble burying have been somewhat unclear. Thus, for example, imipramine and mianserin suppressed marble burying, but at doses that also suppressed general activity [15]. Ichimaru et al. [111] found that marble burying was selectively suppressed by clomipramine but not by desipramine. The effects of SSRIs in this paradigm have been more uniform. Njung'e and Handley [163,164] and Ichimaru et al. [111] found that fluvoxamine, zimeldine, and citalopram dose-dependently inhibited marble-burying behavior without suppressing general activity.

Gyertyan [96] suggested that marble burying in mice is not burying behavior per se, but rather digging/burrowing behavior, a species-typical response in mice that occurs in the absence of any observable aversive or threatening stimulus. In this sense, digging behavior might be more analogous to compulsive behavior in humans and thus mouse digging may represent a model of obsessive-compulsive disorder [96]. Although some of the pharmacological data reviewed above might be consistent with this view, insofar as certain antiobsessional compounds (e.g., clomipramine) were effective in suppressing marble burying, further work is needed before this hypothesis can be supported.

2. Fear-Potentiated Startle

The magnitude of rats' startle reflex to an acoustic stimulus can be potentiated by presenting the acoustic startle stimulus together with a cue (e.g., light) that has previously been paired with shock (for a review, see Ref. 44). An anxiolytic drug effect in this paradigm is most clearly indicated by a specific reduction of potentiated startle (i.e., a reduction of startle on light-noise trials versus a reduction of startle on noise-alone trials) [44]. A number of benzodiazepine anxiolytics, including diazepam, flurazepam, and midazolam, produced selective, dose-dependent reductions of fear-potentiated startle [43,107], whereas a number of other agents, including cinanserin and p-chloroamphetamine, did not block potentiated startle [21,46]. However, false positives have been reported for morphine [44] and haloperidol [108].

Although the mixed anxiolytic-antidepressant drug alprazolam reduced fear-potentiated startle [108], studies using standard antidepressant compounds such as imipramine, amitriptyline, and fluvoxamine have failed to demonstrate reliable effects on fear-potentiated startle [21,108,119].

In contrast, fear-potentiated startle is reliably suppressed by $5HT_{1A}$-type anxiolytics. Buspirone, gepirone, and ipsapirone each blocked potentiated startle across a wide range of doses [46,121,145,161]. In addition, the magnitude of these anxiolytic effects were similar to those of benzodiazepine anxiolytics. The selective $5HT_{1A}$ agonists flesinoxan and 8-OH-DPAT also produced dose-dependent anxiolytic effects on fear-potentiated startle [119,120].

Thus, the fear-potentiated startle test seems to be sensitive to the anxiolytic effects of both benzodiazepine and $5HT_{1A}$ compounds, but insensitive to traditional antidepressant agents. Given the positive effect found with the mixed anxiolytic-antidepressant drug alprazolam, however, more extensive work using chronic administration of traditional antidepressants may ultimately reveal anxiolytic effects of some of these compounds as well.

3. The "Conflict" Tests

Although there are many variants of conflict tests, they generally fall into one of two categories. In the Geller-type conflict tests, well-trained rats are tested on alternating schedules of unpunished (reward only) and punished (reward + shock) responding [84].

In Vogel-type conflict tests, untrained, water-deprived rats are allowed to drink from a water spout where every *n*th lick is accompanied by shock [234]. In both types of tests, responding is suppressed by shock and "anticonflict" (i.e., anxiolytic) effects are indicated by increases in punished responding, whereas nonspecific effects (e.g., hyperphagia, dipsogenesis) are indicated by increases in unpunished responding. Additional tests are sometimes conducted to assess treatment effects on pain perception (e.g., flinch/jump thresholds to shock). (For a review, see Ref. 183.)

A variety of benzodiazepine-type anxiolytics produce reliable anticonflict effects, whereas other compounds such as neuroleptics, analgesics, and stimulants do not. This anticonflict effect of standard anxiolytic drugs has been demonstrated in a variety of species, including humans [134], and the relative potency of various anxiolytics in suppressing conflict behavior in animals agrees well with their relative potency in treating human anxiety disorders [28]. Thus, the conflict test appears to satisfy many of the criteria of a correlational model. In addition, insofar as conflict between opposing drives may be associated with human anxiety, conflict models may also have some degree of isomorphism and homology.

The mixed anxiolytic-antidepressant drug alprazolam produced reliable, dose-dependent anticonflict effects in rats across a wide range of doses [55,85,102,125,196, 213]. Furthermore, the anticonflict effect of alprazolam was apparent after both acute and chronic administration.

A number of standard antidepressant drugs such as the TCAs imipramine, desipramine, and amitriptyline all produced significant anticonflict effects after chronic or subchronic administration, but not after acute administration [25,76,77]. Chronic bupropion, mianserin, and trazodone also produced significant anticonflict effects in this test [26], as did the MAOIs phenelzine and pargyline [27,77]. These findings suggest that conflict tests may be quite sensitive to the anxiolytic effects of antidepressant drugs under chronic drug regimens.

In contrast to their reliable sensitivity to the anxiolytic effects of benzodiazepine and antidepressant drugs, the conflict tests have shown erratic sensitivity to the anxiolytic effects of $5HT_{1A}$ drugs such as buspirone [110]. Furthermore, when anxiolytic effects of $5HT_{1A}$ drugs are detected, their magnitude is often smaller than that of standard anxiolytics such as diazepam and typically occur over a much narrower dose range [50,51,148,152, 180,203,204,235,241,244]. Howard and Pollard [110] studied the effects of buspirone in the conflict test under a wide variety of experimental conditions and failed to find a robust anxiolytic effect under any condition. Other researchers have reported no significant effects of buspirone in the conflict tests or even dose-related *decreases* in punished responses [14,34,82,125,147,201,217,238].

In contrast to the inconsistent anticonflict effects of buspirone-type anxiolytics in rats in the conflict tests, these drugs have very reliable anticonflict effects in pigeons [4,159,205]. The reasons for these species differences are unknown.

Thus, conflict tests appear to possess broad utility for detecting the anxiolytic effects of benzodiazepine and antidepressant drugs, although the exact conditions under which reliable anxiolytic effects of $5HT_{1A}$ compounds can be detected have not been determined.

4. Shock-Induced Ultrasonic Vocalization

Another ultrasonic vocalization model has been developed in which adult rats are given multiple, inescapable foot shocks [52]. Testing occurs after this "training" period. During the test period, foot shocks are again administered and the duration of ultrasonic vocaliza-

tions is measured during intershock intervals. The duration of these vocalizations is used as the index of anxiety. Shock-induced ultrasonic vocalizations are dose-dependently suppressed by benzodiazepine-type anxiolytics (diazepam and chlordiazepoxide), but are generally unaffected by nonanxiolytic agents [52,162]. The test also appears to be quite sensitive to $5HT_{1A}$-type drugs such as buspirone, ipsapirone, gepirone, and 8-OH-DPAT, all of which dose-dependently suppress shock-induced ultrasonic vocalizations [5,6,52,155, 197,199,216,240].

A variety of antidepressant drugs, including the TCAs imipramine, amitriptyline, doxepin, clomipramine, and the MAOI tranylcypromine, also reduced shock-induced ultrasonic vocalizations. Antidepressants that are more selective inhibitors of NE or DA reuptake, such as maprotiline and bupropion, were not effective [52]. SSRIs such as paroxetine, citalopram, sertraline, and fluvoxamine suppressed shock-induced vocalizations to varying degrees [52,200,206], although the effects of fluoxetine have been inconsistent [5,200]. Nevertheless, the overall pattern of these results seems to point toward inhibition of 5HT reuptake as one mechanism for the inhibition of shock-induced ultrasonic vocalizations in adult rats. In addition, the model appears to have broad utility for studying the mechanisms of action of benzodiazepine and $5HT_{1A}$-type drugs.

III. SUMMARY AND CONCLUSIONS

In summary, the models reviewed in this chapter show at least some sensitivity to a variety of agents known to produce anxiolysis in humans (i.e., the benzodiazepines, antidepressants, and $5HT_{1A}$ compounds). All of the models show good sensitivity to benzodiazepine anxiolytics. Light–dark exploration, social interaction, elevated plus maze, shock-probe marble burying, and the conflict tests have also shown some sensitivity to antidepressants and $5HT_{1A}$ compounds, but to varying degrees. Ultrasonic vocalization appears to be the most broadly sensitive measure. Fear-potentiated startle, although sensitive to both benzodiazepine and $5HT_{1A}$ anxiolytics, has thus far failed to detect the anxiolytic effects of traditional antidepressants.

While the majority of these models showed at least some sensitivity to antidepressant and $5HT_{1A}$ compounds, the anxiolytic effects of these drugs were often more variable than the effects of benzodiazepine anxiolytics. In addition, there were a number of instances in which antidepressant and $5HT_{1A}$ agents produced effects opposite to those of standard anxiolytics, suggestive of an anxiogenic action. There are several possible explanations for these inconsistencies which have more general implications for animal models of anxiety and anxiolytic drug action.

A drug may have very reliable effects in an animal model of anxiety, but unless that drug also has reliable antianxiety effects in humans, it cannot be used to validate the animal model. Conversely, a drug that has inconsistent or unreliable anxiolytic effects in humans cannot be used to invalidate an animal model of anxiety. In this regard, there is little clinical evidence that $5HT_{1A}$ agents, other than buspirone, produce reliable antianxiety effects in humans, and even the effects of buspirone appear to be more variable than the effects of benzodiazepine anxiolytics [172,207,208,236]. A number of clinical trials [166,207] suggest that the efficacy of buspirone across different human anxiety disorders [1] is not as robust as the benzodiazepines (for a recent summary of comparative clinical findings see Ref. 2, Table 1, p. 224). These clinical data are certainly not definitive but, if anxious humans respond more variably to buspirone than to benzodiazepine anxiolytics,

one might expect the effects of $5HT_{1A}$ agents in animal models of anxiolytic drug action to be more variable than the effects of benzodiazepines.

The clinical efficacy of antidepressant drugs in the treatment of anxiety disorders is far more convincing, but there is still some variation in efficacy (for a review, see Ref. 233). There is also some disagreement about whether specific antidepressants are required for particular anxiety disorders (e.g., agoraphobia, panic), or are superior to benzodiazepine anxiolytics for these disorders. Furthermore, anxiety and depression in humans often overlap so that interpretation of a therapeutic drug effect as being either anxiolytic or antidepressant can sometimes be difficult. Perhaps the most important clinical finding in this literature, however, is that unlike classical benzodiazepines, the anxiolytic effects of traditional antidepressants in humans are normally delayed (2 to 4 weeks), and the initial (acute) response may sometimes be an exacerbation of anxiety [2]. Thus, acute antidepressant treatment in an animal model of anxiety may be of questionable relevance to its pharmacological validation.

Chronicity may be equally relevant to the effects of $5HT_{1A}$ compounds in these models. Whereas chronic administration of $5HT_{1A}$ or antidepressant drugs often resulted in reliable, anxiolytic effects in a variety of animal models [26,54,60,76,89,123,131, 135,185,191,204, 215,241], acute administration resulted in less reliable anxiolytic effects or even anxiogenesis [89,99,156,211,213].

Another possibility is that different animal models represent qualitatively different types of anxiety or fear, only some of which are reliably inhibited by $5HT_{1A}$ agents or antidepressants. Thus one could speculate that the social interaction test primarily reflects a type of social phobia, which is reliably suppressed by $5HT_{1A}$ agents and certain antidepressants, whereas the elevated plus-maze test reflects a type of acrophobia, which is not as reliably suppressed by $5HT_{1A}$ agents or antidepressants. This would imply that animal fears can be pharmacologically dissected, which in turn would support the pharmacological dissection of human anxiety. Although such speculation seems to be consistent with some of the animal data reviewed in this chapter, at this time there is no convincing clinical evidence that specific anxiety disorders are differentially affected by benzodiazepine, $5HT_{1A}$, or antidepressant anxiolytics [2,233].

Thus, a number of factors, including clinical effectiveness, chronicity, and model type, may alter the correspondence between the effects of benzodiazepines, $5HT_{1A}$ agents, and antidepressants in animal models of anxiety. On the whole, however, the data summarized above suggest that there is enough correspondence between drug effects across these tests that future paradigmatic studies may ultimately establish their validity as general models of antianxiety drug action. While this ideal may not be attained by all models, it should be remembered that class-specific models can also serve as a valuable tool for studying the mechanisms by which either benzodiazepine, $5HT_{1A}$, or antidepressant drugs produce their anxiolytic effects.

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Provocation of Anxiety States in Humans and Its Possible Significance for the Pathogenesis of These Disorders

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I. INTRODUCTION

Panic attacks are the hallmark of panic disorder. However, they also occasionally occur in other mental disorders and in up to 35% of the general population [1]. Spontaneous panic attacks are incidental and unpredictable and, therefore, difficult to study [2]. Earlier studies focused on information about spontaneous panic attacks obtained from patients. However, this information, as Griez and Schruers [2] pointed out, is essentially subjective and entirely retrospective, and thus not very reliable and difficult to quantify. Another approach to the study of panic attacks has been the monitoring of various physiological changes during spontaneously occurring attacks. This monitoring confirmed the presence of major physiological changes, such as heart rate increase and finger temperature changes during self-reported panic attacks [3,4]. Nevertheless, monitoring physiological variables and biochemical changes during spontaneously occurring panic attacks is technically quite difficult. In addition, as Balon and colleagues [5] pointed out, changes in physiological measures have not always correlated well with subjective anxiety. Thus, even though monitoring various changes during spontaneously occurring panic attacks would be clearly the most scientifically and methodologically appropriate approach, it has not been applied in studies of pathophysiology of panic attacks.

Some of the early studies of anxiety states noticed various abnormal physiological and metabolic findings in subjects with panic anxiety. For instance, two studies [6,7]

reported that anxious patients produce more lactate with exercise than do control subjects, with the abnormality being greater in chronic neurocirculatory asthenia than in acute one [7]. Cohen and White pointed out the abnormal oxygen consumption and excessive lactate production during exercise in anxiety states in several articles [8]. This led Pitts and McClure [9] to test their hypothesis that anxiety during anxiety states might be directly related to the rise in blood lactate. They found that the majority of patients with anxiety neurosis in their study panicked during the infusion of sodium lactate. Their experiment ushered in a new era of studying panic anxiety—provocation of panic attacks in the laboratory. It should be noted that although Pitts' and McClure's study [9] is considered the beginning of the era of anxiety provocation in the laboratory, Wearn and Sturgis [10] used epinephrine to reproduce the symptoms of irritable heart in anxiety patients as early as 1919.

Provocation of anxiety in the laboratory allows for relatively easy monitoring of various physiological, biochemical, and lately even imaging changes during laboratory-induced panic attacks. Thus, for decades, this has been one of the main biological approaches to the study of panic anxiety. During the 1970s and 1980s, sodium lactate has been the most frequently studied provocative agent [11]. Other agents, such as β-carbolines [12], caffeine [13], carbon dioxide [14], cholecystokinin-tetrapeptide [15], epinephrine [16], flumazenil [17], fenfluramine [18], isoproterenol [19], *m*-chlorophenylpiperazine [20], norepinephrine [21], yohimbine [22], and other nonpharmacological paradigms (hyperventilation, catastrophic misinterpretation) [23] have also been used for provocation of anxiety in the laboratory.

This chapter will review the most frequently used paradigms of anxiety provocation in humans. Similar to the interest in other biological tests (e.g., dexamethasone suppression test), the interest in anxiety provocation in the laboratory has waxed and waned. Although provocation of anxiety states in humans has undergone significant development during the last decade, the findings from the previous decades provide the most important knowledge base. Thus, for each paradigm, we will review briefly the most important older findings and then focus on an update from the last decade.

II. SODIUM LACTATE

In a carefully designed double-blind study, Pitts and McClure [9] observed that 13 of 14 patients, but only 2 of 10 healthy controls, developed anxiety during the infusion of 0.5 M sodium lactate. Glucose in saline did not produce any attacks. Patients rated lactate-induced anxiety as very similar, if not identical, to their real-life attacks. Several other studies replicated the finding of lactate-induced anxiety in panic disorder during the late 1960s and early 1970s [24–26]. Kelly and colleagues [25] reported findings similar to those of Pitts and McClure [9]. Most of their patients with chronic anxiety neurosis (16 of 20) and 1 of 10 controls panicked during an infusion with 0.5 M sodium lactate. They also reported that one patient panicked during the saline lead-in infusion, but not during the lactate infusion (anticipatory anxiety?). Forearm blood flow and heart rate were higher during lactate than during saline infusion. Bonn and colleagues [26] used 1 M sodium lactate in a fairly large sample of 66 subjects. They emphasized the striking hypophosphatemia during lactate infusions, and reported that propranolol could not modify the symptoms produced by lactate. They were also the only ones who used lactate infusion as a treatment modality. They described marked improvement in subjects with intractable anxiety after twice-weekly "flooding" with sodium lactate. Other studies in the early 1980s

established that sodium lactate provoked anxiety in panic disorder patients [27,28] and less frequently in healthy controls [28].

A. Studies Focused on Reliability and Validity

The provocation of panic attacks in patients with defined anxiety neurosis or panic disorder has been reproduced in various laboratories around the world, under various conditions, and with different concentrations of sodium lactate (0.5 or 1.0 M) [11,24,25,26,29–37). Panic attacks in response to lactate infusion have also been demonstrated in patients with infrequent panic attacks not meeting the DSM-III criteria for panic disorder [38]. However, in one study [39], patients with a low frequency of panic attacks (one or less per month) did not respond positively to lactate. Though Ehlers and colleagues [32] argued that patients and controls respond to lactate similarly, most studies demonstrated that panic disorder patients respond to lactate differently than healthy controls (70–100% of patients showed a panic response versus 0–40% of controls in various studies). Cowley and Arana [11] thus calculated that the lactate-induced panic had a sensitivity of 67% and specificity of 89% in differentiating patients with panic disorder from nonpsychiatric subject controls (thus, in a group of subjects with a 50% prevalence of panic disorder, the positive predictive value of the test would be 86%).

Several studies addressed the issue of whether lactate-induced panic attacks and naturally occurring attacks were symptomatically similar or comparable. Liebowitz and colleagues [29] found that both lactate-induced and naturally occurring panic attacks were symptomatically similar. In a study by Balon and colleagues [40], patients rated both dextrose (presumably spontaneous) and lactate-associated attacks between moderately and very much similar to their usual attacks. Lactate attacks were rated slightly more similar, possibly because they were rated as more severe and were associated with more somatic symptoms of anxiety. Aronson and colleagues [37] also noted a high degree of similarity between lactate-induced and naturally occurring panic attacks. In the largest single cohort of lactate-infused panic disorder subjects [41], the vast majority of subjects rated the lactate-induced panic attacks as very similar to their typical panic attack. The overall severity level was very similar, and both the physical and anxiety symptoms were overwhelmingly rated as similar. However, a substantial minority of the lactate nonpanickers also rated the lactate infusion experience as similar to their "usual" panic attacks. Three symptoms—afraid in general, dyspnea, and desire to flee—were the most predictive and revealing of panic response to lactate [41].

Various studies examined the diagnostic specificity of lactate infusions. Only 1 of 7 obsessive-compulsive disorder but 26 of 48 panic disorder patients panicked during sodium lactate infusion in a study by Gorman and colleagues [42]. Similarly, only 1 of 15 patients with social phobia and 10 of 20 panic disorder patients panicked during lactate infusions in a study by Liebowitz and colleagues [43]. Sodium lactate induced flashbacks in all seven patients with post-traumatic stress disorder (PTSD), six of whom also met the criteria for panic disorder [44]. Sodium lactate also induced panic attacks in six of these PTSD patients [44]. Interestingly, sodium-lactate-induced flashbacks in six of seven PTSD subjects without comorbid panic disorder, but no panic disorder or healthy subjects reported flashbacks or other intrusive PTSD symptoms during lactate infusion in a study by Jensen and colleagues [45]. In the same study [46], five of seven subjects with PTSD experienced sudden onset of anxiety sufficient to meet the criteria for panic attacks. These panic attacks were phenomenologically similar to lactate-induced panic attacks induced in

panic disorder patients. Cowley and colleagues [47] reported that patients with generalized anxiety disorder reacted more like panic disorder patients during lactate infusions. This conclusion was criticized by Liebowitz and Hollander [48], who pointed out that only 1 of 9 generalized anxiety patients panicked with lactate in the Cowley and colleagues study [47] compared to 9 of 22 panic disorder patients.

Other diagnostic categories outside the realm of anxiety disorders have also been studied. Alcoholic patients with panic disorder had fewer panic attacks in response to lactate than nonalcoholic patients with panic disorder [49] in a study by George and colleagues. Alcoholics with panic attacks had a significantly higher rate of lactate-induced panic than alcoholics without panic attacks in a study by Cowley and colleagues [50]. In a study of bulimic patients by George and colleagues [51], none of the bulimic patients panicked during lactate infusion. In a study by Lindy and colleagues [52] 4 of 18 bulimics (one with a history of panic disorder) had a panic attack during lactate infusions. Finally, Pohl and colleagues [53] reported that four of eight bulimics experienced a panic attack during lactate infusions. All bulimics in this study had concurrent panic attacks. Cowley and colleagues [54] reported similar rates of panic response to lactate in patients with both primary depression and secondary panic attacks and patients with panic disorder. In another study [55], Cowley and colleagues also found that patients with major depression without panic attacks had a much lower response to lactate than did panic disorder patients. McGrath and colleagues [56] reported significantly higher response to lactate in depressed patients with panic attacks than in depressed patients without panic attacks. These findings were confirmed by Cowley and colleagues [57], Buller and colleagues [58], and Targum [59]. Sandberg and colleagues [60] reported that 7 of 13 women with marked premenstrual dysphoric changes and none of seven controls panicked while infused with sodium lactate. Finally, George and colleagues [61] reported that sodium lactate administration elicited intense emotional response—rage, panic, greater changes in speech, breathing, and motor activity—in perpetrators of domestic violence compared to nonviolent control subjects.

The majority of studies suggest that lactate-induced panic is associated with a recent history of panic attacks rather than the diagnosis of panic disorder. It seems to be a state rather than a trait marker. Lactate has a moderate sensitivity and good specificity in distinguishing subjects with panic attacks from controls and from patients without coexisting panic attacks [11].

B. Studies Focused on Etiology

Various theories on the etiology of panic hypothesize most frequently the involvement of the noradrenergic and serotonergic systems. Noradrenergic theories often focus on either α- or β-receptor systems. Other theories suggest abnormalities in chemoreceptor sensitivity and sudden changes in peripheral biochemistry, such as acute respiratory alkalosis. Most of the challenge studies focusing on the possible etiology of lactate-induced panic were performed during the 1980s.

1. Noradrenergic Mechanism

The role of the noradrenergic mechanism in the etiology of lactate-induced panic has been entertained by several authors [30,37,62–65]. Liebowitz et al. [30,63] hypothesized that lactate-induced panic involves central noradrenergic discharge, with inconsistent peripheral manifestation, and that a peripheral catecholamine surge is not the mechanism of lactate-induced panic. In their study, lactate-induced panic attacks were regularly accom-

panied by biological changes consistent with hyperventilation and central noradrenergic activation (elevated heart rate and lowered PCO_2 and bicarbonate levels). Elevations of plasma norepinephrine and cortisol were irregular, and changes in lactate, pyruvate, pH, phosphate, epinephrine, and diastolic blood pressure were not consistent during panic attacks. The findings on epinephrine levels during lactate infusions in various studies have been contradictory [27,30,31].

Patients who panicked during lactate infusions showed higher mean plasma 3-methyl-4-hydroxyethylene glycol (MHPG), a major metabolite of peripheral and central norepinephrine, and a higher anxiety rating at baseline in a study by den Boer and colleagues [66]. On the other hand, Pohl and colleagues [64] did not find plasma MHPG elevation at baseline, nor during lactate or isoproterenol infusions, in panic disorder patients and controls. Carr and colleagues [31] also found neither a significant increase of MHPG during lactate-induced panic nor any correlation between various biochemical and neuroendocrine variables and lactate-induced panic. Urinary output of homovanillic acid and 4-hydroxy-3-methoxymandelic acid (catecholamine metabolites) was decreased in patients with panic attacks and in controls during lactate infusions in a study by Clow and colleagues [35].

Propranolol, a β-receptor blocking agent, does not block panic attacks or prolong the time to panic during lactate infusions [62]. This finding casts doubt on a β-adrenergic hypothesis of panic as an explanation for lactate-induced panic. Intravenous propranolol reduces lactate-induced panic only negligibly, whereas intravenous diazepam is quite effective [37,65]. Clonidine pretreatment did not lower prelactate anxiety, but blocked lactate-induced panic in 4 of 10 subjects in a study by Coplan and colleagues [67].

Several studies focused on the response to lactate after successful treatment with various medications and its significance for the etiology of panic anxiety. Rifkin [68] demonstrated blockade of lactate-induced panic with tricyclic antidepressants. In a study by Fyer and colleagues [69], 7 of 13 patients panicked with lactate before treatment with tricyclic antidepressants, but none of them panicked with lactate while treated. This suggests that lactate vulnerability can exist in clinically well, unmedicated patients and may be a trait characteristic. Successful alprazolam therapy also blocked lactate vulnerability in studies by Liebowitz and colleagues [63] and Cowley and colleagues [70]. In another study, Liebowitz and colleagues [71] also reported that pretreatment with diazepam (5 mg i.v.) significantly attenuated the effects of lactate-induced panic in panic disorder patients, although it did not block the lactate-induced panic in a majority of patients. Gorman et al. [72] reported a detailed analysis of patients reinfused with lactate after treatment with various antipanic medications. They suggested that the nine symptoms that responded to drug therapy were associated with hyperventilation, and that antipanic drugs may have a specific effect in blunting hyperventilation. Yeragani and colleagues [73] also reported that tricyclic antidepressants appeared to increase the threshold for lactate-induced panic. Pohl and colleagues [74] found that panic disorder patients treated with either imipramine or diazepam had significantly less lactate-induced anxiety than placebo-treated patients when reinfused with lactate. Imipramine also decreased post-treatment panic attack frequency and diazepam decreased the perceived severity of post-treatment lactate-induced panic. Interestingly, in a study by Keck and colleagues (75), valproate blocked reinduction of panic symptoms on lactate rechallenge in 83% of patients who initially experienced panic symptoms on initial infusion. Thus, in summary, several studies demonstrated successful blockade of lactate-induced panic with various psychotropic drugs. However, none of the above-mentioned treatment studies provide any definitive clues as to the etiology of lactate-induced panic. The success of blockade can be explained by several mechanisms

(e.g., a decrease in the respiratory rate and blockade, or downregulation of various receptors and decreased sensitivity to repeated lactate challenge).

The findings linking the mechanism of lactate-induced panic to noradrenergic systems are weak. Other provocative techniques seem to be better suited for addressing the involvement of the α-adrenergic (yohimbine) or the β-adrenergic (isoproterenol) system in the etiology of panic anxiety. The involvement of another neurotransmitter, serotonin, in the etiology of lactate-induced panic was suggested by Lingjaerde [76] and others. Lingjaerde [76] theorized that lactate may stimulate serotonin reuptake in central serotonergic neurons and thereby induce anxiety by reducing the inhibitory serotonergic influence on the locus coeruleus.

2. Neuroendocrine Findings

Various endocrine organs are involved in central and peripheral neurotransmitter regulation. Abnormalities of the hypothalamic-pituitary-adrenal axis (HPA) during stress led several authors to study the abnormalities of this axis during lactate-induced panic. Levin and colleagues [77] measured cortisol and corticotropin during lactate-induced panic and did not find elevation of either hormone. Plasma β-endorphin decreased slightly, but significantly, during lactate infusions in panic disorder patients, depressed patients, and controls [78]. This findings suggests that the HPA axis is not activated by lactate-induced panic, as ACTH and β-endorphin arise from the same precursor molecule in the anterior pituitary gland. Appleby and colleagues [27] and Carr and colleagues [31] also did not find evidence for pituitary-adrenal activation during lactate-induced panic. Similarly, Peskind and colleagues [79] reported that neither sodium lactate nor hypertonic sodium chloride increased cortisol or ACTH in panic disorder patients and controls. However, plasma arginine vasopressin increased during both infusions, a response typical for acute hypernatremia. Untreated and treated panic disorder patients have a plasma vasopressin response to lactate similar to normal subjects [80], a finding suggesting intact posterior pituitary function in these patients. Kellner and colleagues [81,82] suggested that the lack of pituitary-adrenocortical activation during lactate-induced panic is due to an immediate rise of atrial natriuretic hormone which suppresses both ACTH and cortisol release. Den Boer et al. [66] also found no single neuroendocrine variable (cortisol, β-endorphin) to correlate with lactate-induced panic.

Thus, the neuroendocrine findings during lactate-induced panic do not clearly support any single hypothesis for induction of such panic.

3. Respiratory and Biochemical Findings

Shortness of breath and hyperventilation are frequent symptoms of spontaneous and lactate-induced panic attacks [83,84]. Lactate is metabolized to bicarbonate and subsequently to carbon dioxide and water. Carbon dioxide then easily passes the blood-brain barrier. Centrally perceived hypercarbia may cause hyperventilation and panic [85]. However, D-lactate, which is poorly metabolized to carbon dioxide, does induce panic [86]. The role of carbon dioxide in the induction of anxiety and in the etiology of panic attacks will be discussed in detail later.

Panicking patients have a lower PCO_2 than nonpanicking patients during lactate infusions [30]. Carbon dioxide pressure decreases during the lactate infusion [87] and lactate causes metabolic alkalosis. However, metabolic and respiratory alkalosis develops in all subjects during lactate infusions, whereas only hyperventilation-induced hypocapnia differentiates panickers and nonpanickers. Patients who had an acute panic attack during

lactate infusion had a greater increase in minute ventilation over baseline than nonpanicking patients or controls in a study by Gorman and colleagues [83].

Gaffney and colleagues [36] similarly described hyperventilation during lactate-induced panic, together with hypernatremia, hypocalcemia, and decreased bicarbonate levels. They suggested that sodium lactate produces panic by mimicking the physiology of spontaneous panic. Gorman and colleagues [88], in a study addressing the proposed theory of Grosz and Farmer [89], infused panic patients with lactate and with sodium bicarbonate, a sodium lactate metabolite. They reported a similar rate of panic during both infusions, although lactate appeared to be a stronger stimulus. Lactate induced a decrease in PCO_2 in panickers and nonpanickers, whereas bicarbonate decreased PCO_2 only in panickers. D-Lactate also produced hypocapnia [14]. Venous PCO_2 normalized after treatment with tricyclic antidepressants [90]. These findings suggest ventilatory dysregulation as a possible cause of panic anxiety. However, it is not clear whether this mechanism is peripheral or central. Maddock and colleagues [91] noted exaggerated increases in serum lactate in panic disorder (PD) patients following hyperventilation during glucose infusion; however, the lactate response was not correlated with heart rate, PCO_2, and other variables. Coplan and colleagues [92] infused nonhuman primates with lactate and saline and measured their cerebrospinal fluid (CSF) and venous blood lactate, pH, PCO_2, PO_2, and bicarbonate. Despite the development of characteristic peripheral biochemical effects of infused sodium lactate (increased lactate and bicarbonate levels, metabolic alkalosis), no increase in central lactate or carbon dioxide were observed. However, according to Dager and colleagues [93], lactate appeared to cross the blood-brain barrier, and lactate concentrations in cisternal fluid increased three times during the intravenous lactate infusions in baboons. In another study, Dager and colleagues [94] observed significantly higher brain lactate levels (using proton magnetic resonance spectroscopy) in panic disorder patients panicking during lactate infusion. The mechanism of the brain lactate rise was not clear. Hyperventilation may have a role in producing higher lactate levels in these subjects [94].

Data from several studies [30,85,95] did not support the role of lactate-induced hypocalcemia initially suggested by Pitts and McClure [9] and Fink et al. [24]. Although a significant decrease in plasma inorganic phosphate during lactate infusions was observed by Liebowitz and colleagues [30], no consistent change in phosphate levels accompanied lactate-induced panic. Inorganic phosphate also decreased during lactate infusions in studies by Gaffney and colleagues [36] and Aronson et al. [37], and during D-lactate infusions [86]. A significantly lower inorganic phosphate level at baseline was found in patients who panicked during subsequent lactate infusion when compared with nonpanicking patients in two studies [86,96]. However, other authors [97] were not able to replicate this finding. Hyperventilation is the most probable cause of phosphate decrease during the infusions. Baseline hypophosphatemia suggests an abnormal metabolic state.

Other biochemical changes, such as an alteration in the body's NAD+/NADH ratio, are speculative and were discussed by other authors [30].

4. Cognitive Theories

Many authors [98,99] suggested that lactate produces panic through conditioning and cognitive mechanisms. According to this theory, the large volume of hyperosmolar fluid infused produces various symptoms and cues panic. Peskind and colleagues [79] were able to produce the same high incidence of panic and equivalent increases in panic symptoms in panic disorder patients by infusing them with sodium lactate or hypertonic saline (3% NaCl). This suggests that a rapid increase in sodium or osmolality may be involved in

panic induction. Gorman and colleagues [86] infused patients with sodium D-lactate, which is poorly metabolized. D-Lactate produced panic, hypocapnia, and alkalosis, thereby indicating hyperventilation. Even though lactate metabolism may not be necessary for panic induction, D-lactate may work through some other, as yet unknown, mechanism. As discussed before [91–94], it is not clear whether lactate crosses the blood-brain barrier, although some studies [93,94] reported increased CNS or CSF lactate concentrations during intravenous lactate infusions. The perception of various cues may be influenced by the severity of anxiety, especially before the infusion. Some authors, therefore, focused on the role of baseline anxiety on subsequent response to lactate. Liebowitz and colleagues [30] found a greater baseline autonomic arousal in subsequent panickers. Cowley and colleagues [100] reported that patients who had a typical panic attack with lactate had higher baseline symptom ratings, but these did not fully account for the differences in response to lactate. They felt that baseline measures may influence how a patient reaches the threshold for panic. Two other studies [37,101] reported similar findings. Yeragani et al. [102] studied the effects of infusion-induced panic anxiety on subsequent preinfusion anxiety and infusion-induced panic attacks during repeated infusions. Although there was a decrease of preinfusion anxiety from one infusion to the next, there was no evidence of a significant decrease or increase in the frequency of subsequent panic attacks. In a study by Targum [39], patients with high frequency of panic attacks (at least one attack a week) reacted positively to lactate infusions, whereas patients with low frequency of panic attacks (one or fewer a month) did not respond to lactate infusions. The author felt that the current heightened anticipatory state (influenced by recent spontaneous attacks), rather than a putative underlying trait, predominates in the provocation of panic. Interestingly, cognitive behavioral therapy decreased the vulnerability to subsequent lactate infusion in a study by Shear and colleagues [103]. It seems plausible that cognitive misperception may play a significant role in triggering panic attacks during lactate infusions.

5. Miscellaneous

Many other hypotheses of panic etiology have been tested. Gorman and colleagues [104,105] addressed the popular idea that hypoglycemia causes panic attacks. They infused two sets of panic disorder patients with 0.5 M sodium lactate, and they did not find any evidence of low blood sugar levels during lactate-induced panic attacks [104] nor significant changes in insulin levels [105]. However, coadministration of glucose resulted in a reduced sensitivity to the panicogenic effects of lactate in a study by George and colleagues [106], which could be explained by the effect of glucose on the adrenergic system, catastrophic misinterpretation, and other possibilities.

There is no support for the involvement of endogenous opiates in the pathogenesis or control of panic attacks [107]. Cowley and Dunner [108] found a lack of significant association between lactate response and presenting clinical variables. Patients with a panic response to lactate were more likely to report a definite history of panic attacks in at least one first-degree relative in their study. Balon and colleagues [109] found a higher prevalence of anxiety disorders among first-degree relatives of controls who panicked during lactate infusion than in those who did not panic. Two of these controls subsequently developed panic attacks and, in one of them, panic attacks began soon after the infusion [110]. Both of these findings [108,109] suggest some genetic vulnerability to lactate-induced panic. George et al. [111] reported a marked reduction of parasympathetic nervous activity (vagal tone) in six healthy volunteers during lactate administration and hyperventilation. They hypothesized that this reduction facilitates sympathetic activity (tachycardia).

Several other studies focused on autonomic regulation in panic disorder patients during lactate infusions. Yeragani and colleagues [112] suggested an exaggerated cardiac vagal withdrawal in PD patients during lactate infusions. Panic disorder patients had a greater cardiac and respiratory reactivity than healthy controls with lactate infusions during sleep when the influence of cognitive factors is minimal or absent [113]. There were also substantial differences in autonomic regulation during lactate infusions between PD patients and healthy controls in a study by Sloan and colleagues [114].

C. Summary

Sodium lactate is a specific (89%) and reliable provocative technique of panic attacks, similar to naturally occurring panic attacks, and accompanied by similar physiological changes. Lactate-induced panic is blocked by antipanic drug treatment. The presented studies clearly demonstrate that the pathogenesis of lactate-induced panic is still unknown. Various theories of lactate-induced panic, such as hypocalcemia, hypophosphatemia, hypoglycemia, hypersensitive β-adrenergic receptors, sudden shifts in pH, alkalosis, stimulation of central noradrenergic centers, decreased α_1-adrenergic receptor density, endogenous opioid dysregulation, reduction of inhibitory serotonergic influence on the locus coeruleus, hypothalamic-pituitary-adrenal axis stimulation, decrease in parasympathetic activity (vagal tone), increase in tribulin, alteration of NAD+/NADH ratio, hyperventilation, chemoreceptor sensitivity, carbon dioxide sensitivity, fluid expansion, and cognitive theories (nonspecific stress, interceptive phobic cues) [115] have been entertained. Ventilatory dysregulation (stimulation of ventilation) and cognitive misinterpretation of peripheral cues may be the most plausible explanations at present. However, as Griez and Schruers [2] pointed out, "In contrast to the growing body of data on the validity of lactate infusion as a laboratory model of panic, no convincing progress has been made in answer to the question of why patients panic from lactate."

III. CARBON DIOXIDE

Shortness of breath is one of the prominent symptoms of panic attacks. Accompanying hyperventilation leads to an increased exhalation of carbon dioxide and lowering of arterial concentration of carbon dioxide (CO_2). Therefore, one would assume that increased inhalation of CO_2 would decrease anxiety. Carbon dioxide did actually reduce anxiety in some studies. Griez and van den Hout [116] reported that double inhalation of a mixture of 35% CO_2 and 65% oxygen reduced anxiety levels in 12 healthy volunteers. These authors also used this technique, together with exposure, in the treatment of "phobophobia" [117]. Wolpe [118] strongly advocated the use of CO_2 inhalation in the treatment of anxiety.

However, most studies report an anxiogenic effect of inhaled CO_2. Cohen and White [8] found that the rebreathing technique, which increases CO_2 levels, produced anxiety attacks in anxiety neurosis patients. In another study [119], 7 of 12 (58%) panic disorder patients panicked during increased ventilation stimulated by 5% CO_2, and three of them also during room–air hyperventilation. The rate of CO_2-induced panic was similar to the rate of lactate-induced panic in this study. Gorman et al. [84] later expanded their sample to 31 patients and reported that 39% of them panicked with 5% CO_2 and 23% with room–air hyperventilation. They also administered 7% CO_2 to some subjects who did not panic during the 5% CO_2 challenge. Most of the 5% CO_2 nonpanickers panicked with 7% CO_2. These findings were replicated by the same group [120] in a methodologically improved

study, which also confirmed a better discrimination between patients and controls with 7% CO_2. The 5% CO_2 also provoked panic in 8 of 14 panic disorder patients in a study by Woods et al. [121].

van den Hout and Griez [122] demonstrated that inhalation of 35% CO_2 and 65% oxygen resulted in autonomic symptoms highly reminiscent of natural or lactate-induced panic in normal subjects. When CO_2 inhalation was preceded by intake of a β-blocker (60 mg of propranolol), fewer symptoms occurred than when preceded by a placebo. The same procedure provoked short-lived autonomic panic symptoms in both panic disorder patients and normal controls [123], but high subjective anxiety was elicited only in patients. Other researchers [115,124–126] also reported that this procedure provoked higher anxiety in panic patients than in controls. Zandbergen and colleagues [127] reported significantly higher anxiety during 35% CO_2 challenge in PD patients, when compared with the response during this challenge in normal persons and during hyperventilation in both patients and normal subjects. They suggested that panic disorder patients are specifically hypersensitive to an increase in CO_2 pressure. Anxiety ratings increased markedly during rebreathing both in patients and controls in a study by Woods and colleagues [128], and anxiety increases were significantly greater in patients than in healthy subjects. Ventilatory response to carbon dioxide was similar in patients and controls. On the other hand, Lousberg and associates [129] reported that panic patients showed significantly higher ventilatory response than normal controls using the same rebreathing technique. A number of other studies [130–134] confirmed the anxiogenic effect of various concentrations of CO_2. The role of CO_2 as a laboratory model for initiating panic has thus been clearly established.

A. Studies Focused on Diagnostic Specificity

As with lactate, several studies tried to address the diagnostic specificity of CO_2 challenge [14,84,135–145]. Patients with other anxiety disorders such as social phobia, generalized anxiety disorder, and obsessive-compulsive disorder did not panic, whereas, in a study by Gorman and colleagues [84], 39% of panic disorder patients panicked during 5% CO_2 challenge.

Patients with social phobia were the most frequently studied group besides panic disorder patients. All three patients with social phobia and 67% of panic disorder patients panicked during subsequent challenge with 7% CO_2 in the aforementioned study [84]. On the other hand, 36% of social phobics compared with 50% of panic disorder patients panicked during 35% CO_2 challenge in another study by Gorman and colleagues [14]. The difference in the frequency of panic between patients with social phobia and panic disorder patients was even larger (18.7% vs. 62.5%) during single inhalation of 50% CO_2 in a study by Rapee and colleagues [135]. Papp and colleagues [136] reported panic rates of 72% for PD patients and 30% for social phobia patients in a study with 35% CO_2. Interestingly, 5.5% CO_2 did not differentiate well specific phobias and panic disorder in a study by Antony and colleagues [137]. In a study by Verburg and colleagues [140], a comorbid depressive disorder appeared to increase the vulnerability of panic disorder patients to 35% CO_2. In studies by Perna and colleagues [141,142], 35% CO_2 challenge differentiated well between panic disorder and generalized anxiety disorder [141] and between panic disorder and obsessive-compulsive disorder [142]. Patients with comorbid panic and generalized anxiety or obsessive-compulsive disorder responded to 35% CO_2 similarly as patients with panic disorder. Nine of 14 women with premenstrual dysphoria without panic disorder panicked during 35% CO_2 challenge, compared with none of 12

controls [143]. Interestingly, anxiety after 35% CO_2 inhalation was significantly stronger in the early follicular phase than in the midluteal phase in patients with panic disorder [144]. Finally, Ware and colleagues [145] used the 35% CO_2 challenge as a diagnostic tool in the evaluation of suspected panic disorder. Carbon dioxide seems to be somewhat less panic-disorder-specific than lactate. The sensitivity to CO_2 in panic disorder patients may be concentration-dependent, and Gorman and colleagues [14] suggested that increasing the concentration/dose of CO_2 may compromise diagnostic specificity. Further studies could help to better elucidate the sensitivity and specificity of this technique.

B. Studies Focused on Etiology

Results of animal studies present some theoretical basis for the mechanism of anxiety induction with CO_2. For instance, hypercapnia caused a rapid increase in the firing rate of the locus coeruleus neurons of rats [146]. Gorman and colleagues [119] theorized that the similarity in response to lactate and CO_2 in their patients was suggestive of the involvement of CO_2 (lactate $\rightarrow$ bicarbonate $\rightarrow$ carbon dioxide) in triggering anxiety by stimulation of the locus coeruleus. CO_2-induced panic was associated with an exaggerated ventilatory response and increases in plasma norepinephrine and diastolic blood pressure [84], suggesting that patients may have hypersensitive CO_2 receptors. On the other hand, the complex physiological and biochemical measurements (MHPG, plasma cortisol, prolactin, growth hormone) obtained in a study by Woods and colleagues [120] did not elucidate the anxiogenic effect of CO_2, and Gorman and colleagues [14] were not able to find a significant group difference in anxiety level, physiological, or biochemical variables in response to 35% CO_2. However, 35% CO_2 may be too high a concentration [14].

Various studies addressed biological changes during CO_2 challenge and their relationship to possible etiology. Martinez and colleagues [147] observed significantly greater blood pressure reactivity in PD patients than in nonpanickers or controls, but they did not observe any overall difference in hemodynamic responses between patients and controls. Sinha and colleagues [148] noticed decreased cortisol levels after CO_2 challenge. The results of a study by Welkowitz and colleagues [149] suggested that CO_2-induced panic is a robust biological effect that occurs independent of cognitive set changes, thus the probability of cognitive misperception seems to be small. Tryptophan depletion caused a greater anxiogenic response and an increased rate of panic attacks after 5% CO_2 challenge in PD patients [150]. This finding suggests involvement of the serotonin system in the etiology of CO_2-induced panic. Interestingly, premedication with cholecystokinin tetrapeptide (a panicogenic agent, see below) decreased panic symptoms upon 35% CO_2 challenge in healthy volunteers [151]. Dager and colleagues [152] reported that hyperventilation disproportionately increased brain lactate in PD patients. Finally, two studies [153,154] suggested increased hypersensitivity to 35% CO_2 in first-degree relatives of PD patients. These findings suggest a degree of genetic vulnerability.

Several studies also reported on the effect of treatment on CO_2-induced anxiety and its relation to etiology. Venous pH, venous PCO_2, and bicarbonate were normalized in panic patients after treatment with tricyclic antidepressants or clonidine [90]. Beckett and colleagues [155] reported blockade of rebreathing-induced panic attack with clonazepam in a patient with panic disorder. Alprazolam markedly attenuated anxiety increases during rebreathing of 5% CO_2 in eight panic disorder patients [128]. The authors hypothesized that alprazolam may antagonize CO_2-induced anxiety by decreasing noradrenergic function through stimulation of benzodiazepine receptors located on noradrenergic neurons.

Pretreatment with alprazolam, but not with clonidine, significantly reduced the rebreathing-induced increase in anxiety [156]. The anxiogenic effect of rebreathing 5% CO_2 was significantly reduced in six patients after long-term imipramine treatment [157], a finding consistent with an increase of noradrenergic function in CO_2-induced anxiety. Furthermore, alprazolam [158] and similarly clonazepam [159] blocked the 35% CO_2-provoked panic in PD patients. Various antidepressants (mostly imipramine) or cognitive-behavioral therapy (CBT) reduced CO_2 sensitivity in PD patients in a study by Gorman and colleagues [160]. Three other antidepressants, toloxatone (monoamine oxidase type A inhibitor) [161], fluoxetine [162], and citalopram [163] also significantly reduced 35% CO_2 reactivity in PD patients. CBT was successful in reducing the sensitivity to 35% CO_2 in PD patients in a study by Schmidt and colleagues [164]. Thus, treatments usually successful in the treatment of PD are also successful in blocking the effect of CO_2 in PD patients.

Other authors have suggested additional physiological, behavioral, and cognitive explanations of CO_2-induced panic. Griez and van den Hout [165] reported that CO_2 produced largely sympathomimetic responses in the cardiovascular system of healthy subjects. van den Hout and Griez [166] indicated that the occurrence of peripheral panic symptoms in healthy volunteers after CO_2 inhalation in their study could be attributed to the sudden decrease in arterial PCO_2. These authors [167] also suggested that hypocarbia alone is not sufficient to provoke anxiety in patients. van den Hout and colleagues [115] observed a decrease in anxiety with the increased number of CO_2 challenges and suggested that their data support a behavioral explanation of the anxiogenic effect of CO_2. Subjects with panic attacks who were given no explanation reported a greater proportion of catastrophic cognition and greater panic than those with panic attacks who received a full explanation [135]. Another possible contribution of psychological factors to the laboratory induction of panic was presented in a study by Sanderson and coauthors [168]. Patients who believed that they had control over CO_2 administration reported a smaller amount of anxiety and less intense anxiety than the patients who believed that they could not control the CO_2 administration.

The etiology of CO_2-induced panic remains unknown. Hypersensitivity of carbon dioxide receptors, increase in noradrenergic function, or cognitive misinterpretation of peripheral symptoms induced by CO_2 are the most plausible explanations. It is interesting that benzodiazepines, respiratory depressants, and various breathing techniques are effective in treatment of panic.

C. Summary

Carbon dioxide is a valid and relatively convenient model of panic anxiety. It provokes anxiety similar to naturally occurring panic and its anxiogenic effect is successfully blocked by antianxiety agents. The specificity of this model may be dose- or concentration-dependent. The etiology of CO_2-induced panic anxiety remains unknown. The issue of oversensitivity of chemoreceptive areas in PD subjects is still open to debate [2]. Klein's suffocation alarm theory of panic [169], according to which PD patients have a hypersensitive suffocation monitor that predisposes them to experiencing a panic attack under certain conditions, provides an elegant explanation of CO_2 and some other challenges, but not of all anxiety provocation tests.

IV. YOHIMBINE

Yohimbine is an α_2-adrenergic antagonist. It interacts with adrenoreceptors that are selectively stimulated by clonidine, but it can also interact with α_1-receptors. It has been well

established that α_2-adrenoreceptors regulate sympathetic flow in intact animals and humans. Increased noradrenergic function may play a pivotal role in the pathophysiology of anxiety states [170]. The nucleus locus coeruleus (LC) is a collection of noradrenergic neurons in the brain stem, and electric stimulation of this nucleus in monkeys results in fearful behaviors, whereas destruction of this nucleus reduces such responses [171,172]. The same investigators have demonstrated that activation of LC by yohimbine produces an increase in LC firing and produces fearful behaviors.

In humans, yohimbine produces symptoms of anxiety and an increase in heart rate and blood pressure [173]. Charney and colleagues [174] gave 30 mg of yohimbine to 10 healthy subjects, and this resulted in increases in subjective anxiety, blood pressure, and plasma MHPG. In this study, both diazepam and clonidine significantly antagonized yohimbine-induced anxiety. In another study, Charney and colleagues [175] administered 20 mg of yohimbine orally to 20 healthy controls and 39 drug-free patients with agoraphobia and panic attacks. This resulted in a significantly greater anxiety and somatic symptoms, such as palpitations, hot and cold flashes, restlessness, and tremors in patients compared with controls. Patients with frequent panic attacks also had a significantly higher plasma MHPG response to yohimbine than did normal persons. Gurguis and Uhde [176] and Gurguis and colleagues [177] reported that in their study panic disorder patients had a greater anxiogenic response to 20 mg of yohimbine than controls.

Charney and Heninger [178] reported that alprazolam treatment of panic disorder patients was associated with a decrease in baseline MHPG levels and blunting of the yohimbine-induced increase. They speculated that the antipanic mechanism of action of alprazolam may be due to an interaction between benzodiazepine-sensitive and noradrenergic neural systems. Charney and Heninger [179] also speculated that the regulation of noradrenergic activity is aberrant in some patients with PD. They [179] suggested that the increased dynamic range of noradrenergic activity observed as an increased sensitivity to both yohimbine and clonidine may be due to abnormalities in regulatory input to noradrenergic neurons or dysfunction in the α_2-adrenergic receptor-effector-coupling mechanism. Rasmussen and colleagues [180] found that yohimbine, administered to 12 drug-free patients with obsessive-compulsive disorder and 12 healthy subjects, had no significant effect on obsessive-compulsive symptoms. Southwick and colleagues [181] studied administration of yohimbine in PTSD patients. Yohimbine induced significant increases in core PTSD symptoms, and some of the patients experienced yohimbine-induced flashbacks and panic attacks (some patients had comorbid panic disorder). They speculated that uncontrollable stress produced substantial increases in noradrenergic neuronal function. Southwick and colleagues [182] also reported that 4 PTSD subjects who took over-the-counter oral yohimbine experienced marked exacerbation of anxiety/panic and PTSD-specific symptoms.

In summary, yohimbine appears to be an anxiogenic agent, and the yohimbine model of anxiety has helped understand the pathophysiology of anxiety to a considerable extent. However, it should also be noted that there are some inconsistencies with this model. Some studies did not find a baseline difference in MHPG levels between panic disorder patients and controls [183]. Lactate-induced panic and caffeine-induced anxiety are not accompanied by increases in plasma-free MHPG [64,80,184]. Although the yohimbine model of anxiety is attractive, it is also possible that the increase in sympathetic flow can result in symptoms of anxiety through an additional β-adrenergic mechanism, as isoproterenol induces symptoms of panic anxiety in PD patients [19,185]. Last, but not least, a clinical caveat: yohimbine probably should not be used for treatment of sexual dysfunction associated with medications in anxious patients.

V. CAFFEINE

Caffeine (1,3,7-trimethylxanthine) is a widely used psychotropic substance. Caffeine is associated with an increase in self-rated symptoms of anxiety in several studies [186,187]. Caffeine in doses of 600 mg/day has been reported to result in caffeinism, characterized by anxiety, sleep disturbances, and nervousness [188]. Caffeine is also known to produce symptoms of panic anxiety in patients with anxiety disorders [189].

Boulenger and colleagues [186] found that PD patients, but not depressed patients or normal controls, had levels of self-rated anxiety and depression that correlated with their degree of caffeine consumption. They also found that panic disorder patients had increased sensitivity to the effects of one cup of coffee. Furthermore, PD patients reported more frequent discontinuation of coffee intake because of untoward side effects compared with controls. A proportion of PD patients reported reduction in panic attack frequency after caffeine consumption reduction [190]. Eighty-four percent of anxious patients were low-caffeine consumers, compared with 41% of medical inpatients in a study by Lee and colleagues [187]. Uhde and colleagues [191] administered three different oral doses of caffeine (240, 480, and 720 mg) to eight normal controls and two panic patients. There was a dose-related increase in measures of anxiety state in all subjects. Two normal controls developed unequivocal panic attacks after receiving 720 mg of caffeine. Charney and associates [184] reported on the robust increases in subject-rated anxiety and nervousness in 11 healthy subjects after oral administration of 10 mg/kg of caffeine. They [189] also found that PD patients had a significantly greater anxiogenic response to caffeine (10 mg/kg), compared with controls, and the levels of anxiety were significantly correlated with plasma caffeine levels in patients. Caffeine increased plasma cortisol levels equally in both groups. Mathew and Wilson [192] studied the effects of 250 mg of caffeine in eight patients with generalized anxiety disorder, nine PD patients, and nine normal controls. The anxiety disorder patients did not show any evidence of increased anxiety and panic after caffeine. Both patients and controls who received caffeine had significant decreases in cerebral blood flow. This study suggests that small doses of caffeine may not induce symptoms of panic anxiety.

Several authors [193] proposed antagonism of adenosine effects as the mechanism of action of caffeine. Several other mechanisms may be involved in the anxiogenic response to caffeine. These include inhibition of phosphodiesterase, increased brain catecholamine activity [194], also consistent with findings in Charney and colleagues' study [184], or antagonism of benzodiazepine and adenosine receptor function [195,196]. Caffeine's anxiogenic effect may thus be related to several neurobiological systems, namely noradrenergic, dopaminergic, adenosinergic, and even GABAergic. Alprazolam blocks caffeine-induced anxiety [13]. Findings from a study by Orlikov and Ryzov [197] on increased kynurenine (metabolite of tryptophan) during caffeine-induced anxiety in 15 male volunteers suggest the possibility of serotonergic system involvement.

The caffeine model of anxiety, although inconclusive as to the mechanism of its anxiogenic effects, appears to be a useful research tool.

VI. ISOPROTERENOL

The role of catecholamines in the pathophysiology of anxiety has been studied for almost a century. In a study by Wearn and Sturgis [10] epinephrine reproduced the symptoms of "irritable heart," a syndrome with functional cardiac symptoms and anxiety. In subsequent

studies, epinephrine provoked anxiety attacks in patients with anxiety disorders, but not in subjects with other psychiatric disorders [16,198]. Norepinephrine also induces anxiety attacks in panic disorder patients [21].

Isoproterenol is a sympathomimetic amine, structurally very similar to epinephrine. Unlike epinephrine, isoproterenol lacks α-adrenoceptor agonist effect and acts almost exclusively on β-adrenoceptors. In early uncontrolled studies, isoproterenol provoked intense anxiety states in patients with a history of anxiety symptoms [199], and produced anxiety attacks in subjects with a history of panic attacks [200]. In both studies, the anxiety states quickly subsided after the intravenous injections of the β-blocker propranolol.

In a large double-blind study, Pohl and colleagues [185] found that PD patients became much more anxious during isoproterenol infusions and were much more likely to panic than normal controls (66% vs. 9%). In a follow-up experiment, 14 of the patients in this study were reinfused with isoproterenol and placebo after treatment with tricyclic antidepressants [201]. Thirteen of these 14 patients panicked before treatment, and only two after treatment. The anxiogenic effects of isoproterenol in PD patients suggests that β-adrenoceptors may play a role in the pathophysiology of panic. One inconsistency in the β-adrenergic model is that β-blockers are relatively ineffective for panic anxiety [202], although they are effective for performance anxiety. However, this finding might be explained by the upregulation of β-receptors that occurs when β-blockers are given for an extended time. Panic disorder patients may experience early improvement with propranolol that is not sustained [203]. Beta-blocker use for performance anxiety is generally episodic, and since the drugs are only given before scheduled public performances, β-receptor upregulation would not occur.

The exact mechanism of isoproterenol-induced anxiety is not known, as isoproterenol (and epinephrine) does not readily cross the blood–brain barrier and some of the central effects may be mediated by isoproterenol's peripheral effects. If isoproterenol exerts its anxiogenic effects outside the CNS, there are several possible explanations. One is the possibility that, since provocative models of anxiety all have peripheral effects and symptoms, drug-induced anxiety is a function of the psychological effects that occur secondary to a provocative infusion (i.e., cognitive perception of the experience). Another potential hypothesis is that PD is a peripheral disease of autonomic arousal, and that the subjective symptoms of anxiety are a secondary phenomenon.

Isoproterenol is another reliable model of anxiety induction. As with the other provocation techniques, it induces anxiety similar to naturally occurring panic, and its effects are blocked by antianxiety treatment. The relation of this model to CNS hypotheses of anxiety is unknown.

VII. CHOLECYSTOKININ

Cholecystokinin is the most abundant neuropeptide in the CNS and also happens to be a panicogenic agent [15,204]. De Montigny [205] demonstrated that cholecystokinin tetrapeptide (CCK-4) can induce severe anxiety and brief panic attacks in healthy volunteers. Cholecystokinin has been shown to induce panic attacks [206]. PD patients are more sensitive to the effects of CCK-4 than normal controls [207]. The panicogenic effect of CCK-4 is dose-dependent [208,209].

The anxiety-provoking properties of CCK-4 are not limited to panic disorder only. Women with premenstrual dysphoric disorder showed greater anxiety and panic response to CCK-4 [210]. Similarly, though CCK-4 did not provoke flashbacks in eight PTSD

patients in a study by Kelner and colleagues [211], it induced panic in seven of these patients.

Infusion of CCK-4 did not activate the hypothalamic-pituitary adrenal axis and did not increase plasma MHPG levels in one study [212]. However, CCK-4 increased the secretion of ACTH, but not cortisol in another study with PD patients [213]. Interestingly, CCK-B receptor agonist pentagastrin not only induced anxiety in healthy subjects, but also significantly increased ACTH and cortisol levels, blood pressure, and heart rate. The effect of CCK-4 on respiration is not totally clear and may be dose dependent [214,215]. Bradwejn and colleagues [214] found that a fairly high dose of CCK-4 stimulates, among other things, respiration in healthy volunteers. However, in a study by Schruers and colleagues [215], a lower dose of CCK-4 (10 μg) did not increase any of the respiratory parameters in healthy volunteers. CCK challenge has similar behavioral effects as to the CO_2 challenge [216].

The behavioral effects of CCK-4 in healthy volunteers were blocked by CI-988, a CCK-B receptor antagonists [217]. However, this CCK-B receptor antagonist failed to block the effects of CCK-4 in panic disorder patients [218]. Interestingly, clonidine also failed to block the panicogenic effect of CCK-4 in PD patients [219]. However, CCK-B receptor antagonists CI-998 and L-365,260 were not effective in the treatment of panic disorder [220,221]. Interestingly, in a study by Bradwejn and Koszycki [222], PD patients successfully treated with imipramine showed reduced sensitivity to the panicogenic effect of CCK-4. Similarly, fluvoxamine [223] significantly decreased the sensitivity of PD patients to CCK-4, while placebo was without effect.

Cholecystokinin tetrapeptide is an interesting anxiogenic agent. Its anxiogenic properties seem to be dose-dependent and not quite diagnosis-specific. The anxiogenic effect of CCK-4 could be blocked by various agents. Interestingly, its effect seems to be age dependent [224]. The mechanism of action of CCK-4 is unclear. Rehfeld [225] outlined some of the questions that need to be answered to elucidate the mechanism of action of CCK-4 (e.g., whether endogenous CCK-4 exists, whether the panicogenic effect is mediated only through CCK-B receptors, and whether measurements of CSF CCK peptides are useful).

VIII. SEROTONERGIC CHALLENGES—mCPP AND FENFLURAMINE

The involvement of serotonergic neurons in the pathophysiology of anxiety has been entertained especially after the finding of effectiveness of serotonergic antidepressants in various anxiety disorders. Meta-chlorophenylpiperazine (mCPP) was found to be anxiogenic in panic disorder patients [226], obsessive-compulsive (OCD) patients [227–229], and in higher doses in healthy volunteers [226]. However, mCPP has produced similar anxiety increases and behavioral changes in PD patients and healthy controls in controlled studies [230,231]. Kahn and colleagues [232] also suggested that mCPP had pronounced side effects that might induce anxiety as a cognitive side effect.

The finding of OC symptom exacerbation in OCD patients has also not always been replicated [233,234] and may be dose-dependent [235]. Interestingly, mCPP increased in feeling calm and strange in schizophrenic patients and healthy volunteers in a study by Maes and Meltzer [236]. Methylphenidate, but not mCPP, produced psychotic symptoms in first-episode schizophrenia patients [237]. However, mCPP did decrease anxiety, hallucinations, and anger, and increased agitation, somatic concerns, and impaired understandability [237].

Fenfluramine, an indirect serotonin agonist, has been studied in anxiety [18] and other disorders. The anxiogenic effect of d-fenfluramine is unclear. It decreased simulated public speaking anxiety in a study of healthy volunteers [238], and increased anxiety and confusion in another study of healthy volunteers [239]. D-Fenfluramine caused anxiety similar to generalized anxiety in PD patients, but reduced anxiety following 7% CO_2 challenge [240]. In a study by McBride and colleagues [241], it did not significantly exacerbate OC symptoms in OCD patients.

The mCPP or fenfluramine challenges suggest possible involvement of the serotonergic system in the pathophysiology of anxiety. However, their exact mechanism of action remains unclear. These challenges are strictly a research and experimental tool. One clinical caveat: mCPP provocation of anxiety in some sensitive patients may be the mechanism of action of anxiety associated with some antidepressants, as mCPP is one of their metabolites (e.g., nefazodone).

IX. BENZODIAZEPINE-RECEPTOR CHALLENGES

Several studies [17,242] reported provocation of panic anxiety with flumazenil, a benzodiazepine antagonist, in some PD patients. However, in a study with lactate-sensitive PD patients [243], flumazenil did not induce panic attacks in PD patients. Flumazenil did not provoke a high rate of panic attacks in social phobia (2 of 14 patients, none of 14 controls) [244]. In a study by Randall and colleagues [245], flumazenil also did not produce an increase of anxiety in PTSD patients. Flumazenil remains another experimental challenge strategy. The mechanism of action is not clear. The theory of altered GABA-receptor function in panic disorder needs to be further explored.

X. CONCLUSION

Most of the provocative strategies discussed in this chapter have been replicated as methods for inducing anxiety in panic disorder patients. Some of them, for instance, the benzodiazepine-receptor or serotonergic challenges, are more controversial and their further use remains questionable. These challenges provoke anxiety that is similar to naturally occurring panic anxiety, and they all have some threshold specificity. The methods vary, but they share some possible common pathophysiology or mechanism: all may affect the noradrenergic system and all could potentially be explained by cognitive misinterpretation of peripheral symptoms. Arguing against the latter is that many of the techniques occasionally provoke signs of anxiety in normal controls, and normal controls with a family history of anxiety disorders may be at greater risk for this.

The provocative strategies are reversed by clinical treatment and, thus, clinically validated. As Woods et al. [128] pointed out, all provocation techniques appear to constitute useful neurobiological probes into the pathophysiology of panic anxiety. However, even though our knowledge of the biology of anxiety has increased enormously, the etiology of panic is still an enigma. These provocative techniques also seem to be an effective model for confirming the diagnosis of panic disorder [145] or the development and testing [103] of more specific and effective treatments. For instance, the CCK-B antagonist L-365,260 failed to block lactate-induced panic [246] and was later found to be ineffective in the treatment of panic disorder [221]. The results of provocation studies also tell us that these substances should be used carefully in other situations in anxious patients (e.g.,

cholecystokinin in GI tests; caffeine consumption in panic disorder; yohimbine in sexual dysfunction associated with antidepressants).

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31

Pharmacotherapy of Anxiety Disorders

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I. INTRODUCTION

Many treatments for patients with anxiety disorders have become available in the last decade. The findings of randomized controlled trials and the advent of better tolerated and safer drugs together have led to considerable changes in prescribing practice. Newer classes of drug such as the selective serotonin reuptake inhibitors (SSRI) have largely replaced traditional treatment approaches with monoamine oxidase inhibitors (MAOI) or tricyclic antidepressants (TCA). This chapter reviews the pharmacological treatment of panic disorder, social anxiety disorder, generalized anxiety disorder, and post-traumatic stress disorder. It gives greatest emphasis to the findings of double-blind placebo-controlled studies, and reflects changes in practice by focusing on treatments that are effective and acceptable in clinical practice.

II. PANIC DISORDER

A. Selective Serotonin Reuptake Inhibitors

Two recent reviews have examined the efficacy and tolerability of SSRIs in the management of patients with panic disorder [1,2]. In many countries, prescription of an SSRI is regarded as the most appropriate first-line treatment: in others, cognitive therapy may be recommended, despite the limited availability of trained cognitive therapists. Typically, about 60% of patients will become free of panic attacks after short-term treatment with an SSRI, continuation treatment being associated with further improvement. The side-effect profile of SSRIs in anxiety disorders is similar to that seen in patients with depression, the more common side effects being nausea, sweating, diarrhea, and delayed ejacula-

tion or anorgasmia. The sexual side effects of SSRIs and other antidepressants have been reviewed elsewhere [3].

1. Zimelidine

In the first randomized controlled trial ($n = 44$) of an SSRI in panic disorder, zimelidine was found to be more efficacious than either placebo or imipramine [4]. Unfortunately, zimelidine was later associated with the development of a disorder similar to Guillain–Barre syndrome, and was withdrawn from clinical use [5].

2. Citalopram

There are few placebo-controlled studies of citalopram, although it has been available in some European countries for many years. An 8-week treatment study ($n = 475$) compared three dose's of citalopram to placebo and clomipramine, and found that the lowest daily dose of citalopram (10–15 mg) was not different from placebo, but higher doses (20–30 mg and 40–60 mg) were associated with significantly greater reductions in panic attack frequency and measures of anxiety and depressive symptoms [6]. Patients who completed the acute study and were expected to benefit from longer term treatment could then be included in a further 1-year double-blind continuation study [7]. In the 279 patients who entered this treatment phase, there were again no differences in efficacy between the lowest dose of citalopram and placebo, but patients who received higher doses of citalopram or who continued with clomipramine had the best chances of responding. There were few differences in tolerability between citalopram and clomipramine. Although patients who received higher doses of citalopram made similar improvements to those with clomipramine, the daily dose of clomipramine in both treatment phases was low (60–90 mg) and further studies are needed to establish the relative efficacy of the two drugs.

3. Fluoxetine

Fluoxetine is used widely as a treatment for depression, but has been studied only infrequently in panic disorder. The first evaluation involved both acute and continuation treatment phases [8,9]; a second study examined the effect of dose increase in early nonrespondents [10].

In the acute phase of the placebo-controlled study ($n = 243$) patients were randomized to receive either placebo or one of two daily doses (10 or 20 mg) of fluoxetine for 10 weeks [11]. Fluoxetine was associated with greater reductions in panic attack frequency, phobic symptoms, and functional impairment. These changes were most noticeable with the higher dose; the 10-mg daily dose was linked to a significant reduction in panic attack frequency, but did not differ from placebo on measures of anxiety symptoms, work impairment, anticipatory anxiety, or overall functioning. In the continuation phase ($n = 88$), patients who had responded to fluoxetine were randomly allocated either to continue with fluoxetine or to switch to placebo, both groups being followed up for 24 weeks. Patients continuing with active treatment showed significant reductions in panic attack frequency and phobic avoidance, but switching to placebo significantly worsened anxiety and depressive symptoms [9].

In the second placebo-controlled study ($n = 180$), patients who had not responded after 6 weeks of treatment had the dose increased (to a maximum of 60 mg per day in the fluoxetine group) [10]. The proportion of patients who became panic-free increased in both groups (from 29% at 6 weeks to 42% at endpoint with fluoxetine, from 16 to 28% with placebo), suggesting that dose increases may be useful in some patients.

The relative efficacy of fluoxetine and moclobemide (a reversible inhibitor of monoamine oxidase type A) has been evaluated in an 8-week treatment study involving 399 patients: at study endpoint 70% of fluoxetine-treated patients were panic-free, compared to 63% with moclobemide, there being no difference in overall response assessed by the Clinical Global Impression Scale (CGI) [11].

4. Fluvoxamine

This has been evaluated in a number of placebo-controlled and comparator-controlled treatment studies. In the first ($n = 50$), both fluvoxamine and clomipramine were efficacious, 15 of 26 clomipramine-treated and 14 of 24 fluvoxamine-treated patients being free from symptoms at study endpoint [12]. In a second randomized, controlled trial comparing fluvoxamine with the noradrenaline reuptake inhibitor maprotiline, fluvoxamine was associated with a reduction in anxiety and depressive symptoms and a decrease in the number of panic attacks, whereas maprotiline had little effect. At study endpoint, 10 of 20 patients treated with fluvoxamine had improved substantially, compared to only 5 of 24 patients who received maprotiline [13]. A third placebo-controlled study compared fluvoxamine with ritanserin (a postsynaptic $5HT_2$-receptor blocker), but only fluvoxamine was efficacious, with a significant reduction in panic attacks and improvements in anxiety and depressive symptoms and avoidance behavior [14].

Further placebo-controlled studies have confirmed the efficacy of fluvoxamine. In an 8-week study ($n = 50$), treatment with fluvoxamine (mean daily dose at endpoint, 206.8 mg) was associated with reductions in panic attacks from week 3, and anxiety and depressive symptoms from the sixth week. At study endpoint, 61% of the fluvoxamine group were free of panic attacks, compared to 22% of those receiving placebo, but the panic attacks that remained were no less severe [15]. In another placebo-controlled trial ($n = 188$) fluvoxamine was also significantly more efficacious than placebo, with greater reductions in panic attack frequency and Clinical Anxiety Scale scores, from the second week of treatment [16]. These studies are partly supported by the findings of a third placebo-controlled investigation ($n = 46$) in which fluvoxamine produced a significantly greater reduction in the number of limited symptom attacks, but not full panic attacks [17].

The relative efficacy of fluvoxamine in panic disorder has been evaluated through comparisons with imipramine [18,19], brofaromine [20], and cognitive therapy [21,22]. An 8-week multicenter double-blind placebo-controlled study ($n = 148$) found that fluvoxamine (mean daily dose at endpoint, 171.4 mg) was less efficacious than imipramine (164.7 mg) [18]. However, patients in the placebo group had significantly fewer panic attacks at baseline than those in the two active treatment groups, and further analysis using data from only one center found evidence for efficacy of both imipramine and fluvoxamine [19]. In a small study ($n = 30$) comparing fluvoxamine (daily dose, 150 mg) with the reversible monoamine oxidase inhibitor brofaromine, both treatments were efficacious. Ninety-three percent of brofaromine-treated patients were much or very much improved, compared to 87% in the fluvoxamine group, but only a minority had reductions of 50% or more on the Hamilton Anxiety Scale [20].

The first comparison of fluvoxamine ($n = 75$) with cognitive therapy found that it was superior to both cognitive therapy and placebo: at the end of the 8-week study, 90% of the fluvoxamine group had achieved moderate to marked improvement, compared to 50% with cognitive therapy and 39% with placebo [21]. In the fluvoxamine group, 81% became free of panic attacks, this figure being less with cognitive therapy (53%) or placebo

(29%); depressive symptoms improved in parallel with changes in panic attacks and anxiety symptoms [23]. However treatment with cognitive therapy produced more beneficial changes in "abnormal personality traits," which were relatively resistant to fluvoxamine or placebo [24]. By contrast, a placebo-controlled 13-week study ($n = 190$) in primary care found that cognitive behavior therapy was more effective than fluvoxamine; the greatest improvements were seen with cognitive behavior therapy and fluvoxamine in combination [22,25].

5. Paroxetine

The efficacy of paroxetine in short- and long-term treatment has been investigated in a series of placebo-controlled multi-center trials, some of which have also included an active comparator drug. In the first study ($n = 120$) [26], patients received either a flexible dose (10–60 mg/day) of paroxetine or placebo in addition to cognitive behavior therapy. There was a significantly greater reduction in panic attack frequency in patients receiving paroxetine, compared to those taking placebo, from the sixth week of treatment: however, the majority (64%) of patients who responded to paroxetine were still experiencing panic attacks at the end of the study. The optimal dosage of paroxetine in the acute treatment of panic disorder is around 40 mg/day, this having been established in two placebo-controlled studies [29]. In the first of these, 425 patients received either placebo or 10, 20, or 40 mg/day of paroxetine; only the 40 mg daily dose was significantly more efficacious, with 86% of paroxetine treated patients becoming free of panic attacks compared with 50% on placebo [27].

The relative efficacy of paroxetine has been examined in three studies. In one investigation [28], 367 patients were randomized to receive placebo, paroxetine (daily dose, 20–60 mg), or clomipramine (daily dose, 50–150 mg). More patients in the paroxetine group experienced a complete resolution of panic attacks by the end of the 12-week study, and paroxetine treatment showed a significantly earlier onset of action than clomipramine. Patients who completed acute-phase treatment could then continue with treatment for a further 36 weeks. Panic attacks in the active treatment groups continued to decrease during the maintenance study: at the study endpoint, 85% of paroxetine-treated patients were free from panic attacks, compared to 72% with clomipramine and 59% with placebo [29]. A further placebo-controlled comparison ($n = 131$) has evaluated the relative efficacy of paroxetine, clomipramine, and cognitive therapy. After 12-weeks, both drug treatments were superior to cognitive therapy or placebo in reducing anticipatory anxiety agoraphobia avoidance, and associated disability: furthermore, significantly more paroxetine-treated patients were panic-free (65%) than with cognitive therapy (40%) or placebo (34%) [30]. However, another comparator-controlled study produced less encouraging results: when compared to treatment with placebo or alprazolam there was no advantage for paroxetine, probably because of unusually high response rates among patients receiving placebo (data on file, SmithKline Beecham Pharmaceuticals).

Further evidence for long-term efficacy comes from an investigation that employed a "relapse prevention" design. In this study ($n = 175$), patients who responded to acute treatment were allocated randomly either to continue with paroxetine or to switch to placebo: only 5% of patients who continued with paroxetine relapsed, compared to 30% in patients who switched to placebo [31].

6. Sertraline

The results of several large double-blind placebo-controlled studies of sertraline have been published [32–34]. In two studies of identical design, which together included 342 patient

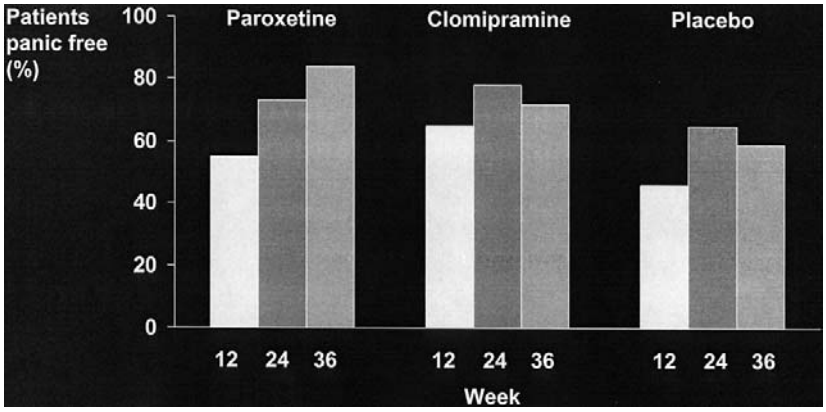

Figure 1 Efficacy of paroxetine and clomipramine in long-term treatment of panic disorder ($p = 0.004$, paroxetine vs. placebo at week 36). (From Ref. 28).

[32,33], sertraline (50–200 mg/day) was significantly more efficacious than placebo in reducing the "panic attack burden" (a product of the number and severity of panic attacks), from the second week of treatment. Significantly more patients became free of panic attacks with sertraline (59.3%) than with placebo (46.3%). In addition, there were greater improvements with sertraline in quality-of-life measures, including items relating to mood, work, social, family and leisure activities, and in overall satisfaction with life. In one study [33], adverse events were as frequent with sertraline and placebo; in the other [32], significantly more patients stopped treatment with sertraline because of adverse experiences.

In a third double-blind, placebo-controlled 12-week study ($n = 178$) that compared three fixed daily doses (50, 100, or 200 mg) [34], sertraline was superior in reducing the number of panic attacks and limited symptom attacks, and in improving anxiety symptoms. Although there were advantages for sertraline on scores on the CGI scale earlier in the study, there was no difference from placebo at the end of treatment. The number of patients dropping out from the study were similar for active and placebo treatment, but significantly more patients discontinued sertraline because of adverse events (mainly dry mouth and delayed ejaculation). The authors concluded that there was no advantage in increasing the dose of sertraline above 50 mg per day [34].

The relative efficacy of sertraline, compared to other treatments for panic disorder, is uncertain. A double-blind placebo-controlled study that compared sertraline to imipramine, yet to be published, found some advantages for sertraline over placebo, but none for imipramine, probably because of high placebo response rate [35].

7. Summary

Most double-blind placebo-controlled studies of SSRIs in acute and continuation treatment of patients with panic disorder have found evidence for efficacy. However, the magnitude of improvement during treatment is variable—the proportion of patients becoming free from panic attacks ranges from 36 [26] to 86% [27], highest response rates with SSRIs being seen in those studies that also show greater improvements on placebo [36]. Similar variation is seen in measures of global anxiety [36]. Differences in study design do not allow exact comparison of the efficacy of different SSRIs, and there is still a need for head-to-head comparisons. Although a meta-analysis of 27 placebo-controlled, random-

ized trials concluded that SSRI treatment was more effective than treatment with either imipramine or alprazolam [37]; this was published in 1995 and therefore is out of date. The relative side-effect burden with differing treatments is also unclear, although a recent review suggests SSRIs are better tolerated than other pharmacological treatments in panic disorder [38]. The relative efficacy of SSRIs and psychological approaches remains unclear, but combination treatment may produce optimal results [22].

B. Benzodiazepines

The efficacy and tolerability of benzodiazepine anxiolytics in the treatment of panic disorder have been reviewed elsewhere [2]. The efficacy of alprazolam has been established in both short- and long-term treatment, whereas clonazepam, lorazepam, and diazepam have been evaluated only in short-term treatment studies.

1. Alprazolam

The short-term efficacy of alprazolam (6–10 mg/day) was established in the first phase ($n = 526$) of the Cross-National Collaborative Panic Study. After 8 weeks of treatment, significantly more alprazolam-treated patients (55%) were panic attack–free than among those receiving placebo (32%): there were also significant advantages for alprazolam over placebo in reducing anticipatory anxiety and associated disability [39]. In the second phase of the study ($n = 168$), patients were allocated randomly to receive alprazolam (6–10 mg/day), imipramine (150–250 mg/day), or placebo for 8 weeks. At study endpoint, both active treatments (70% panic-free) were significantly more efficacious than placebo (50%): alprazolam had an earlier onset of action than imipramine [40]. Two subsequent placebo-controlled studies have confirmed the efficacy of alprazolam in short-term treatment [41,42].

Alprazolam may also be efficacious in continuation treatment. In an extension of the Cross-National Study in which patients who completed phase II ($n = 181$) could continue with treatment for a further 6 months, the efficacy of all three treatments was maintained [43]. An 8-month treatment study ($n = 106$) also found that alprazolam, imipramine, or placebo were helping patients become free of panic attacks [44].

2. Clonazepam

The short-term efficacy of clonazepam has been confirmed by the findings of three placebo-controlled treatment studies. In a 6-week study ($n = 71$), both clonazepam (0.5–5.0 mg/day) and alprazolam (1–10 mg/day) were significantly more efficacious than placebo: at endpoint the proportion of patients who were panic-free was 50%, 46%, and 14%, respectively [45]. The second investigation ($n = 32$) confirmed the efficacy of clonazepam over 4 weeks of treatment [46]. A subsequent fixed-dose treatment study ($n = 413$) found that higher daily doses of clonazepam were all more efficacious than placebo in reducing phobic avoidance and anticipatory anxiety; at study endpoint 73% of the group who received 1.0 mg per day were panic-free compared to 55% of those receiving placebo [47].

3. Other Benzodiazepines

Both diazepam and lorazepam have been efficacious in short-term controlled studies. In a placebo-controlled comparison ($n = 241$) of diazepam (60–100 mg/day) and alprazolam (6–10 mg), both active treatments were significantly more efficacious in reducing panic attack frequency, anticipatory anxiety, agoraphobic avoidance, and overall disability [48].

Finally, a 6-week treatment study showed that lorazepam had similar efficacy to alprazolam [49].

4. Summary

Benzodiazepines are efficacious in short-term treatment of panic disorder. Treatment studies indicate that the treatment effect for benzodiazepines (i.e., the difference between active treatment and placebo groups) is 20 to 32% for alprazolam and 22 to 36% for clonazepam. There is much less evidence that benzodiazepines are efficacious in longer term treatment. Side effects are common and can be troublesome. About 80% of patients will experience sedation or drowsiness, and ataxia, fatigue, slurred speech, memory disturbances, and sexual problems are not uncommon [2]. Approximately one-third of patients undergoing long-term treatment develop dependence, and discontinuation symptoms can be most distressing. As such, benzodiazepines are best reserved for short-term treatment when other approaches have not proved unhelpful.

III. SOCIAL ANXIETY DISORDER

The treatment of patients with social phobia has received much attention in recent years, possibly because of increasing awareness of the burden imposed by this potentially lifelong condition. The effects of most treatments have been evaluated in short-term placebo-controlled, randomized trials, so there is little information on the potential value of continuation treatment.

A. Selective Serotonin Reuptake Inhibitors

1. Citalopram

There are no published studies with citalopram, but the efficacy of other SSRIs in this condition suggests that citalopram is likely to be efficacious. Studies with the enantiomer escitalopram are believed to be underway.

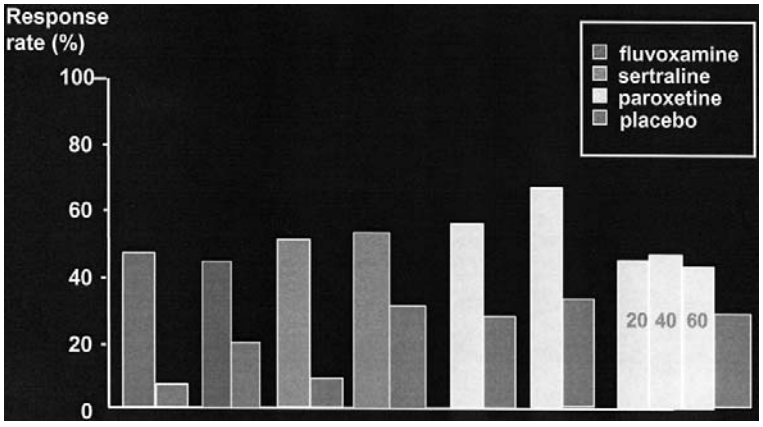

Figure 2 Social anxiety disorder: controlled treatment studies of SSRIs. (From Refs. 51–57,67.)

2. Fluoxetine

No placebo-controlled or comparator-controlled studies of fluoxetine have been published. There has been one double-blind, placebo-controlled study of fluoxetine in children with elective mutism, which has some similarities to social phobia [50]. Children who had not responded to placebo treatment were randomly allocated to either continue with placebo or switch to fluoxetine at a dose of 0.6 mg/kg/day. There were significant advantages for fluoxetine on parents' ratings of mutism and global change, but not on ratings made by teachers or health professionals, and most children remained substantially impaired at the end of the treatment period.

3. Fluvoxamine

The effects of fluvoxamine have been investigated in two double-blind, placebo-controlled studies. In the first, single-center, study ($n = 30$) patients received either a fixed daily dose of fluvoxamine (150 mg) or placebo for 12 weeks: a substantial improvement was seen in 7 (46%) of the 15 patients who received fluvoxamine, but in only one patient (7%) who received placebo. Fluvoxamine treatment had significant advantages on measures of social and anticipatory anxiety, but did not differ from placebo in phobic avoidance [51]. In a subsequent multicenter, 12-week flexible-dose placebo-controlled study ($n = 92$), there were significant advantages for fluvoxamine (mean daily dose at endpoint, 202 mg) on all social phobia rating scales from week 8 and at endpoint there were significantly more responders with fluvoxamine (42.9%) than with placebo (22.7%) [52].

4. Paroxetine

Three large double-blind, placebo-controlled multicenter studies have evaluated efficacy in short-term treatment [53,54] (data on file, SmithKline Beecham Pharmaceuticals). In the first, conducted in North America ($n = 187$), patients were randomly allocated to receive placebo or flexible daily doses (10–50 mg) of paroxetine for 12 weeks. At the end of the study, significantly more paroxetine-treated patients were rated as much or very much improved (paroxetine 55%, placebo 23.9%): there were also significant advantages for paroxetine in measures of social anxiety symptoms and phobic avoidance [53].

A second multicenter double-blind treatment study ($n = 290$) conducted in Europe and South Africa produced similar findings. Patients were assigned randomly to receive placebo or flexible doses of paroxetine (mean daily dose at endpoint, 34.7 mg) over 12 weeks. Significantly more patients who received paroxetine responded to treatment (65.7% vs. 32.4%), and paroxetine was associated with significantly greater reductions in social anxiety symptoms and phobic avoidance and greater improvements in work, social, and family life [54]. A third, placebo-controlled study, which employed fixed daily doses of paroxetine (20, 40, or 60 mg), found that there was little to be gained in increasing the dose of paroxetine above 40 mg per day, because higher doses were not associated with greater improvement, but rather linked to a greater side-effect burden (data on file, SmithKline Beecham Pharmaceuticals).

There are at present no published large-scale studies of paroxetine in long-term treatment but an investigation of open-label acute treatment, followed by double-blind placebo-controlled discontinuation, found that paroxetine was efficacious in preventing relapse of illness [55].

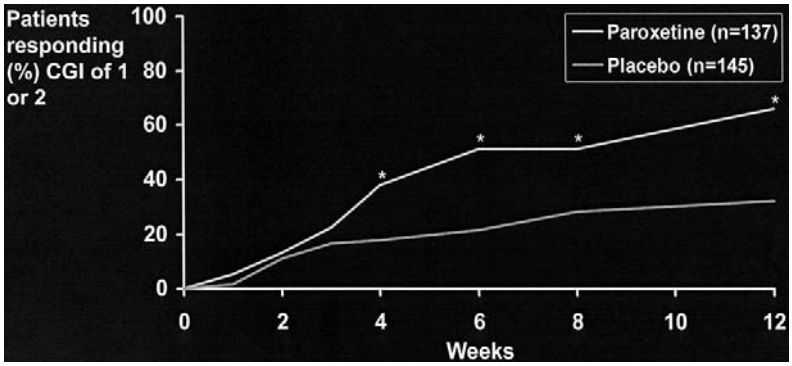

Figure 3 Paroxetine in generalized social anxiety disorder ($p < 0.001$ vs. placebo). (From Ref. 54).

5. Sertraline

Three placebo-controlled studies have examined the effects of sertraline in the acute treatment of patients with social phobia [56–58]. A preliminary, small, double-blind crossover study ($n = 12$) of treatment with flexible daily doses of sertraline (50–200 mg) found a significant improvement in social anxiety symptoms with sertraline, but not with placebo [56]. In a larger ($n = 233$) placebo-controlled parallel-group flexible-dose 12-week treatment study, there were significant advantages for sertraline in measures of social anxiety symptoms and phobic avoidance, and in the proportion of patients who responded to treatment (sertraline, 52%; placebo 29%) [57]. The investigation of sertraline in social phobia is a 24-week double-blind placebo-controlled treatment study in primary care, in which patients were allocated randomly to receive sertraline alone, exposure therapy alone, a combination of sertraline plus exposure, or placebo: the greatest response rate (46%) was seen with combination treatment [58].

6. Venlafaxine

There is at present little evidence for the efficacy of venlafaxine in social phobia. In a 12-week open-label study, 8 out of 17 patients (47%) showed clinically relevant improvement during venlafaxine treatment [59].

B. Tricyclic Antidepressants

Tricyclic antidepressants have not been studied extensively in patients with social phobia there is little evidence to suggest they may be valuable. While early open-label studies suggested that treatment with clomipramine could be efficacious, these studies were uncontrolled and included patients with a variety of anxiety disorders [60–62]. A more recent 8-week open study of imipramine in 15 patients showed a response rate on the CGI scale of only 20% [63].

C. Monoamine Oxidase Inhibitors

Randomized controlled trials have shown the irreversible MAOI phenelzine to be efficacious in social anxiety disorder. Its efficacy has been evaluated in double-blind, placebo-

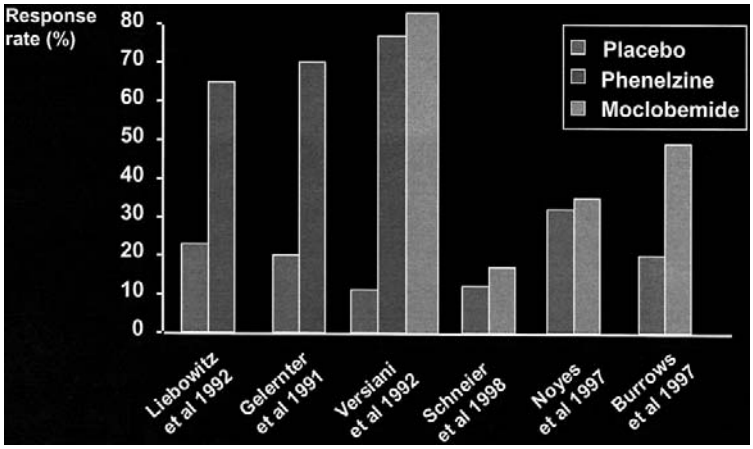

Figure 4 Social anxiety disorder: controlled treatment studies of MAOIs.

controlled studies involving over 200 patients [64–66]. In these studies, phenelzine was consistently more effective than placebo. However, phenelzine has a rather poor side-effect profile, causing weight gain and hypotension; the need for a restricted diet because of the interaction of the drug with tyramine-containing foodstuffs and sympathomimetic medicines precludes phenelzine from being a first-line treatment [67].

There are inconsistent efficacy data for the selective and reversible MAOI moclobemide. Early placebo-controlled studies found it to be more efficacious than placebo [66,68], particularly at higher doses, but later studies suggest that moclobemide treatment conveys little benefit [69,70]. In a large ($n = 523$), 12-week controlled study of moclobemide, with doses ranging from 75 to 900 mg/day, response rates using the CGI scale were only 32 to 38% for moclobemide, compared with 33% for placebo [69]. Although moclobemide is licensed for the treatment of social phobia in some countries, the overall evidence for clinical efficacy of moclobemide does not support its use as a first-line treatment. Furthermore, at higher doses it behaves more like an irreversible MAOI, and patients must observe similar dietary restrictions to those needed when taking phenelzine.

Three 12-week placebo-controlled treatment studies with the reversible MAOI brofaromine have produced encouraging results, although the development of the drug has been curtailed. In the first study, there was a response rate (a score of 10 or less on the Hamilton Anxiety Scale) of 73% with brofaromine, compared to 0% with placebo [71]. In a second study, response rates ("very much" or "much" improved on the CGI item) were 78% for brofaromine and 23% for placebo [72]. In the third investigation ($n = 102$), the response rates were 50% for those receiving brofaromine and 19% in those allocated to placebo [73].

D. Benzodiazepines

There are few data on the efficacy of benzodiazepines in the treatment of social phobia, but two short-term, placebo-controlled studies have evaluated alprazolam and clonazepam. For alprazolam, there was only modest efficacy (evaluated by the fear questionnaire) over a 12-week treatment period in a study of 65 patients (38% response rate vs. 20% for placebo [64]. In a study of 75 patients, clonazepam was significantly superior to placebo

on the basis of the CGI scale, over a treatment period of 10 weeks (78% response rate vs. 20% for placebo [74]. Benzodiazepines are probably best reserved for patients who do not respond to other treatment approaches. They have marked sedative effects and are not recommended in patients with comorbid alcohol abuse or dependence. Furthermore, they are largely ineffective in treating depression, which is a disadvantage in the management of patients with social phobia, who often have significant comorbid depressive symptoms.

E. Buspirone

The azapirone anxiolytic drug buspirone produces only a modest improvement in patients with social phobia [75,76]. A small ($n = 30$) placebo-controlled, double-blind treatment study showed no significant differences between placebo and buspirone at an average dose of 30 mg/day [77]. However, it may be a useful adjuvant treatment in some patients who have made a partial response to an SSRI [78].

F. Beta-Blockers

Beta-blockers are often prescribed to professional performers in an attempt to control performance-related anxiety manifest by autonomic symptoms such as tachycardia, tremor, and sweating [79]. However, placebo-controlled trials with atenolol have found it not to be helpful in the treatment of generalized social phobia [65,80,81]. Beta-blockers should be avoided in patients with asthma and, because of the limited evidence for efficacy, are not an appropriate treatment [82].

G. Gabapentin and Prebagalin

A double-blind placebo-controlled treatment study with the anticonvulsant drug gabapentin suggests that it could be helpful in the overall management of patients with social phobia [83]. After 7 days of single-blind placebo treatment, patients were allocated randomly to receive either placebo or flexible doses (300–360 mg/day) of gabapentin. Outcome measures included the LSAS, Fear Questionnaire (FQ), Brief Social Phobia Scale (BSPS), and CGI. A total of 82 patients were enrolled: 69 entered double-blind treatment, but only 39 completed the study. There were significant advantages for gabapentin over placebo on the LSAS, FQ, BSPS, and CGI, especially in patients with more severe symptoms at baseline. There were more withdrawals because of adverse events with gabapentin than with placebo, the more common events being nausea, dizziness, somnolence, insomnia, nervousness, and facial edema [83]. A recent fixed-dose (150 mg or 600 mg/day) placebo-controlled study ($n = 135$) indicates that at the higher dose pregabalin is efficacious in generalized social phobia, but more research into its efficacy and tolerability is required before its potential value in treatment can be assumed [84].

H. Summary

SSRIs are efficacious in short-term treatment of patients with social phobia. Their efficacy in long-term treatment is not yet established fully nor is their relative efficacy compared to other treatment approaches, including drug treatment with phenelzine and psychological treatment with cognitive behavior therapy. Monoamine oxidase inhibitor drugs are useful in many patients, but the tolerability of phenelzine is poor and stringent dietary restrictions restrict its use in clinical practice: it is probably best reserved for second-line or third-

line treatment in patients who have not responded to an SSRI. Moclobemide has proven efficacy, although the evidence is somewhat conflicting: higher doses are needed, and it is best to follow a traditional MAOI exclusion diet. Benzodiazepines may be useful in the early phases of treatment while waiting for an SSRI to become efficacious, or possibly as an adjuvant approach in partial responders. Finally, the evidence for gabapentin and pregabalin suggests these compounds are efficacious in social phobia, but the relative efficacy and tolerability of these compounds compared to an SSRI has not been established.

IV. GENERALIZED ANXIETY DISORDER

A. Selective Serotonin Reuptake Inhibitors

As has been demonstrated, selective serotonin reuptake inhibitors have proven efficacy in panic disorder and social anxiety disorder. Accumulating data, most relating to paroxetine, show that SSRI treatment can also be efficacious in patients with generalized anxiety disorder(GAD).

1. Citalopram

No controlled trials with citalopram have yet been presented or published. An early randomized controlled trial comparing two dose ranges of citalopram and imipramine in 472 primary care depressed patients found that both treatments produced reduced mean scores on the HAMD anxiety factor [85]. A more recent study comparing citalopram and paroxetine in 104 patients with DSM-IV major depression or mixed anxiety-depressive disorder found that both treatments were associated with significant reductions in the HAM-A total scorce [86]. By contrast, the results of a 24-week double-blind placebo-controlled treatment study comparing citalopram and sertraline in 323 patients with DSM-IV major depressive disorder indicate that citalopram was more efficacious than placebo in reducing mean scores on the HAMD anxiety cluster ($p < 0.01$), there being no significant difference on this measure between sertraline treatment and placebo [87].

2. Fluoxetine

There are no published treatment studies in adults with DSM-IV GAD. An open pilot treatment study in 16 children and adolescents (9–18 years) with mixed anxiety disorders showed that fluoxetine might be of only limited benefit [88]. Double-blind treatment studies of depressed patients indicate that fluoxetine is as efficacious as imipramine, clomipramine, or amitriptyline in relieving anxiety symptoms in depression, but the efficacy of fluoxetine in comorbid depression and GAD is not proven [89].

3. Fluvoxamine

The efficacy of fluvoxamine as a treatment for GAD has not been established. However, in a small ($n = 30$) open study of patients with comorbid major depression and GAD, fluvoxamine showed significant improvement in both anxiety and depressive symptoms [90]. This finding requires replication in patients with GAD before the efficacy of fluvoxamine can be assumed.

4. Paroxetine

The efficacy of paroxetine in short-term treatment of GAD has been evaluated in four randomized double-blind controlled studies [91]. The first evaluation of paroxetine was

in a comparator-controlled trial involving 81 patients with a DSM-IV diagnosis of GAD, paroxetine being compared to imipramine and 2′-chlorodesmethyl-diazepam in an 8-week treatment study [92]. Paroxetine showed superior efficacy to 2′-chlorodesmethyldiazepam and similar efficacy to imipramine. Paroxetine treatment differed significantly ($p < 0.05$) from 2′-chlorodesmethyl-diazepam from week 4 onward, while imipramine only did so at study endpoint. The second investigation was an 8-week, dose-finding study involving 566 patients, performed in the United States. In this study, paroxetine treatment (20 or 40 mg/day) was significantly superior to placebo ($p < 0.001$) in reducing both the mean HAM-A total score and the mean scores on HAM-A items 1 (anxious mood) and 2 (tension). Response rates were 68 and 81% with paroxetine 20 mg and 40 mg/day, respectively, compared with 52% of patients in the placebo group (observed case data). By the end of the study, the mean change from baseline on a health-related quality-of-life questionnaire (EuroQoL-5D) and visual analog scale was significantly greater for both paroxetine-treatment groups, indicating a significant improvement in quality of life. The third treatment study was an 8-week, flexible-dose study conducted in 326 U.S. patients with GAD. Paroxetine (20–50 mg/day) was significantly superior to placebo ($p < 0.05$) in reducing mean HAM-A total score and mean scores on HAM-A items 1 and 2 and was generally well tolerated. A fourth study of similar design conducted in 372 patients in Europe has revealed similar reductions in HAM-A total score and HAM-A items 1 and 2.

The effects of treatment may extend beyond symptom reduction and improved quality of life. A small ($n = 29$) uncontrolled pilot study showed paroxetine treatment was associated with a reduction in maladaptive personality traits [93] with significant decreases in harm avoidance ($p = 0.0001$) and novelty seeking ($p = 0.006$), and a significant increase in self-directedness ($p = 0.0004$) The placebo-controlled paroxetine treatment studies also showed that as symptoms of GAD resolve there is an associated improvement in symptom-related disability, assessed using the patient-rated Sheehan Disability Scale (SDS) which covers impairment in social, work, and family life. At endpoint in all three studies, a statistically significant difference between paroxetine and placebo was seen in the SDS total score (flexible-dose study 1 difference $= -1.8$; $p = 0.037$; study 2 difference $= -2.4$; $p = 0.001$; fixed-dose study for 20 mg/day regimen, difference $= -3.1$; $p < 0.001$; for 40 mg/day regimen, difference $= -3.6$; $p < 0.001$) [94].

Paroxetine has recently been found efficacious in long-term treatment, there being significantly fewer relapses with paroxetine (11%) than with placebo (40%) in a 6-month relapse-prevention study [95].

5. Sertraline

No clinical trials of sertraline in the treatment of GAD have been reported. Double-blind treatment studies indicate that sertraline is efficacious in relieving anxiety symptoms within depression and the symptoms and impairment associated with panic disorder, social phobia, post-traumatic stress disorder, and obsessive-compulsive disorder, but its efficacy in GAD cannot be assumed.

The findings of the randomized controlled trials with paroxetine indicate that SSRI treatment can be efficacious in GAD. There are many further research needs, such as establishing the comparative efficacy and acceptability of differing treatments, in both short- and long-term treatment; examining the effects of combining SSRI treatment with psychological approaches such as cognitive-behavioral therapy; and evaluating the effectiveness of treatment in wider clinical practice.

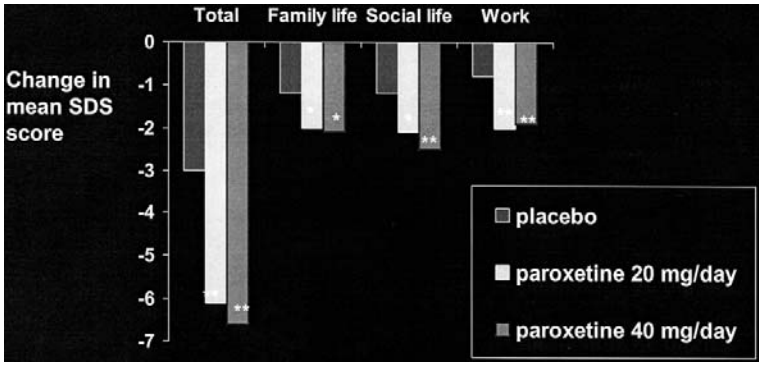

Figure 5 Fixed-dose study of paroxetine in generalized anxiety disorder (*$p < 0.027$ vs. placebo for pairwise comparisons; **$p < 0.001$ vs. placebo, $^{+}$adjusted for treatment and site.

B. Other Antidepressant Drugs

1. Nefazodone

Nefazodone inhibits the reuptake of both serotonin and noradrenaline, although its strongest action is antagonism of the $5HT_{2a}$ receptor. Studies in patients with major depression and associated anxiety indicate that nefazodone can significantly reduce anxiety symptoms [96], and a small open-label evaluation suggests that it can improve the symptoms of GAD [97].

2. Venlafaxine

This serotonin-noradrenaline reuptake inhibitor has proven efficacy in the treatment of depression and GAD. A preliminary study [98] in depressed outpatients indicated that once-daily venlafaxine was efficacious in relieving anxiety symptoms, and suggested it might therefore have a role in the management of patients with GAD. This supposition was confirmed by the findings of two studies of short-term treatment of GAD with venlafaxine [99,100]. In one study, venlafaxine was significantly superior to the active comparator buspirone in reducing anxiety symptoms, and there was some evidence that venlafaxine had an earlier onset of action [99]. Three other placebo-controlled studies of venlafaxine in the short-term treatment of GAD have been presented [101,102]. Efficacy is reported in two of the three studies of venlafaxine compared to placebo, but until these studies are published fully it is difficult to make a detailed assessment.

The long-term efficacy of venlafaxine extended-release capsules has been shown through the results of a 6-month randomized, double-blind, placebo-controlled, parallel-group study [103]. These 6-month results were replicated in a second placebo-controlled study [104]. The single relapse-prevention study, over a period of 4 months did not show efficacy for venlafaxine compared to placebo. Evidence supporting a dose-response relationship for venlafaxine is reported in the 6-month studies, with higher doses associated with a better response.

3. Mirtazapine

The primary mechanism of action of mirtazapine is through antagonism of presynaptic alpha-2 receptors and heteroceptors. No studies of mirtazapine in GAD have been reported,

although a small placebo-controlled study of outpatients with primary anxiety disorders indicated that it could reduce anxiety symptoms and improve overall functioning [105]. A meta-analysis of eight double-blind controlled treatment studies in patients with major depression suggests that mirtazapine is efficacious in reducing anxiety-related items of the Hamilton Rating Scale for Depression [106].

4. Buspirone

Buspirone is a azapirone anxiolytic drug, with partial agonist properties at $5HT_{1a}$ receptors, which has proven efficacy in the treatment of patients with GAD [107]. An early study [108] established that buspirone had comparable efficacy to diazepam in patients with generalized anxiety, and a meta-analysis of eight controlled treatment studies indicates that buspirone has comparable efficacy to benzodiazepines in the management of GAD [109]. It also appears efficacious in reducing associated depressive symptoms in patients with GAD, but is not an accepted treatment for patients with major depression [110]. However, not all studies with buspirone have been positive [111,112].

5. Benzodiazepines

A systematic review of randomized controlled trials has established that benzodiazepines are an effective and rapid treatment for many patients with GAD [113]. However, the benzodiazepines are far from ideal anxiolytic drugs. The untoward effects of benzodiazepines include sedation, memory disruption, and psychomotor impairment, with an associated increased risk of traffic accidents. Other problems include the development of tolerance, abuse, and dependence, and withdrawal symptoms on stopping the drug. As such, many authorities counsel that benzodiazepines should be reserved for short-term use (up to 4 weeks), and prescribed at low dosage [114]. Others have argued that benzodiazepines are clearly efficacious, and withholding treatment on the basis of potential risk of dependence is unjustified and detrimental to overall well-being [115].

V. POST-TRAUMATIC STRESS DISORDER

A. Selective Serotonin Reuptake Inhibitors

The number of published or presented placebo-controlled or comparator-controlled studies of SSRIs in the treatment of post-traumatic stress disorder (PTSD) has increased rapidly in recent years.

1. Fluoxetine

Three double-blind placebo-controlled studies with fluoxetine have been performed. In the first study [116], 64 patients (31 combat veterans and 33 civilian patients) with PTSD were randomly allocated to receive placebo or fluoxetine, up to a maximum daily dose of 60 mg, for 5 weeks. By the end of the study there were significant advantages for fluoxetine over placebo, in terms of overall PTSD psychopathology, largely in the civilian patients. Certain symptom clusters, such as hyperarousal and hostility, showed little change with fluoxetine treatment. In a second placebo-controlled flexible-dose 12-week treatment study of fluoxetine in civilian patients ($n = 53$), fluoxetine was associated with significantly greater reductions in overall PTSD psychopathology, and with a greater likelihood of response (fluoxetine, 85%; placebo, 62%) [117]. A parallel investigation in combat

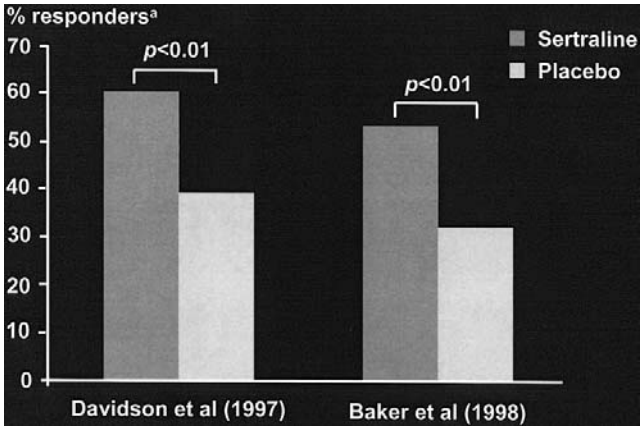

Figure 6 Sertraline in short-term treatment of PTSD. ([a] Responder = CAPS-2 30% decrease and CGI = 1 or 2 at endpoint.

veterans using an identical protocol found no difference between fluoxetine and placebo, indicating that patient background is an important treatment consideration in PTSD [118].

2. Paroxetine

Three large multicenter placebo-controlled treatment studies, together involving about 1200 patients, have been undertaken. The findings of the two flexible-dose studies (20–50 mg/day) and one fixed-dose study (20 or 40 mg/day) together show that paroxetine is efficacious in reducing PTSD symptoms, with significant advantages over placebo on the reexperiencing/intrusion, avoidance/numbing and increased arousal symptom clusters. Treatment was efficacious in all patient groups regardless of trauma type or gender [119].

3. Sertraline

Two double-blind, flexible-dose, placebo-controlled, 12-week treatment studies with sertraline have been performed [120,121]. In the first study ($n = 208$) (mean sertraline daily dose at endpoint, 125 mg), there were advantages for sertraline in three measures of overall psychopathology, and no significant differences in the rate of discontinuation from treatment because of adverse experiences [120]. In the second study ($n = 187$), there were significantly greater improvements with sertraline in three and four primary efficacy measures (CAPS-2 severity score, CGI-S, and CGI-I) and on all secondary measures, and again the rate of discontinuation because of side effects was similar in the two groups [121]. Preliminary data suggest that continuation of sertraline treatment is efficacious in preventing symptomatic relapse.

B. Tricyclic Antidepressants

Two placebo-controlled studies have evaluated the efficacy of TCA treatment in combat veteran patients with PTSD. The first was an 8-week study ($n = 46$) of amitriptyline, which found it to be efficacious, with significant advantages over placebo on global measures of PTSD symptoms, anxiety, and depression: the presence of comorbid depression, panic disorder, or alcohol abuse reduced the chance of response [122]. A further analysis showed

that response was greater with less severe degrees of trauma, with less severe symptoms, and when there was no premorbid neuroticism [123]. The second placebo-controlled 8-week treatment study ($n = 60$) of a TCA involved imipramine (up to 300 mg/day), with phenelzine (up to 75 mg/day) as an active comparator. Imipramine was marginally less efficacious than phenelzine (global response 65% and 68%, respectively), the MAOI showing greater efficacy in reducing intrusive and avoidant symptoms [124].

C. Other Antidepressants

Two placebo-controlled treatment studies with brofaromine have been published. A small ($n = 45$) flexible-dose European study found that brofaromine was efficacious in a predominantly civilian group of patients, greater efficacy being seen in those whose symptoms had lasted more than 12 months. At study endpoint, 55% of brofaromine-treated patients no longer fulfilled diagnostic criteria for PTSD, compared to 26% of those who had received placebo; insomnia was the most troublesome side effect [125]. A larger ($n = 118$) U.S. study found some evidence for efficacy of brofaromine: although there were no significant drug–placebo differences on measures of PTSD symptoms, overall response rates were greater with drug treatment (60%) than with placebo (39%) [126].

The potential efficacy of trazodone or nefazodone in PTSD has been assessed in open-label treatment. In a small case series ($n = 6$), trazodone treatment (up to 400 mg/day) produced only marginal benefit in reducing PTSD symptoms, with no associated improvement in social or occupational functioning [127]. Nefazodone has been evaluated in two case series. In the first, there were considerable improvements in most measures in 10 combat veterans treated with doses up to 600 mg per day for 12 weeks [128]. These promising findings are supported by a second report, in which 36 combat veterans showed improvements in measures of PTSD symptoms, anxiety, and depression, over 8 weeks [129].

D. Summary

Placebo-controlled treatment studies indicate that SSRIs, amitriptyline, imipramine, phenelzine, and brofaromine are efficacious in PTSD. The evidence for differing SSRI treatment varies; only paroxetine has been found efficacious in both genders, regardless of trauma type, across all key symptom clusters. More research is needed into the effects of long-term treatment, and into the relative efficacy and tolerability of different treatment approaches.

VI. CONCLUSIONS

Modern management of patients with anxiety disorders will focus on better-tolerated treatments, with efficacy that has been proven in both short- and long-term treatment studies. Although numerous double blind placebo-controlled trials have shown that the SSRIs are efficacious in a range of anxiety disorders, many patients do not respond to treatment. The magnitude of improvement in those who do respond is often disappointing, so there is still a need to develop novel psychopharmacological approaches with greater efficacy than SSRI treatment. In addition, many patients with anxiety disorders are exquisitely sensitive to the side effects of psychotropic drugs, and new treatments with a more acceptable side-effect profile are needed. Finally, many patients prefer psychological treatment approaches, fearing possible side effects of drug treatment or the risk of becoming depen-

dent. There have been few well-designed controlled studies where the effects of drug and psychological treatments are compared. This is a substantial deficit, as clinicians cannot obtain a full picture of the relative strengths and weaknesses of different treatment approaches in these chronic and severe disorders.

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Pharmacotherapy of Mixed Anxiety/Depression Disorders

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I. INTRODUCTION

The choice and the clinical management of the pharmacological treatment of psychiatric conditions characterized by the co-occurrence of anxiety and depression is mostly influenced by the model adopted to explain the co-occurrence of symptoms that appear to belong to different diagnostic domains. The two models currently accepted to explain the occurrence of psychiatric comorbidity are the categorical and the dimensional one. According to the categorical model, as first described by Roth et al. in the 1960s [1], anxiety and depression are discrete syndromes that may co-occur in the same patient at a certain time. Actually, anxiety may occur either as a "subthreshold" or subsyndromal condition (i.e., few or isolated symptoms not structured in a full syndrome) or as full syndrome with all the clinical features that characterized the specific clinical diagnosis (e.g., panic disorder, obsessive-compulsive disorder, generalized anxiety disorder). More than 30% of anxiety disorder patients receive an additional diagnosis of depression (major depression or dysthymia) [2], 20% had generalized anxiety disorder (GAD) [3], and 20 to 30% of this group experience panic attacks [4]. Similarly, depression may be present as a full syndrome or as a subthreshold condition.

The clinical observation that anxiety and depression frequently co-occur at a subsyndromal level, together with data derived from genetic and pharmacological studies on these comorbid conditions, have stimulated the popularity, in recent years, of the dimensional model as opposed to the categorical one.

According to the dimensional model, the different clinical conditions (e.g., anxiety and depression) are not discrete entities but a "spectrum" of syndromes characterized by anxiety and depressive symptoms in different combinations and with different degrees of severity [5].

The concept of spectrum, as opposed to that of full syndromal discrete diagnoses, implies the consideration of clinical conditions that are not severe or definite enough to fulfill the diagnostic criteria but that may be relevant in terms of prevalence in the population and challenging in terms of clinical management. It has been estimated that the prevalence of subthreshold depression and anxiety in psychiatric populations is about 7% and 22%, respectively [6]. Furthermore, the co-occurrence of depression and anxiety symptoms (even though at a subsyndromal level) has been associated with greater severity of illness, higher levels of functional impairment [7], chronicity [8], and delayed [9] or poor [10] response to antidepressants.

The International Classification of Diseases (ICD-10) [11] provides a code for mixed anxiety and depression disorder. In the DSM-IV [12], the diagnostic category of mixed anxiety depressive disorder (MAD) and of minor depressive disorder have been proposed as research diagnostic criteria, suggesting the need for a more dimensional approach to explain the presence of comorbid conditions. The essential feature of MAD is a persistent or recurrent dysphoric mood lasting at least 1 month. The dysphoric mood is accompanied by additional symptoms that also must persist for at least 1 month and include at least four of the following: (1) concentration or memory difficulties; (2) sleep disturbance; (3) fatigue or low energy; (4) worry—being easily moved to tears; (5) hypervigilance in anticipating the worst; (6) hopelessness or pessimism about the future; (7) low self-esteem or feelings of worthlessness. Actually, many questions about this diagnostic category remain unanswered. The prevalence of MAD in the general population, the etiopathogenetic mechanisms underlying this illness, its natural course, and the response to treatment are still uncertain. In addition, the clinical characteristics that may allow discrimination between MAD, major depressive disorder (MDD), or anxiety disorders are not clearly defined yet [13]. Furthermore, the matter appears to be complicated by methodological and assessment issues. As an example, it cannot be ruled out that the frequently observed overlap of depressive and anxiety symptoms could be due to the use of nonspecific assessment instruments (e.g., the Hamilton Depression Rating Scales for Anxiety and for Depression). In addition, general practitioners sometimes are not able to distinguish between anxiety and depressive disorders because this diagnosis requires psychiatric training. This has led to the assumption, quite common in the general practice, that psychiatric patients usually experience an admixture of symptoms instead of specific conditions [14].

However, the traditional view that anxiety and depression are discrete clinical conditions has been dramatically challenged in the last few years, particularly when the dichotomy between antidepressant and anxiolytic compounds has been surpassed. The finding that some antidepressants show specific antianxiety properties and are effective in the treatment of anxiety disorders even when not complicated by the occurrence of depressive symptoms dates back to the early 1980s, when tricyclic antidepressants (TCAs) in monotherapy were found to be effective in the treatment of specific anxiety disorders [15–17]. In the last decade, it has become clearer that the boundaries between antidepressant and antianxiety activities were fallacious, as serotonergic antidepressants showed efficacy in anxiety disorders where benzodiazepines (BDZs) were not equally effective [18].

From a clinical perspective, compared with depressed patients without anxiety symptoms, those with symptoms of both disorders (either at subsyndromal or at full-

syndromal level) have more severe depressive symptoms, higher levels of psychosocial impairment, higher health-care costs, and are less likely to respond well to treatment [19,20]. Prospective studies on depressive patients have shown that the co-occurrence of panic attacks was correlated with a poor outcome [21,22], and patients with depression and comorbid obsessive-compulsive disorder (OCD) have been found to be more frequently resistant to treatment, even when selective serotonin reuptake inhibitors (SSRIs) are used [23]. Moreover, depressed patients with a baseline high level of anxiety were less likely to respond to the treatment with tricyclic antidepressants (TCAs) than patients with no anxiety symptoms [7]. In addition, in a post-hoc analysis of more than 250 cases of "anxious-depression," Filteau et al. [24] found that patient responders to SSRIs had higher baseline anxiety/agitation levels than those responders to norepinephrine reuptake inhibitors (NRI). On the other hand, high baseline levels of anxiety did not appear to reduce the overall antidepressant response but rather to delay the onset of the clinical response to sertraline or imipramine in patients with MDD [25]. It is likely that a longer duration of antidepressant treatment is necessary to obtain remission of symptoms in patients with higher levels of anxiety [26]. A meta-analytic study comparing fluoxetine, placebo, and TCAs failed to demonstrate that high levels of anxiety during the course of a major depressive episode affect the response to antidepressants, suggesting that the choice of the antidepressant to be administered for the treatment of major depression should not be based on the presence or absence of anxiety [27,28]. In a recent multicenter study on patients with recurrent MDD, baseline anxiety levels were not related to the antidepressant response to two antidepressants with different pharmacodynamic profile (bupropion or sertraline), thus confirming that the baseline anxiety levels are not a clinical variable on which the choice of an antidepressant should be based [26].

In general, the most commonly used antidepressants [TCAs, SSRIs, and monoamine oxidase inhibitors (MAOIs-)] also exert anxiolytic properties, and there is evidence indicating that these agents are more effective in the treatment of patients with symptoms of both depression and anxiety then classical anxiolytic compounds [29].

The decision process for the choice of an antidepressant for the treatment of depression complicated by anxiety symptoms should include several clinical considerations. First, the ideal compound should induce a rapid improvement of anxiety symptoms, minimal side effects, and minimal risk of developing abuse or dependence. The antidepressants currently used, despite the different degrees of selectivity, act on different neurotransmission systems, producing both clinically beneficial and adverse effects [30]. Furthermore, the fact that the symptoms related to mixed anxiety/depressive syndromes usually have a chronic course suggests that a monotherapy (with a compound exerting both antidepressant and anxiolytic activity) will be better tolerated than combination therapy [31].

In this chapter we will review the main literature on the pharmacological treatment of comorbid anxiety and depression (either at syndromal or at subsyndromal level). In the last few decades, many studies have been published on this issue. However, the main limitation of these studies is that the diagnosis, in many cases, is not clearly assessed (according to DSM or ICD). Co-occurring syndromal conditions are often confused with isolated anxious or depressive symptoms, and the diagnosis of MAD according to DSM-IV research criteria is not usually considered.

We will focus our attention primarily on the research that considered full syndromal or subthreshold anxiety–depression comorbidity. The pharmacodynamic profile and clinical differences among the different antidepressant compounds currently used in the treat-

ment of depression and anxiety conditions will be briefly reviewed, and practical guidelines for the treatment of these conditions will be derived from the studies available to date on adult and elderly populations.

Pharmacokinetic aspects in the case of combination treatments are rarely considered, whereas they appear to be critical for the assessment of the efficacy and tolerability of pharmacotherapy in complex comorbid conditions.

II. TREATMENT IN THE ADULT POPULATION

A. Benzodiazepines

Benzodiazepines (BDZs) in combination with antidepressants are often used in the general practice for the treatment of anxious/depressive syndromes at subsyndromal levels. BDZs are effective, at least in the short-term, for the treatment of anxiety and panic attacks, with rapid resolution of the anxiety symptoms, but with the risk of developing psychological and physical dependence. This potential complication, among other efficacy considerations, prevents us from using these compounds as first-choice therapy in mixed anxiety/depression conditions. Furthermore, their antidepressant activity is controversial [32]. On the other hand, there is some evidence that BDZs, in particular clonazepam, may induce depressive symptoms [33,34]. They may also induce sedation and, when administered at high doses, may be associated with the development of dependence, with the occurrence of withdrawal symptoms when the treatment is discontinued. Moreover, multiple daily dosing with some short-acting BDZs may affect patient compliance, and missed doses may lead to a worsening of anxiety [29]. Given all these potential implications, the use of BDZs in patients with depression and comorbid anxiety is not advised, except as short-term adjunctive treatment to an antidepressant [35]. The rapid action of BDZs in relieving anxiety symptoms may improve patient compliance and thus continue therapy long enough to experience antidepressant treatment effects. After 3 to 6 weeks, when the antidepressant action is fully reached, BDZ therapy should be slowly tapered, in order to prevent rebound anxiety or withdrawal symptoms [36]. In a recent meta-analytic study involving 9 clinical trials on a total of 679 major depressives, the combination therapy group (antidepressants plus BDZs) was 37% less likely to drop out than the antidepressant group alone, and more likely to show a clinical response (defined as 50% or greater reduction in the depression scales from baseline) after 4 weeks of treatment [37].

In fact, during antidepressant treatment (with venlafaxine or SSRIs) [38] for anxiety disorders or mixed anxiety-depression syndromes, patients may discontinue the treatment or show unstable compliance because the latency of the clinical efficacy is 2 or more weeks. In these cases, the addition of BDZs at the early stages of the treatment together with an appropriate clinical intervention (i.e., explaining to the patient that the clinical effect of the treatment will require a few weeks) will improve compliance significantly and thus the success of the treatment.

B. Tricyclic Antidepressants and Monoamine Oxidase Inhibitors

1. Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are compounds with a relatively broad spectrum of pharmacodynamic profiles and clinical activities, and are effective in a wide range of de-

pressive pictures. Rather than being selective drugs limited in their efficacy strictly to depressive symptoms, TCAs exert their efficacy also in anxious depression [39].

TCAs appear to be at least equivalent to the BDZs in the short term and more effective in the long-term treatment of MAD, with particular efficacy in improving the cognitive symptoms of anxiety [19,32]. However, they may induce psychomotor activation [40]. Amitriptyline appeared to be superior to placebo and diazepam in the treatment of mixed anxiety/depression conditions [41]; similar findings were found for imipramine versus chlordiazepoxide and placebo [42]. Imipramine and clomipramine have been suggested to be more useful in case of panic disorder (PD) complicated by comorbid depression. In these cases, the treatment should be started with low doses and gradually titrated upward in order to avoid the risk of exacerbating panic attacks during the early phases of treatment [20].

Clomipramine is particularly indicated in depressed patients with comorbid OCD [43]. In general, OCD patients require longer periods of treatment and higher doses in comparison to what is required by other clinical conditions.

Although TCAs have quite good antianxiety properties, their use is sometimes restricted by the fact that they may induce serious side effects, and because of the potentially dangerous interaction with other compounds, such as MAOIs, ethanol, SSRIs, antipsychotics, oral contraceptives, and anticholinergic drugs [44]. For all these reasons, TCAs are no longer considered a first-choice treatment in MAD.

2. Monoamine Oxidase Inhibitors

Monoamine oxidase inhibitors (MAOIs) are often used for the treatment of more severe and/or atypical forms of MDD, for major depressive episodes complicated by the presence of clinically significant anxiety symptoms, and for treatment-resistant depression [45]. Phenelzine was clearly found superior to placebo and to amitryptiline in depressed patients with anxiety features [46]. At least one study has reported that MAOIs are more effective than TCAs in treating patients with atypical depression who also suffer from panic attacks [45]. A within-treatment analysis indicated that depressed patients responded better to MAOIs than did those with prevalent anxiety symptoms [47]. However, the use of MAOIs is limited by their side-effect profile and by the high potential for adverse drug–drug interactions [44]. Moclobemide, a reversible inhibitor of monoamine oxidase-A, provides an alternative to the traditional irreversible MAOIs. This compound appears to be quite effective in treating both agitated and retarded forms of depression [48], but more evidence is needed to establish its efficacy in mixed anxiety/depressive syndromes [31].

C. Selective Serotonin Reuptake Inhibitors

Several studies have shown that selective serotonin reuptake inhibitors (SSRIs) are as effective as TCAs in treating depression and anxiety, although traditionally TCAs have been favored over SSRIs in patients with mixed syndromes. According to the results of three large meta-analytic studies, fluoxetine [27,49] and paroxetine [50] are as effective as TCAs in reducing anxiety and agitation in depression, and another study has shown that sertraline is as effective as clomipramine in anxious depression [51]. When compared to TCAs, SSRIs show a better tolerability profile [27,52] and a good efficacy in major depression with severe anxiety symptoms. Furthermore, these compounds are effective in anxiety disorders without depression, with a specific activity on anxiety symptoms, compulsions, and avoidance behaviors. The dual (i.e., antianxiety and antidepressant) ef-

fect of these compounds, together with data derived from biological studies, have supported the hypothesis that serotonin (5HT) dysfunctions play a central role in the pathogenesis of mixed anxiety/depression syndromes. Given their pharmacokinetic characteristics, SSRIs are suitable for once-daily dosing, are generally well tolerated even in the long term, and have the compelling benefit of being safe in overdose [53].

1. Fluoxetine

Results from two meta-analytic studies (involving 19 trials and 3183 patients and 31 trials and 4737 patients) [54] showed that fluoxetine and TCAs have comparable efficacy in terms of antidepressant response and induce psychomotor agitation at the same rate. Fluoxetine represents a good treatment for PD patients with depression and for patients with medical conditions and comorbid anxiety and depression [55].

In randomized, double-blind studies [56,57], fluoxetine (20–60 mg/day) showed a similar efficacy to that of imipramine and amitriptyline in the treatment of patients with major depression and anxiety symptoms (as evidenced by the scores of HRDS items psychic anxiety, somatic anxiety, agitation). Data from a 10-week double-blind trial on 284 patients with major depression indicate that fluoxetine (20 mg/day), is as effective as other SSRIs in patients with both high or low baseline anxiety [58]. These data confirm previous evidence showing that 20 mg/day of fluoxetine is the optimal dose in the treatment of depression, with or without prominent anxiety features [59].

2. Sertraline

Some studies have shown the efficacy and safety of sertraline in the treatment of mixed anxiety and depression syndromes. In a sample of 38 patients suffering from mixed anxiety depressive disorders (diagnosed according to ICD-10), during the first week of treatment there was a 40% decrease in anxiety symptoms (as measured by the HAM-A), while the decrease in depression symptoms was 20% at week 2 and 50% at week 3. These results confirm the clinical observation that the efficacy on depressive symptoms has a longer latency than the efficacy on anxiety [60].

Considering the three symptom clusters [using the Inventory of Depressive Symptomatology-Clinician rated (IDS-C)] of anxiety, depression, and anhedonia in a sample of 140 patients with MDD, sertraline showed a "sequential" therapeutic effect acting first on anxiety, then on depression, and later on anhedonia [61].

A controlled, double-blind, randomized study did not show any statistically significant difference in efficacy and onset of action of fluoxetine, sertraline, and paroxetine during acute treatment of MDD with severe anxiety symptoms (with the exception of a statistically significant greater improvement in the anxiety/somatization factor score for both fluoxetine- and sertraline-compared to paroxetine-treated patients) [62]. In addition, this study did not support the assumption derived from previous reports [58,63] of a better tolerability for one agent over the other. However a fixed-dose study on 363 depressed patients suggested that fluoxetine 60 mg/day, when compared to placebo, may increase agitation, anxiety, and insomnia [64].

3. Fluvoxamine

As far as the control of anxiety symptoms in general is concerned, a meta-analysis showed an advantage of fluvoxamine over TCAs [65], while in head-to-head comparisons with other SSRIs, fluvoxamine was equivalent to fluoxetine [66], sertraline [67], and citalopram [68], and slightly superior to paroxetine [69] in reducing anxiety in patients with MDD.

In an 8-week, open-label trial on 30 patients with MAD, fluvoxamine showed moderately strong effectiveness in improving anxiety and depression with a greater efficacy on depressive patterns [70]. Fluvoxamine also showed a good efficacy profile in cases of comorbid OCD and MDD [71].

In a 12-week open trial on 30 patients, fluvoxamine, showed a positive effect on both depressive and anxiety symptoms in MDD subjects with one or more co-occurring anxiety disorders [72], and in a 8-week, open trial on 70 patients, fluvoxamine improved both PD and depressive symptoms [73].

4. Paroxetine

Paroxetine showed similar efficacy to maprotiline in depressed outpatients [74]. This SSRI provides effective and well-tolerated monotherapy for depression, PD, OCD, social phobia (SPh) [75], and GAD [76].

According to the results of a 12-week, double-blind parallel group trial on 1002 patients with a primary diagnosis of depression who also experienced symptoms of anxiety, this compound has shown similar efficacy to that of clomipramine, but a lower incidence of anticholinergic side effects [77]. A 6-week randomized, double-blind, placebo-controlled study in outpatients with anxious depression showed that the clinical efficacy of paroxetine and imipramine was similar at week 6, although paroxetine appeared to have an earlier onset of action on anxiety symptoms [78].

A 6-week study on 275 patients comparing mirtazapine and paroxetine in MDD showed a similar efficacy (although mirtazapine showed a faster onset of action in anxiety, as evidenced by a reduction at HAM-A total scores) and good tolerability. However, paroxetine-treated patients reported more frequently the occurrence of nausea, vomiting, and tremor, while mirtazapine-treated patients reported more weight gain and flulike symptoms [79].

5. Citalopram

Citalopram appears to exert good efficacy on depression and anxiety with a low incidence of side effects [80]. A comparison with sertraline, in a double-blind, randomized study on 323 patients with MDD showed that citalopram has a faster antidepressant activity, that this clinical effect is significantly related to a more pronounced antianxiety effect, and that the compound has a better tolerability profile leading to a significant improvement in compliance [81].

D. Miscellaneous Agents

1. Venlafaxine

Venlafaxine is an antidepressant classified as serotonin norepinephrine reuptake inhibitor (SNRI). Its mechanism of action consists of the inhibition of both norepinephrine (NE) and 5HT reuptake [82]. This compound has recently been approved for the treatment of GAD [83].

Venlafaxine has shown considerable efficacy in alleviating anxiety (as showed by a reduction in anxiety/somatization cluster scores at HAM-D) in patients with MDD in a randomized, placebo-controlled trial [84]. A meta-analytic study on 358 patients suffering from MDD with severe anxiety symptoms at baseline showed that venlafaxine, in comparison to placebo, induced a highly significant improvement in both depression and

anxiety symptoms (anxiety/somatization factor and anxiety psychic item score on HAM-D) [85].

The slow-release preparation of this compound appears to have a faster onset of action, especially at higher doses. In addition, venlafaxine has been shown to improve significantly both the psychic and somatic manifestations of anxiety in 359 MDD patients [86].

As previously stated, the combination with a BDZ may prove useful in the early phases of treatment because the venlafaxine antianxiety clinical effect requires days to weeks to become evident.

2. Mirtazapine

Mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA) whose direct enhancement of NE and $5HT_{1A}$-receptor-mediated 5HT neurotransmission is thought to be responsible for its antidepressant activity, whereas the $5HT_2$ and $5HT_3$ blockade may account for the low incidence of anxiety and agitation [87]. Clinical trials have shown that mirtazapine is similar to TCAs in both its pharmacodynamic properties (interaction with NE and 5HT transmission) and overall clinical efficacy [88]. In three double-blind, randomized, controlled studies on patients with MDD and severe anxiety symptoms, comparing mirtazapine with citalopram (Mir: $n=136$; Cit: $n=133$), paroxetine (Mir: $n=127$; Par: $n=123$), and fluoxetine (Mir: $n=60$; Flu: $n=63$), mirtazapine consistently appeared to reduce anxiety symptoms more than any of the other antidepressants, with a faster onset of the effect on anxiety symptoms and a sustained activity throughout the course of treatment [89]. The results from a meta-analysis of eight randomized, double-blind, placebo-controlled clinical trials showed that the rapid improvement in anxiety symptoms seen in 161 depressed patients treated with mirtazapine could have important implications when choosing antidepressant therapy for depressed patients with prominent anxiety symptoms [30].

More than 700 patients with moderate-to-severe symptoms of depression have also been tested to estimate the antianxiety effects of mirtazapine versus amitriptyline [90]. Both drugs proved to be equally effective, as shown by the variations on anxiety and somatization factor scores of the HAM-D scale (items 10–13, 15, and 17). However, mirtazapine induced fewer side effects and adverse reactions in comparison to imipramine (76% vs. 87%), and a lower dropout rate (4.9% vs. 9.1%).

In an 8-week open-label study, a sample of 10 patients suffering from MDD with comorbid GAD showed a significant improvement on both HAM-D and HAM-A total scores [91]. Excessive drowsiness and weight gain are sometimes limitations of the tolerability profile for this compound. In the event of the occurrence of these undesirable effects, administration at night and at lower dosage is advisable.

In conclusion, the clinical antidepressant efficacy of mirtazapine appears to be similar to that of classical antidepressants with a more specific effect on anxiety and somatization symptoms and a better tolerability profile.

3. Buspirone and Gepirone

These two $5HT_{1A}$ agonists share some efficacy in the treatment of comorbid anxiety and depression [92]. Buspirone has been reported to have a significant effect on the treatment of GAD with co-occurring depressive symptoms [93], a situation that occurs in almost 50% of patients with a primary diagnosis of GAD [32]. More recently, it has been suggested that buspirone may have a significant clinical effect in other anxiety disorders

and comorbid conditions, even though more controlled trials are needed to confirm this observation [94]. The idea that buspirone could be useful in the treatment of GAD patients when discontinuing benzodiazepines is still controversial [95].

Gepirone has also been shown to be effective in the treatment of GAD with [92] and without [96] comorbid depression. However, the observation that SSRIs and venlafaxine may show a better efficacy profile than $5HT_{1A}$ agonists makes the role of buspirone and gepirone uncertain in the treatment of comorbid anxiety–depression conditions.

4. Nefazodone

Nefazodone acts as a weak inhibitor of NE and 5HT reuptake, while it is a potent 5-HT_2 antagonist. In a meta-analysis of six randomized, placebo-controlled, double-blind trials on patients suffering with MDD with high levels of anxiety, nefazodone showed a significantly faster effect (within the first week of treatment) on agitation than imipramine or placebo [97].

In an open 8-week trial on 40 patients with PD and comorbid MDD or dysthymia, nefazodone showed efficacy in reducing both acute anxiety symptoms and depressive symptoms [98].

E. Antipsychotics

Severely depressed patients who responded poorly to antidepressant treatment or with high levels of anxiety and agitation may be treated with antipsychotics. Three patients suffering from unipolar recurrent depression complicated by high levels of anxiety have been treated with atypical antipsychotics (quetiapine, risperidone, and olanzapine) with good response [99]. This observation appears to confirm the hypothesis that in patients with recurrent chronic depression and high levels of anxiety, low doses of atypical antipsychotics may improve function through an antianxiety or antidepressant effect that is independent from their antipsychotic efficacy [100]. This effect may occur with typical antipsychotics as well, but concern about the induction of tardive dyskinesia has limited the usefulness of these compounds [101].

Given their lower risk for tardive dyskinesia and extrapyramidal side effects (EPS), atypical antipsychotics (e.g., risperidone, olanzapine), devoid or with few EPS, have been successfully employed in the treatment of refractory obsessive-compulsive disorder, with promising results [102,103].

III. TREATMENTS IN THE ELDERLY POPULATION

In the elderly, as in adults, the co-occurence of depression and anxiety is often at a subsyndromal level (MAD). Risk and morbidity factors for comorbid depression and anxiety in late life may be different from those for depression without anxiety and from those in younger adults. Elderly depressives with comorbid anxiety disorders or symptoms present with more severe psychopathology, higher impairment of the global functioning, a more difficult course of illness, and a decreased or delayed treatment response [104]. In addition, some anxiety disorders show an increased prevalence rate in the elderly. The 12-month prevalence rate for GAD has been estimated from 7 to 9%, and this disorder has been considered the most prevalent anxiety disorder in patients over 65 years of age [105]. On the other hand, 91% of elderly with GAD show depression [106], which usually has an earlier onset [107]. In a recent study, van Balkom et al. [108] showed that not only MDD,

but also BDZ use and chronic somatic diseases are significantly more prevalent in elderly patients with an anxiety disorder than in patients without comorbid conditions.

The ideal treatment in these situations should take the form of a monotherapy with a compound effective in the treatment of both anxiety and depression. An effective dosage of the drug should be achievable through once-daily administration to improve compliance. The compound should have a good tolerability profile and should be safe in case of overdose, since the risk of suicide is higher in depressed patients with anxiety than in those without prominent anxiety [35].

The main problems faced in the management of the pharmacological treatment of mixed anxiety–depressive syndromes in the elderly are related to the clinical, biological pharmacodynamic, and pharmacokinetic peculiarities of this population [109,110] (Table 1).

In the elderly, symptoms of depression and anxiety are often accompanied by somatic complaints, which may dominate the clinical picture, sometimes masking the mood symptoms. In general, symptoms may be atypical in comparison to those encountered in adults and may include multiple somatic complaints, persistent insomnia, irritability, cognitive impairment, and hypochondriac ideation [111].

The frequent co-occurrence of neurological or medical conditions may cause difficulties in the recognition and diagnosis of depressive and anxiety symptoms [112,113]. A further complication is represented by the fact that in the elderly population there is a considerable overlap among symptoms of depression and anxiety, cognitive decline, and medical syndromes. However, given that the risk of not recognizing and treating depression and anxiety in the elderly is very high in terms of morbidity and increased mortality, clinicians are urged to appreciate anxiety and depressive syndromes in their different and variable forms and combinations so that the pharmacological intervention can be initiated in a timely manner and properly targeted.

In late life there are physiological changes associated with aging, namely, a higher sensitivity to side effects, higher risks for drug–drug interaction phenomena, and compliance problems. All these factors need to be carefully taken into account when selecting a pharmacological treatment [114,115]. For any new medication prescribed, it is important to do a careful physical and laboratory assessment and to consider the pharmacodynamic profile of the compound prescribed, with particular respect to the potential side effects on the central nervous system, including depressogenic or anxiogenic effects.

With respect to pharmacokinetic aspects, differences in the absorption, distribution, metabolism, and excretion for most of the antidepressants and anxiolytics between elderly and younger subjects have been described. Pharmacokinetic parameters are influenced by

Table 1 Clinical–Pharmacological Problems Encountered in the Diagnosis and Treatment of Mixed Anxiety/Depression Disorders in the Elderly

1. Atypical features
2. Cognitive impairment
3. Compliance problems
4. Concomitant medical disorders
5. Environmental factors (interfering with drug response)
6. Bioavailability/metabolic peculiarities
7. Reduced drug tolerability

changes in gastrointestinal motility, renal function, atherosclerosis, muscular mass reduction, and increased body fats. All these factors contribute to determine the bioavailability of the drugs administered and thus they need to be considered carefully when prescribing medication to elderly patients.

From a clinical point of view, TCAs are as effective as SSRIs in elderly anxiety-depressive syndromes [114]. However, imipramine, amitriptyline [116], desimipramine [117], and nortriptyline [118,119] may cause cardiotoxicity and have an increased bioavailability, which leads to higher steady-state plasma concentrations. In addition, the occurrence of anticholinergic effects is particularly dangerous in the elderly because of the higher risk of induction of cognitive dysfunctions and deterioration. TCAs have been reported to cause delirium in 11 to 30% of elderly patients [120]. These side effects may have a significant negative impact on clinical outcome and thus the use of TCAs in subjects with concomitant medical or neurological conditions is discouraged [121].

Clinical evidence suggests the initial use of low doses of nortriptyline and desimipramine (10 mg/day) with a gradual titration upward to 75 mg/day. The dose indicated for other TCAs with more of a sedative effect (such as amitriptyline and dotiepin) is 30 to 100 mg/day. However, the possible occurrence of anticholinergic side effects should be monitored very carefully [122].

SSRIs are at least as effective as TCAs, but have a more favorable tolerability profile [123]. These characteristics make SSRIs more suitable for the treatment of depression and anxiety in the elderly [124]. Potential disadvantages of the SSRIs include the induction of agitation and insomnia (occurring mostly with high doses of fluoxetine), gastrointestinal side effects, and headache. The risk of the induction of adverse events, particularly in the elderly, is usually dose dependent. For this reason, a search for the minimal effective dose of the compound to be administered should be encouraged.

The choice of a TCA versus SSRI in mixed anxiety–depressive syndromes should be based on their anxiolytic profile and on the potential drug–drug interactions. Cytochrome P450 enzymes are the sites of metabolism for SSRIs and their activity is reduced in the elderly. This should always be considered when prescribing more than one compound to an elderly patient. Pharmacokinetic studies suggest that lower doses should be used for citaprolam, paroxetine, and probably sertraline [123,124], particularly in case of renal or hepatic dysfunctions. On the other hand, no clear evidence was found for fluoxetine and fluvoxamine regarding age-dependent pharmacokinetic variations [125].

With respect to efficacy issues, clinical studies revealed that some of the SSRIs are particularly effective in treating comorbid anxiety in depressed patients [126]. The largest clinical database relating to SSRI treatment of patients with depression/anxiety exists for paroxetine [77,127] but other SSRIs have also been shown to be effective.

Fluoxetine (20 mg/day) administered to elderly patients with a diagnosis of MDD appeared to be particularly effective on early awakenings, sexual disturbances, guilt and suicidal thoughts, and psychomotor inhibition [128], but less effective than TCAs on anxiety and insomnia.

In elderly patients with more severe anxiety symptoms, SSRIs with a more sedative profile, such as sertraline or fluvoxamine, should be preferred [129].

Regarding SNRIs, there are data showing that venlafaxine (150 mg/day) and venlafaxine–XR are both effective in reducing anxiety in patients with depression [86,130]. Moreover, venlafaxine has a good tolerability profile, which makes the compound an attractive choice for geriatric patients, where the induction of hypotension is one of the main causes of hip fractures [131]. Venlafaxine has a low plasma protein binding rate

and slightly inhibits the cytochrome P450 system. These two characteristics indicate that the potential for dangerous interactions with other compounds is quite low. However, venlafaxine dosage should be adjusted in elderly patients [132] because of reduced metabolism rate in these subjects [133].

For mirtazapine, data suggest a reduced dose in the elderly because the pharmacokinetic profile of this compound is gender- and age-dependent [134,135]. Females and elderly patients show higher plasma concentrations compared to those of males and adults. The elimination half-life of mirtazapine ranges from 20 to 40 h, with a time to reach the steady state of approximately 1 week [136]. Liver and renal dysfunctions cause a 30–50% decrease in the drug clearance [136].

Some "atypical" antidepressants such as mianserin [122], viloxazine [137], and trazodone [138] have been successfully used in mixed anxiety-depressive syndromes. They induced a significant improvement of several core symptoms of depression and anxiety, as well as an improvement in somatic complaints and sleep disturbances. Overall, the side-effect profile of these compounds is better than that of TCAs, even though mianserin and trazodone may induce orthostatic hypotension.

BDZs in the elderly should be used with caution. They are the most commonly used psychotropic drugs among older people [139,140], but their adverse effects, which appear to increase with age [141,142], limit their benefit in this population. As a consequence, BDZ prescription should be considered only in case of severe insomnia, agitation, anxiety, and somatoform symptoms, and when these symptoms have a central role in the overall depressive picture [111]. The disadvantages associated with BDZ use in elderly patients include daytime sedation or disinhibition (as paradoxical effect), cognitive and psychomotor impairment, dependency, withdrawal and rebound symptoms, ataxia, dysarthria vertigo, and cognitive toxicity in patients with preexisting cognitive decline [143]. Studies on elderly inpatients showed that long-term treatments with BDZs represent one of the most common causes of exacerbation of dementia symptoms. Moreover, the use of BDZs, even at low doses, appears to increase the risk of hip fracture [131]. All these side effects, in particular the psychomotor ones, appear to be particularly evident at the beginning of the treatment and in the first month of continuous use [131,144,145]. BDZs with shorter half-lives are no safer than agents with longer half-life, and are associated with the development of more severe cognitive impairment (e.g., memory deficits), more rapid tolerance, or more severe withdrawal symptoms than those experienced with long-acting compounds [146,147].

Clinicians should consider these risks against potential benefits when prescribing BDZs for elderly patients. These cautions should not be interpreted as if the safest course of action is always to avoid the use of BDZs, but rather suggest careful appraisal of the risks associated with their administration. It is necessary to weigh the hazards of not treating the clinical conditions (e.g., anxiety symptoms, agitation, or insomnia), which are often substantial. In any case, the prescription of BDZs should be kept at low doses, for less than 4 months, and should be strictly monitored. Typical geriatric dosages of short-acting BDZs are the following: oxazepam, 15 to 30 mg/day; lorazepam, 0.5 to 3 mg/day; temazepam, 7.5 to 15 mg/day; alprazolam, 0.25 to 1.5 mg/day; triazolam, 0.125 to 0.250 mg/day. BDZs without active metabolites and with a short or ultrashort life (oxazepam, triazolam) are preferable given the potential risk of accumulation phenomena due to the reduced liver metabolic activity.

The so-called high-potency benzodiazepines (i.e., alprazolam and adinazolam) appear to be quite promising in the treament of comorbid anxiety–depression conditions,

since they have been shown to have both an anxiolytic and an antidepressant activity. The mechanisms underlying this complex clinical profile are not completely known. Alprazolam has been reported to have an effect on corticotropin-releasing hormone (CRH) [148] as well as noradrenergic properties [149], and both these parmacodynamic properties may well account for its antidepressant activity.

However, the limit to their use is related to the risk, in some cases, of exacerbating somatization symptoms, and are not indicated in cases where these features are predominant in the clinical picture. In addition, the risk of the development of abuse or dependence in patients treated with BDZs should be always considered and evaluated in the context of global clinical management. The risk of developing abuse and dependence may be increased in the elderly for pharmacokinetic changes occurring with aging. However, this suggestion has not been confirmed by controlled studies and still remains controversial [150], and recent data support the hypothesis that the development of BDZ abuse and dependence is mostly accounted for by interindividual differences including clinical and personality characteristics of the patients [151].

Many clinicians prefer the use of buspirone for the treatment of chronic anxiety in the elderly [152]. Buspirone does not induce a worsening of depressive symptoms; however, it is not useful for as-needed prescription in the treatment of insomnia or acute anxiety because its clinical effect has some latency. On the other hand, as opposed to what has been described for BDZs, there are few drug–drug interactions and no discernible drug–alcohol interactions, no potential for tolerance, withdrawal, or serious overdose toxicity with buspirone [153].

In conclusion, in the case of mixed anxiety–depressive syndromes occurring in elderly patients, it is of critical importance to differentiate between co-occurrence of depression and anxiety as isolated symptoms (MAD) or full syndromal patterns in order to envisage a more rational and targeted treatment. SSRIs, SNRIs, and NASSAs are preferable to TCAs for efficacy and tolerability profiles both in cases of subsyndromal or syndromal coexistence of anxiety and depression. TCAs can be chosen in cases with melancholic features. However, their use should be carefully monitored, particularly when administered in combination with other compounds, because of the risk of adverse events due to drug–drug interactions. Moreover, their dosage should be adjusted because their pharmacokinetic characteristics are significantly affected by aging.

The use of BDZs should be discouraged in elderly subjects because of the risk of developing confusion and or agitation. Buspirone may be a suitable alternative. In general, a rational management of pharmacotherapy in complex comorbid conditions in the elderly should imply careful attention to the occurrence of drug–drug interaction phenomena (pharmacodynamic and pharmacokinetic interactions with neuroleptics, neurotropics, or drugs for other medical conditions) [111,115]. In case of combination treatment with more than one compound, some side effects may occur despite a good tolerability profile for each individual medication, particularly in case of long-term treatments.

IV. CONCLUSIONS

A critical review of the literature on the treatment of anxiety–depression syndromes led to some theoretical and methodological considerations. First of all, there are some controversial issues: for example, whether high baseline levels of anxiety associated with depression could influence or not the overall clinical outcome [26,35], or whether some compounds with a more specific and selective pharmacodynamic profile (e.g., serotonergic

vs. noradrenergic) could be more useful than others in treating both anxiety and depressive symptoms [24]. From a methodological point of view, in many studies published to date the quality of the association between anxiety and depressive symptoms (full-syndromal or subsyndromal) has not been specified. The new diagnostic category of MAD as proposed by the DSM-IV opens some questions about incidence, etiology, natural course, and treatment response. These issues need to be addressed in order to validate this novel category. Another methodological problem is that most of the studies have been done according to a cross-sectional design, without adequate follow-up periods. Consequently, very little is known about the course of the clinical picture and the long-term effect of the treatments employed. Are SSRIs or other antidepressant compounds actually able to modify the course of mixed anxiety–depression syndromes over time?

Another important issue is relative to the use of BDZs. These compounds are still prescribed for anxiety–depression conditions, even though only some of them have shown some antidepressant activity (e.g., alprazolam) and most of them may exert undesirable side effects (e.g., depressogenic effects). The role of BDZs in the management of neurovegetative symptoms and insomnia in monotherapy or in combination with antidepressants is arguable, and the clinician should always evaluate carefully the risk/benefit ratio of a combined therapy. In some cases, the anxiolytic effect of the antidepressants takes longer to develop and this may hinder treatment compliance in anxious depression and other comorbid conditions. In these cases, the use of BDZs may be advisable for the early stages of the treatment until the clinical effect of the antidepressant compound has been reached [35]. A meta-analytic study showed some superiority of the combination antidepressant–BDZ in terms of drop-out rates and clinical response [37]. However, this study did not elucidate whether the observed advantage of the combination treatment on the global clinical outcome was only due to its effect on sleep and anxiety, or rather to some synergic effects on core depressive symptoms. Moreover, none of the trials included in the meta-analysis lasted more than 8 weeks. Thus, whether a drug combination is more useful than monotherapy in the treatment of comorbid conditions is still unknown. The monotherapy with a drug effective in the treatment of both anxiety and depression (as the SSRIs, SNRIs, and NaSSAs) is generally preferable. The use of one compound as opposed to the use of combination therapies allows the avoidance of drug–drug interaction phenomena, which are particularly risky in the elderly.

The fact that the dichotomy between anxiolytic and antidepressant pharmacological compounds has been definitively overcome appears to be confirmed by the observation that not only TCAs but also the new antidepressants (SSRIs, SNRIs, and NaSSAs) have a significant clinical effect on both anxiety and depressive symptoms and syndromes.

A clear advantage of these new compounds in comparison to BDZs is that they appear to exert an effect on the "core" features of anxiety (the cognitive symptoms and the behavioral changes), while BDZs have a better effect on neurovegetative and somatic symptoms. Thus, these new classes of antidepressants represent a critical step forward in the treatment of co-occurring anxiety/depression compared to TCAs or MAOIs. Furthermore, their pharmacodynamic profile, which implies different degrees of selectivity on the 5HT neurotransmission systems, points out the critical role of this monoamine in the pathophysiology of these clinical conditions. Given higher acquisition costs for these new compounds, formulary directors and pharmacy benefit managers have questioned whether they produced sufficiently superior clinical outcomes to justify their added expense. Some studies estimated the overall treatment costs for SSRIs to be no greater (or even lower)

than those for TCAs, given their better tolerability profile and the consequent improved compliance [154].

Unfortunately, there are more data available on the pharmacological treatment of full-syndromal comorbid conditions (e.g., MDD and GAD, OCD, or PD) than on the treatment of MAD. In our opinion, when treating MAD with antidepressants, a conservative attitude should be kept, particularly when there is no familial loading for anxiety and depressive disorders, and limited impairment in everyday life functioning.

Low doses of SSRIs, SNRIs, and NaSSAs for 4 to 6 weeks could be effective in mild and moderate depressive subthreshold situations. On the other hand, when anxiety and depression are present as full-syndromal disorders, the choice of compound to be administered, the doses, and the duration of treatment should be based on the assessment of the most prevalent symptoms. As an example, in case of OCD with a severe secondary depressive disorder, clomipramine or SSRIs should be preferred to other compounds, despite the observation that in severe depression a TCA should be preferred to a SSRI [155].

In conclusion, the association of anxiety with depression is a common clinical experience in everyday practice. A dimensional approach appears to be more appropriate to explain the clinical and pathophysiological characteristics of these conditions even though there are not univocal models to explain the co-occurrence of anxiety and depression in the same subject. Future studies should focus on a better assessment of the time course of these conditions, including the long-term response to medication. These observations will help the understanding of the biological bases of the occurrence of admixture of symptoms in some clinical situations (e.g., in MAD), and will eventually improve treatment and prevention strategies.

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New and Emerging Therapies for Anxiety

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I. INTRODUCTION

Despite the major progress in the treatment of anxiety disorders in the past decade, there is still a long way to go. For some of the anxiety disorders, prognosis is still moderate or even poor. Moreover, even with the current best treatments in practice many patients recover only partially (i.e., they do not enter remission). A further issue is that all used treatments have side effects that, although not pronounced, in many cases can limit compliance in a significant proportion of patients. For these reasons, the search for the next generation of anxiety therapies is currently quite vigorous, especially as now companies can be reassured that successful treatment for the anxiety disorders will be reimbursed in all countries in the western world.

II. NEW SEROTONIN-TARGETED APPROACHES TO REDUCING ANXIETY

The most direct and, some would argue, safest way of designing new anxiolytics is to build on the finding that serotonin (5HT)-acting drugs are effective anxiolytics. There is growing and perhaps surprising evidence for the efficacy of the selective serotonin reuptake inhibitors (SSRIs) on the broad spectrum of the anxiety disorders. However, it should be remembered that the role of 5HT in anxiety is complex and possibly biphasic [4]. It is conceivable that drugs that either increase or decrease the effects of 5HT are anxiolytic. As yet, there are only limited data pertaining to this issue, but one of the more exciting

recent findings is that the anxiolytic actions of the SSRIs are reversed when 5HT synaptic concentrations are reduced using the tryptophan depletion paradigm. The procedure and theory behind tryptophan depletion is discussed in detail in Bell et al. [5]. The technique has been used to demonstrate that the actions of SSRIs and other 5HT-acting antidepressants are dependent on the presence of 5HT in the synaptic cleft, as relapse is produced by tryptophan depletion [12].

Interestingly, the next group of patients to be studied with tryptophan depletion were those with OCD and it was found that the technique did not produce a relapse [31]. We have just completed a tryptophan depletion study of panic disorder patients who had responded to SSRI treatment and found that tryptophan depletion led to a relapse, as indicated by increased vulnerability to the panicogenic actions of flumazenil [6]. Preliminary data suggest that the same is true in social anxiety disorder. Our ongoing studies show that the anxiogenic response to a social phobic challenge is enhanced after tryptophan depletion in patients successfully treated with SSRIs.

Taken together, it would appear that the anxiolytic action of the SSRIs in some anxiety disorders is similar to that in depression in that an elevation in 5HT concentrations in the synaptic cleft is critical to their therapeutic effect. This fits with the theory of Deakin and Graeff [11] that 5HT acts to inhibit unconditioned anxiety and suggests that other ways of increasing 5HT postsynaptic function might also be anxiolytic. To some extent this is supported by the animal work that found $5HT_{2C}$ agonists to be antipanic in rats [26]. Why OCD should be different is not yet known. It may be that the OCD experiments were not as robust as they could have been, since they did not use anxiety-provoking challenges during the tryptophan depletion. However, if the finding were to remain true, one could then argue that downstream postsynaptic mechanisms underlie activity in OCD and that if these were identified they could give rise to new drug targets.

Another important conceptual issue relates to the mode of action of buspirone. This is a relatively selective $5HT_{1A}$ partial agonist that in animal studies acutely inhibits 5HT neuronal firing. If anxiety is due to excess firing of the 5HT neuronal system, then shutting down the firing rate would be directly anxiolytic in conditions such as generalized anxiety disorder (GAD), but it would worsen anxiety in other conditions such as panic disorder [11]. Interestingly, buspirone itself does not appear to worsen panic disorder but newer analogs such as flesinoxan, which is a full agonist of this receptor, does increase anxiety in these patients [60].

However, the inhibiting action of buspirone at the raphe nuclei is seen after a single dose of the drug whereas, similar to the antidepressants, the therapeutic effects of buspirone take a few weeks to emerge. This suggests that other factors may play an important role. Perhaps, the most obvious target is the postsynaptic $5HT_{1A}$ receptor, which is found in high densities in forebrain areas implicated in anxiety such as the hippocampus, septum, and temporal cortex [49]. Stimulation of these receptors is thought to be responsible for the therapeutic actions of many antidepressants. It may be that some anxiety disorders respond in a similar way. There is provisional evidence that in panic disorder these receptors are downregulated as in depression [50]. It is conceivable then that stimulation of these receptors by an agonist could increase 5HT function and be anxiolytic. The data with flesinoxan mentioned earlier rather go against this theory but the area is still relatively young.

Are there any other ways of improving the clinical value of buspirone? One limiting factor with all the $5HT_{1A}$-agonist drugs is that they have a marked first-pass metabolism

so that poor metabolizers can experience high plasma concentrations that produce unacceptable side effects. One way to overcome this problem is to change the delivery system and a buspirone patch has been reported in children and is being evaluated in adults [9].

Buspirone may also have a role as an augmentation treatment of SSRI partial responders. This has been tried in PTSD with some beneficial effect in 11 of 14 patients [21]. The idea of combining a $5HT_{1A}$ agonist with an SSRI in the same molecule has also been explored [33].

Gepirone is an analog of buspirone that is also a partial $5HT_{1A}$ agonist. It has shown some efficacy in GAD [47] and panic disorder [44]. The trial by Rickels et al. included 198 patients with GAD and showed that the anxiolytic effect was delayed and more adverse effects were noted compared with diazepam. In contrast, rebound anxiety was not experienced upon ceasing gepirone, but it was seen in withdrawal from the benzodiazepine. Of interest is the fact that gepirone is also developed for atypical depression, a condition that is often associated with anxiety.

Flesinoxan was discovered to have a high affinity for $5HT_{1A}$ receptors during initial development as a centrally acting antihypertensive. Phase III pilot trials in patients with panic disorder have shown it to be ineffective. They also show a dose-dependent panicogenic effect [60]. Preclinical studies of the buspirone analogue MM199 have revealed anxiolytic properties [15]. Other $5HT_{1A}$ agonists still in discovery are lesopitron and eptapirone.

If stimulating $5HT_{1A}$ receptors leaves something to be desired in the treatment of anxiety, what about other 5HT receptors as targets (Table 1)?

$5HT_{1A}$ antagonists also have anxiolytic action in rats. A compound, robisartan, is in trials for depression so it may yet be tested in anxiety disorders. It presumably works by disinhibiting the cell body and terminal autoreceptors, therefore increasing 5HT in the synaptic cleft. $5HT_{1B}$ antagonists would also have the same effect on 5HT transmission. Since a number of such compounds have been discovered, we may well have clinical trial data in the next decade.

Deramciclane is a $5HT_{2C}$ antagonist with anxiolytic properties that is now in phase III trials. Animal data show that it does not potentiate the effects of alcohol, yet it improves sleep [7,16]. Another $5HT_{2C}$ antagonist in development for depression, and possibly for anxiety, is agomelatine [61]. This compound has both melatonin agonist and $5HT_{2C}$ antagonist properties in the same molecule and shows anxiolytic properties in animal models.

Table 1 5HT Targets for Novel Anxiolytic Treatments

Type of approach	Examples	Comments
Modify current treatments	Modify SSRIs (e.g., adding $5HT_{1A}$/$5HT_2$ blocking)	Questionable efficacy, better tolerability
$5HT_1$-receptor antagonists	$5HT_{1A}$ and $5HT_{1B}$ blockers	In depression trials, anxiolytic in animal tests
$5HT_2$-receptor antagonists	Deramciclane ($5HT_{2C}$)	In phase II for depression 2C antagonists; anxiolytic in animal tests
Agomelatine	$5HT_{2C}$ antagonist + melatonin agonist	In clinical trials for anxiety; works in animal models

Why the melatonin component should contribute to either the antidepressant or the anxiolytic actions is rather unclear, but studies show that the combination of these two actions produces potentiation of effects.

If the stimulation of postsynaptic 5HT receptors is critical in the action of the SSRIs in the treatment of OCD, then it is likely that the $5HT_2$ rather than the $5HT_{1A}$ subtype is responsible, given the lack of efficacy of the latter agonists in this disorder. There are no selective $5HT_2$ agonists available yet, and indeed such drugs could be hallucinogenic. However, a beneficial effect has been reported in OCD with the hallucinogen psilocybin, which has $5HT_2$-agonist properties [22].

Double-blind reports of trials of the $5HT_3$ antagonist ondansetron have proved rather ineffective in GAD [17] and in panic disorder [14,51]. It remains to be seen if another $5HT_3$ antagonist (zatosetron) may fare better [53].

Finally, we should consider the feasibility of improving on the actions of the SSRIs in the anxiety disorders. One way would be to remove some of the anxiogenic effects apparent early in treatment that are particularly problematic in panic disorder. This may be possible by adding some $5HT_2$-receptor antagonist activity such as is found in nefazodone and mirtazapine. Although neither of these new antidepressants has been extensively tested in anxiety, they are more beneficial than the SSRIs on anxiety symptoms in depression, especially in the early phase of treatment [35,36].

III. NEW GABA-TARGETED APPROACHES

New GABA-targeted approaches are listed in Table 2, where a number of different approaches are considered. Perhaps the most exciting are the GABA-A-receptor subtype agonists, which are predicted to have very focal actions in the brain and, at least in preclinical tests, show excellent separation of anxiolytic and sedative actions [32,39]. There is still some debate as to whether the alpha 2 or the alpha 3 GABA-A-receptor subtype mediates anxiety and it may be that both are involved to differing extents in various forms of anxiety.

Another potential development is that of making partial agonists at one or both of these receptors given the emerging utility of partial agonists in many disorders. Support for the potential utility of GABA-A-receptor partial agonists comes from the early studies

Table 2 GABA and Glutamate Drugs as Potential Anxiolytic Therapies

Type of approach	Examples	Comments
Subtype selective GABA-A-receptor agonists/partial agonists	Pagoclone, α2 and α3 subtype agonists	Some positive data in panic disorder; less side effects than traditional benzodiazepines
Novel GABA modulators	Gabapentin Pregabalin	Some clinical data; may work on Ca channels
Neurosteroids	Pregnenalones, DHEA	? Development in anxiety stopped
Glutamate antagonists	NMDA antagonists	Block conditioned anxiety (e.g. PTSD in animals)
Reduce glutamate release	MGLUrl agonists	Work in animal models; broad range of therapeutic indications

with abecarnil and later ones with pagoclone. Abecarnil was the first new nonbenzodiazepine partial agonist to be tested. It had equivalent efficacy to a benzodiazepine full agonist in a GAD trial and appeared to have fewer side effects and withdrawal problems [45]. However, the development of this drug was ceased.

Pagoclone is a partial agonist in the same series as zopiclone [24], and has similar but less pronounced action on sleep to this full agonist [62]. A recent 11C-flumazenil PET study by our group has confirmed that in humans pagoclone does behave like a partial agonist because it produces less pharmacodynamic action than lorazepam, despite occupying more brain receptors [30]. We have also shown that pagoclone has some efficacy in panic disorder [48].

Other GABA approaches include the use of drugs such as gabapentin and pregabalin, which were developed to be GABA modulators but have since been shown to act on other systems such as calcium channels. Gabapentin is the earlier drug and has been used as an anticonvulsant for many years now. There have been positive trials for this in panic disorder and social anxiety disorder [41,42]. For reasons of potency, pregabalin is now being studied in these disorders and there is already one positive study in social anxiety disorder where the higher dose used was effective [25].

One area of new research that peaked in the 1990s was that of neurosteroids. These are compounds that act at the GABA-A-receptor complex to increase the effects of the natural transmitter GABA. They are effective anxiolytics in animal models as well as being anticonvulsant and hypnotic (for a review, see Ref. 18). However, it appears that the development of the leading compound for anxiety treatment has now ceased and only an anticonvulsant is currently in clinical trials. Why this should be the case is not immediately clear, but it may reflect the fact that the broad action of these agents to promote GABA-A function gives a range of sedative and other side effects.

The potential of glutamate as a target has become obvious in recent years. Glutamate is the major excitatory transmitter in the human brain and so it is critically involved in learning and memory, as well as perception and performance. There is evidence that glutamate contributes to the laying down of traumatic memories [38]. Therefore, it may have a particular involvement in PTSD [40], although it is also likely to be relevant to the learning of avoidance behavior as seen in panic attacks and social anxiety disorder.

In essence, the glutamate theory of anxiety suggests that the embedding of anxiety is a consequence of glutamate-mediated learning. In addition, some authorities believe that simply increasing brain glutamate concentrations will cause anxiety [1]. It is possible to block glutamate transmission at the key receptor involved in learning and memory (the NMDA receptor) by postsynaptic antagonists such as dizocilpine (MK801). These drugs, though, tend to produce problematic alterations in consciousness so they cannot be used as long-term therapy but they can be contemplated as a short-term/immediate intervention. Other antagonists with less problematic side effects have now been discovered and one, memantine, has been used in humans for other indications. It could perhaps be used to test the glutamate theory of anxiety [43].

The multiple groups of metabotropic G-protein glutamate receptors (mGluR) have also been explored recently. Animal studies indicate that mGlu5 antagonists, such as 2-methyl-6-(phenylethynyl)pyridine (MPEP), possess anxiolytic properties [54,56]. Intriguingly, it is also possible to reduce glutamate function by decreasing release through inhibition of presynaptic cell activity. One possible way of achieving this would be with a specific presynaptic glutamate agonist that acts on the glutamate autoreceptor. One such compound appears to be the mGlu2/3 receptor ligand LY354740. It has shown to have

Table 3 Peptides

Type of approach	Examples	Comments
CCK 4 antagonist	PD9	? Clinically effective
Antistress agents	CRF antagonist	Animal tests positive; under trials for depression
Substance P antagonists (NK1-receptor antagonists)	MK 869	Reduced anxiety in depression trials
NPY agonist is anxiolytic	None as yet	Hard to make orally active drug

activity in a number of animal models in which anxiety is prominent, including drug and alcohol withdrawal and conflict tests [27,52]. As there are many other potential indications for this sort of compound, including schizophrenia, the field is one that is likely to grow and it may soon be possible for clinical trials in anxiety to start.

IV. PEPTIDE ANTAGONIST TREATMENTS

A number of peptides have been involved in the brain mechanisms of anxiety (Table 3). The best-studied neuropeptide in anxiety is cholecystokinin (CCK). The tetrapeptide CCK-4 and the synthetic pentapeptide pentagastrin can be used to provoke anxiety in human volunteers and, in lower doses, in patients with panic disorder. Patients with social anxiety disorder show an intermediate sensitivity [13,57]. These actions of CCK-4 are attenuated in patients treated with effective antipanic medications [58]. Taken together, these data were very exciting and suggested that the CCK receptor or endogenous CCK might have a causal role in panic disorder. Based on these theories, several companies made high-affinity antagonists but unfortunately none of the first-generation CCK antagonists, such as CI 988, proved to be effective in panic disorder [59], although they were able to block the anxiogenic actions of CCK-4 itself. There was also a negative trial in GAD [2]. It is possible that pharmacokinetic problems, especially poor brain entry, might have contributed to the poor therapeutic response. Until a compound with good brain entry is discovered and tested, the value of this approach in treating anxiety should remain open.

One of the more exciting areas of research in depression is the search for peptide therapeutics that will attack depression at its presumed source—the central and peripheral stress axis. Corticotropin-release factor (CRF) is one, if not the main, hormone-mediating stress in the brain. CRF production in the nervous system is increased by stress and if CRF is injected into the ventricles of animals it produces many of the behavioral effects of stress. For nearly a decade now, we have known that blocking central CRF receptors could reduce stress-related behavior in animals, although these experiments initially relied on directly injecting a peptide analog of CRF (α-helical CRF) or antisense to CRF directly into the brain [29]. More recently, stable small-molecule antagonists have been discovered and studies with these have confirmed that central CRF mediates many of the behavioral responses to stress in animals (for review, see Ref.20).

One CRF antagonist, the R121919 compound, has been put into pilot trials of human depression with initial promising results [63]. The trials were, however, terminated due to safety concerns, not related to the hormonal actions of the drug. New compounds are in the pipelines of many drug companies so this finding may soon be replicated. Although

depression is the primary target of these new compounds, it seems that the anxiety disorders, especially those with established cortisol abnormalities, such as PTSD, might also be potential targets.

Other peptide antagonists, especially the substance P (NK1 receptor) blockers, have caused a lot of excitement in recent years with the finding that MK869 was effective in depression [3,28,37]. Animal data have revealed substance P to play a role in anxiety-like behavior, depending upon dose and brain region [23]. In the MK 869 depression trial, which was a randomized, placebo-controlled, double-blind comparison with paroxetine in patients with major depression and moderately high anxiety, both drugs significantly improved depression compared with placebo. From week 4 onward, both agents also reduced anxiety, as measured on the Hamilton anxiety scale (HAMA), significantly more than placebo [28]. It appears that the pharmacokinetic properties of this drug mean that it is not an ideal medication. That this research program has not yet come to fruition as a more potent homolog is being tested currently. Therefore, with this compound, resolving the issue of whether NK1-receptor antagonists will prove to be a new class of anxiolytic is still some time away.

However, in light of the exciting findings with MK869, many pharmaceutical companies have produced high-affinity antagonists with high selectivity for the NK1 receptor. Some of these have been tested in anxiety disorders such as social anxiety disorder as well as in depression, but as yet there are no data in the public domain.

Neuropeptide Y (NPY) is colocalized in the central nervous system (CNS) with norepinephrine and it may act in part to modulate the actions of this neurotransmitter. There are a great deal of data linking norepinephrine to anxiety [10,34], but little interest in the development of new direct-acting antinorepinephrine agents. For this reason, NPY might offer a novel way to indirectly downregulate noradrenaline transmission and thus reduce human anxiety [19]. There are three subtypes of NPY receptors the Y-1, 2 and 3, with Y1 being the one most likely related to anxiety. There have been attempts to evaluate NPY function in humans using plasma levels as a proxy for those in the brain. These studies of NPY levels in anxiety disorders have yielded conflicting results [8,55], but a link with norepinephrine has been found in that an increase in norepinephrine release also increases NPY levels in plasma [46]. Making a NPY agonist that is orally active is a challenge, which may explain why there are no reported NPY drugs yet in development.

V. CONCLUSION

The quest for new improved anxiolytics has gathered pace in recent years. Various approaches have been employed in the attempt to produce effective and better-tolerated drugs. Researchers and the industry have tried to capitalize on the success of the SSRIs. The insights that this group of compounds provided into the nature of anxiety have allowed for theorization and design of drugs acting on specific 5HT-receptor types. The success has been limited so far, but a number of promising drugs are currently undergoing trials. Further, better understanding of the neurobiology of the inhibitory neurotransmission in the brain through GABA prompted the research into compounds acting either on specific subunits of the GABA-benzodiazepine receptor complex or as partial agonists to this receptor. These drugs promise to be devoid of the dependence and abuse potential of the classical benzodiazepines, but this is yet to be fulfilled.

New approaches have also flourished. Drugs affecting the excitatory glutamate neurotransmission have shown great promise in animal studies. Finally, drugs interacting with

a variety of neuropeptide receptors, notably those of cholecystokinin, CRF, and substance P, are extensively investigated. It is hoped that at least one of the above approaches will be fruitful and eventually lead to the new generation of anxiolytics.

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34

Scales Used in Depression and Anxiety Research

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I. AIMS AND METHODS

Standardized methods of examination are used in psychiatry to assess objectively and, in some cases, quantify psychopathological phenomena and other clinically relevant domains, making it easier to communicate, verify their status, and analyze statistically [1,2]. They are essential to develop models of psychopathology.

Major areas in which standardized procedures are applied in psychiatry include the following:

Cross-sectional quantitative description of psychopathological abnormalities.
Assignment by a standardized method of individual cases to diagnostic categories.
Quantitative assessment of change over time in psychopathological abnormalities (with or without therapeutic interventions).

Standardized measurement procedures can be categorized on the basis of their methodologies into standardized assessment instruments, systematic behavioral analysis, and objective tests in the narrower sense of the word [3]. The terms standardized assessment instrument or rating scale are applied to structured methods of assessing current and/or past behavior and/or experience, based on lists of characteristics and, in some cases, descriptions of these characteristics. The extent of standardization varies from a simple list of symptoms filled in on the basis of a freely structured exploratory interview to semi- or fully structured interview schedules. These standardized assessment procedures are especially suitable to examine the full spectrum of psychiatric symptomatology; in addition, as they

are less restrictive than other procedures, they are particularly practicable. A variety of interview schedules are available and in general use.

Because they are very practicable, rating scales are often preferred to other methods if the results of patient examinations are to be documented in the context of routine professional care. They are also frequently applied in clinical psychiatric research, such as clinical trials of drugs, studies of longitudinal course, in routine clinical documentation, or in epidemiological studies [4,5], even though, in terms of their level of precision, standardized assessment measures are methodologically inferior to objective tests and systematic behavioral analysis.

II. SCALE CONSTRUCTION, SCORING METHODS AND QUALITY CRITERIA

Standardized methods of assessment, or rating scales, allow description in terms of numerical values of psychological abnormalities of various characteristic forms [6]. Different measurement scales allow the degree of abnormality to be quantified to varying extents. In the simplest instances, such as symptom checklists, scales simply allow for a rating of 0 or 1 to be made for each symptom or complex of symptoms, indicating whether or not it is present. More precise assessment becomes possible if the construction of the scale allows the severity of phenomena to be described using a scale consisting of a series of levels. As there is a danger that different assessors will base their evaluations on different standards, it is important to establish a framework for the assessment by providing anchor points (e.g., by giving examples of situations that would be characteristic for each point on the scale). Overly detailed assessment using an excessively broad scale is not meaningful, as differences at the extreme end of the scale cease to reflect real and significant variations in the phenomena being examined, so that the differentiations being made are not real ones.

In some assessment instruments, the values at which points on the scale are fixed may be varied as required, so that a scale may be constructed that is as finely differentiated as required; examples include visual analog methods of assessing subjective well being [7]. As the measurement of psychological phenomena is essentially imprecise, a relatively coarse scale is usually adequate, especially for comparisons between individuals (Fig. 1).

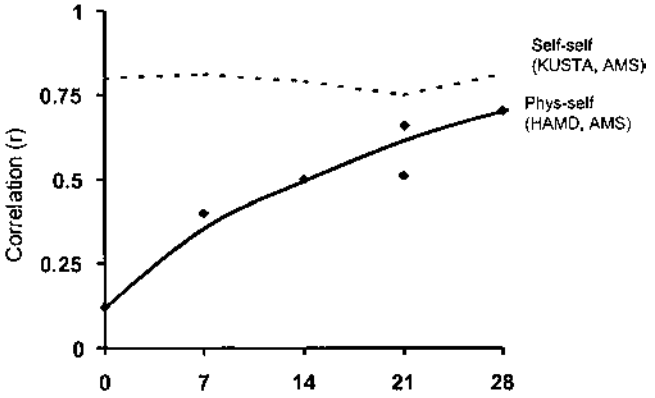

Figure 1 Correlations of different scales during the course of antidepressant treatment (n = 25). (From Ref. 9.)

A fine scale may have advantages for intraindividual comparisons. However, any improvement in measurement precision will generally be achieved not by refining the construction of the scale, but rather by improving methods of measurement [8].

The values for characteristics that belong together (e.g., individual symptoms within a syndrome) can be combined to produce a summary score. The extent to which characteristics belong together to make up a syndrome is determined during the process of test construction (see below) by applying multivariate statistical procedures such as factor and cluster analysis. In some cases, before adding together the figures for each characteristic to produce a summary score, these figures will be weighted to indicate the relative importance of each characteristic within the syndrome. However, if the characteristics have been shown to be relatively independent, theoretical or practical justification is required for any such summation [10].

Measures of psychopathology obtained from standard rating scales generally have the level of measurement of ordinal scales (i.e., they give only a rank order and do not possess the measurement level of an interval scale in which there are equal intervals between points on the scale). A fundamental problem in measurement is that measurement instruments with more detailed scales and higher levels of precision tend to bring with them greater restrictions regarding the phenomena that can be measured. This normally means that increasing quality of measurement is accompanied by increasing abstraction from the theoretical or conventional understanding of the characteristic that is the starting point (the reliability/validity dilemma).

Standardized assessment instruments should meet as far as possible the following quality criteria derived from test theory [11–13]:

1. *Objectivity*: The results should not depend on who carries out the assessment and analyzes the results. Procedure, analysis, and interpretation should be standardized so that, as far as possible, the same results are obtained regardless of who administers, analyzes, or interprets the instrument.
2. *Reliability*: This refers to the reliability with which a standardized assessment instrument records a characteristic. When the measurement is repeated, the same result should be obtained.
3. *Validity*: This is the extent to which the instrument records what it is intended to record. The connection between the results of measurement and any external criteria available for assessing what is to be measured should be as close as possible.
4. *Establishment of norms*: Reference values for different clinical groups and varying groups of normal probands and, where applicable, a representative sample of the general population should be available.
5. *Practicability*: The amount of resources required for administering standardized assessment instruments in terms of time, staff, and material should be as low as possible.

While for psychometric tests in the narrower sense the availability of norms is largely taken for granted, this has been approached with a great deal less rigor for clinical rating scales. Thus, there are only a few psychopathological scales that include norms from representative samples of the general population. Most of the clinical evaluation scales are limited to reference values available for particular diagnostic groups. Referring to such norms or, more precisely, reference values has a substantial impact on the interpretation of results. For example, moderately high scores for the domain of paranoid syn-

dromes have quite a different significance from moderately high scores for depressive symptoms or anxiety symptoms in that depressive and anxiety symptoms are common in the general population, whereas paranoid symptoms are not.

In producing norms for a standardized assessment instrument, the usual starting point is the normal distribution of values. There has to be a relation to the normal distribution in order for it to be possible to derive confidence intervals (see below) and to apply particular statistical tests, such as Pearson's product moment correlation. Two values need to be known to characterize a particular normal distribution: (1) the mean of all scores obtained for the test and (2) a measure of the extent of dispersion of these values, generally expressed in the form of standard deviation. Once these values are known, the standard properties of the Gaussian (normal) distribution allow the proportion of subjects who will have a particular test score to be calculated. Thus, for example, 68% of patients will have a test value that falls within 1 standard deviation either side of the mean, and around 95% a value that is no more than two standard deviations from the mean. On the basis of the norm values, it will therefore be possible to calculate where a proband's score lies in relation to a reference population (Fig. 2).

Norm values for a particular test can be straightforwardly expressed by giving the mean and standard deviation. Once this information is available, a statement may be made about the position of the proband in relation to the reference population. However, a disadvantage of referring to the numerical value of the standard deviation for a particular test is that it is difficult to compare the results obtained by a particular proband for several different tests. To allow comparisons of this sort to be made, a z-value can be calculated; this is the result obtained on a particular test expressed in terms of units of the standard deviation for that test. Results obtained by a particular proband in different tests may also

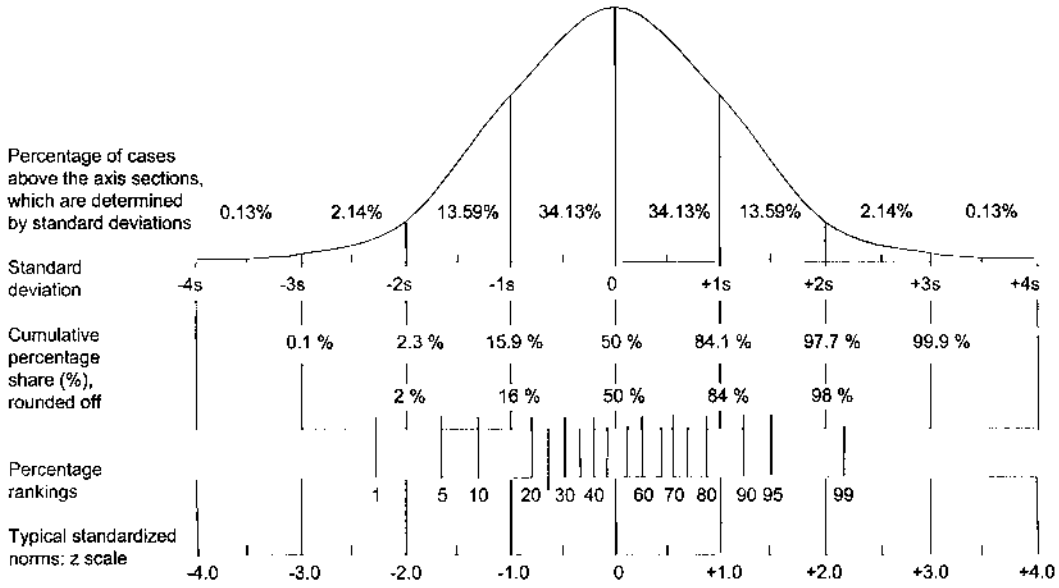

Figure 2 Relationship between some frequently used standardized scales and the normal distribution curve. (From Ref. 6.)

be compared using percentage rankings, by specifying for each test what proportion of a reference population has higher or lower scores for the test.

Various empirical methods may be used to test whether the test quality criteria specified above have been met. Appropriate ways of examining the reliability of a test include test–retest reliability, inter-rater reliability, the split-half correlation coefficient, and internal consistency. To determine test–retest reliability, the same test is given to the same group of people at two different time points. The time between the two applications of the test depends on the interval to which the test is intended to apply. For tests in which the aim is to record enduring personality traits, an interval between applications of the test of between 14 days and 1 year is recommended. For tests where the aim is to record rapidly fluctuating characteristics (such as mood or subjective well-being), a time span between several minutes and a few hours is appropriate. Ideally, identical results should be obtained for each measurement, but of course this is not the case in practice, as measurement errors necessarily occur (related to strong influences caused by the test situation, practice effects, etc.). The correlation between the two values gives the test–retest reliability coefficient. Deciding whether the reliability of a test is sufficiently high depends very much on the purpose of administering the test [11,14,15]. As a rule, a reliability coefficient in excess of 0.8 is required. Methods for which the test–retest reliability is below 0.5 are not generally useful. The measurement accuracy of a test may be different for different diagnostic groups (differential reliability).

Several different procedures also exist for determining the validity of a test (e.g., examination of consensual validity, predictive validity, construct validity, and content validity). Consensual validity is determined by correlating the results of applying the tests to a sample of probands with comparable data obtained by methods other than the application of the test (external criteria). For example, results for the test may be correlated with corresponding scores obtained for the same subjects for another test examining the same psychological characteristics. Whereas with consensual validity test values and external criteria are measured at the same time, predictive validity is determined by investigating whether events predicted on the basis of the test results have actually happened. A classic example is the correlation of test results from an intelligence test with assessment at a later date of actual success at school.

A requirement that needs to be emphasized is that following translation of a scale from one language into another, new validity tests must be carried out with the translated version. This is also true if the scale is modified in any way.

III. STANDARDIZED RATING SCALES FOR THE DESCRIPTION OF DEPRESSION AND ANXIETY

Anxiety and depression scales represent one of the central methodological approaches in clinical research of depression and anxiety (Fig. 3).

Standardized assessment scales relate to past or current behavior and experience. The extent of psychological abnormalities is rated using fixed scales. These rating scales may focus on a single aspect (e.g., anxiety—unidimensional scales) or on several aspects—multidimensional scales) of psychopathology. For each aspect of psychopathology, assessment may be based on a global rating or on different elements within the aspect being assessed (e.g., on individual symptoms of the depressive syndrome). In this latter

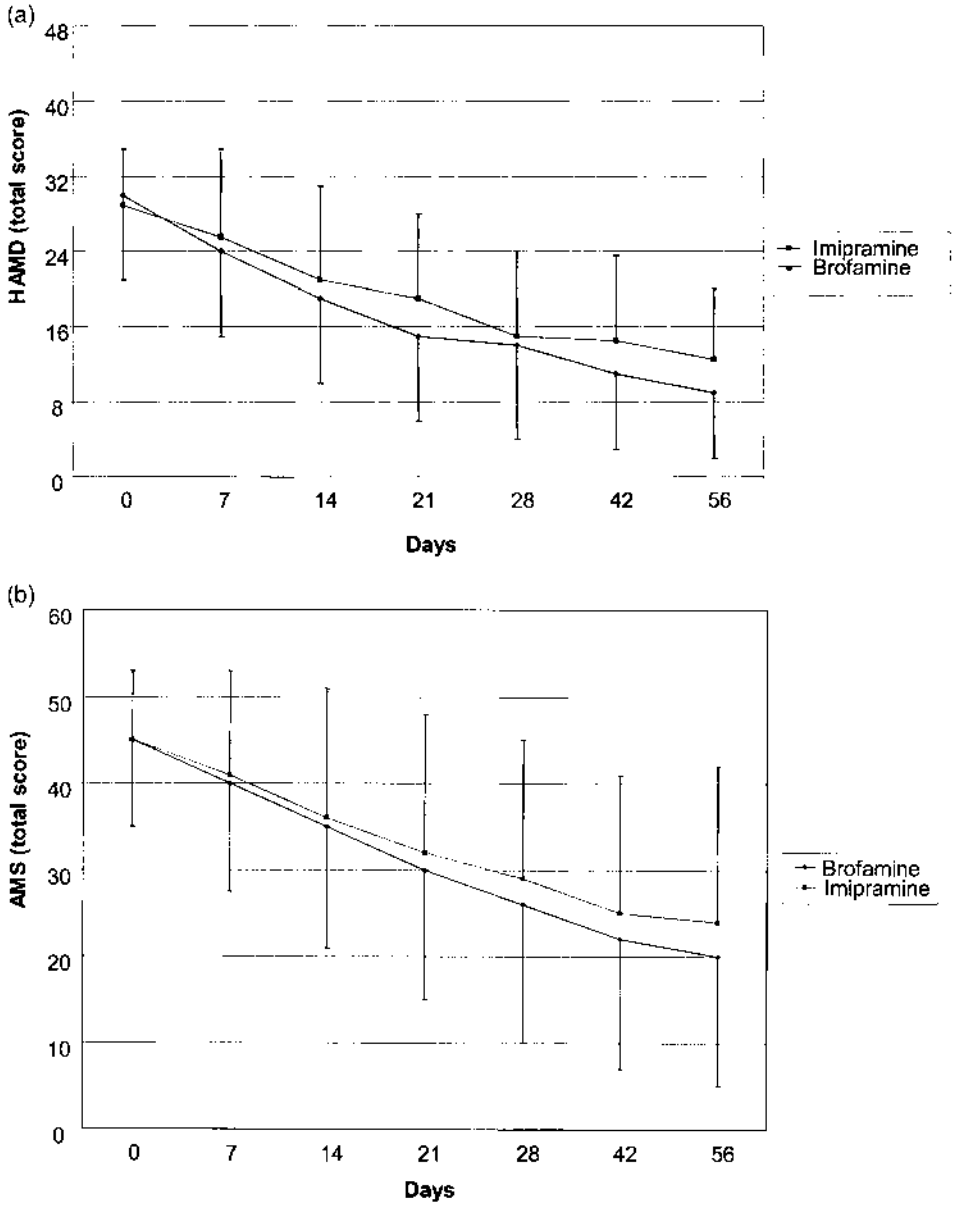

Figure 3 Mean values and standard deviations of the total scores of HAM-D. (a) 21 items and AMS (b) von Zerssen self-rating scale for two treatment groups over an 8-week double-blind period. (From Ref. 16, courtesy of Elsevier Science.)

case, the overall score on the instrument is obtained by adding together values for these different elements.

The level of standardization of standardized assessment scales falls between that of unstructured clinical assessment and that of objective tests. In some of these instruments, standardization is limited to providing guidelines describing items and the categories used

for assessing them and to specifying a method of analysis (generally one or more summary scores are calculated). In other scales, a time frame is also stipulated for the assessment, and in some the framework in which observation takes place is also fixed. In the latter case, the instrument is referred to as a fully structured or standardized interview. Generally, the more extensive the standardization procedures, the greater the reliability of an assessment instrument. However, a highly standardized instrument tends to become less practicable.

Particularly for the simpler rating scales, inter-rater reliability for observer-rated instruments can be improved by systematic joint training of the raters [17]. In principle, fully structured interview methods with extensive individual interviewing ought to produce high inter-rater reliability and, therefore, they have particular advantages in multicenter, multinational studies in which great discrepancies need to be taken into account not only in terms of how mental states are assessed, but also in the psychiatric interview techniques used.

Standardized assessment instruments may be classified on the basis of who carries out the assessment into self-rated and observer-rated instruments. In observer-rated instruments, psychopathological abnormalities are identified by trained assessors (e.g., doctors, psychologists, care staff, lay people trained to administer the instrument) or by significant others (e.g., partner, relatives, friends). The assessment concerns the behavior and/or experience of the patient and is based on the assessor's own observations and/or information given by the patient. Observer-rated scales need to be constructed so that they are appropriate to the level of training of the particular types of interviewer to be involved in their administration.

On the basis of multivariate statistical analysis (factor and cluster analysis), the data obtained from administering rating scales may be used to derive factors. These factors identify groups of individual symptoms that tend to occur together. If we consider that the term "clinical syndrome" generally refers to a group of symptoms that frequently occur in combination, it then becomes apparent that the factors extracted from rating scales relating to mental state are conceptually identical to clinical syndromes.

For some well-developed observer-rated scales, it has been shown that the factor structure also remains relatively stable across different studies, and for many of the factors this is true even with repeated measurements in the course of treatment [18–20]. This invariability of the structure of factors across different samples and time points is an important aspect of the validity of a scale (factorial validity). Different psychiatric diagnostic groups are reflected in different characteristic syndrome profiles when rating scales are applied [21].

It is important to bear in mind that identically named syndromes from different scales may vary greatly in terms of the items included, and the correlation between analogous syndrome scores is not always very great. As scales that measure the same domain (e.g., depressive symptoms) sometimes focus on different aspects of this domain [22], certain questions may be best addressed by using a combination of them.

When observer-rated instruments are administered by professionally trained observers, it is usually assumed that, in making the rating, the observer decides how much weight to put on the information the patient gives (e.g., an observable improvement in general behavior and demeanor is taken into account in the rating even if the patient gives no clear report of this improvement). An advantage of this expert assessment is that it reduces the scope for inaccurate assessments resulting from the distortions in patients' perception of themselves; on the other hand, it introduces the danger of distortions that are related

to the assessment (rater bias). Systematic distortion in the assessor's observations [23] can result from the following factors in particular:

1. *Rosenthal effect*: The assessor's expectations influence the result of the assessment.
2. Tendency on the rater's part to systematically *over-* or *under-rate* the degree of disturbance.
3. *Halo effect*: The result of assessment of one characteristic is influenced by the rater's knowledge of the subject's other characteristics or by the overall impression made by the subject.
4. *Logical errors*: The result of the assessment is influenced by the assessors reporting only those detailed observations that make sense to them in the context of their theoretical and logical preconceptions.

These errors may be partially compensated for by combining observer-rated scales with self-rated scales [3,9,24]. In self-rated instruments, patients can themselves classify past or current behavior and experience on the basis of fixed rating scales. Self-rated scales have the further advantage that their use is very economical for the assessor and eliminates observer bias. However, their use also introduces the disadvantage that conscious or unconscious tendencies to falsify responses (e.g., tendencies to exaggerate or conceal symptoms, the positive response bias, social desirability effects) will have a greater impact on patients and are only partially detectable through use of control scales (so-called lie detector scales).

Apart from a few scales measuring current mental state which, as with the Self-Report Symptom Inventory (SCL-90), record a very broad spectrum of psychopathological symptoms, most self-rated scales focus on specific aspects of disturbance of subjective experience (Table 1). Examples are inventories of physical and systemic complaints [25,26], depressive symptom scales [26–28], or measures of general subjective well-being

Table 1 Examples of Clinical Self-Rated Procedures

Domains	Procedure	Abbreviation	Refs.
Global psychopathology	Self-Report Symptom Inventory	SCL-90	30
		SCL-90R	31
Depression	*Depressivitäts-Skala* (Depressive Symptom Scale)	DS	26
	Befindlichkeits-Skala (Mental State Scale)	Bf-S	26
	Beck Depression Inventory	BDI	32
Anxiety disorders	Self-Rating Anxiety Scale	SAS	33, 31
	State-Trait Angst-Inventar (State-Trait Anxiety Inventory)	STAI	34
Obsessive-compulsive symptoms	*Hamburger Zwangsinventar* (Hamburg Compulsive Inventory)	HZI	35, 36
Alcoholism	*Münchner Alkoholismustest* (Munich Alcoholism Test)	MALT	37

Source: Ref. 6.

[26,29]. One of the advantages of this approach is that the quantity of items is limited, a particular strength where severely disturbed psychiatric patients are concerned.

However, very precise differentiation between different aspects of "subjective state" probably is not generally meaningful [38] in contrast to the detailed measurement of psychological disturbances that may be made by observer assessment. In fact, where results from clinical self-rated scales are compared with observer-rated scales administered by specialists, it seems that the various dimensions of the subjective state that self-rated instruments describe are more similar to one another than the different aspects of psychopathology delineated by clinical observer-rated assessments.

The level of agreement between self-assessment and observer assessment is variable and depends, among other things, on the type of disturbance and on symptom severity [39–42]. Thus, for example, where depressive symptomatology is severe, as at the time of inpatient admission, agreement is substantially more limited than after partial remission of symptoms at the time of discharge. This is probably connected with greater limitation of the capacity for self-observation among the severely depressed, and probably also with the fact that observers tend to recognize very severe depressive symptoms on the basis of nonverbal evidence to a greater extent than with less severe depressive symptoms, where the patient's verbal reports are more important. Compared with patients with endogenous depression, those with neurotic depression show a greater tendency to overstate their symptoms. Degree of agreement between self-rating and observer rating is substantially greater for the amount of change, as measured in longitudinal studies (e.g., in the context of treatment studies), than when psychopathological phenomena are recorded at a single cross-sectional time point [24,43] (Fig. 4).

Multi-methodological diagnostic procedures in which a combination of self-rated and observer-rated scales is applied [5,44] offer the best guarantee of satisfactory description of both subjective and objective psychopathological state.

Measures of subjective well being are of particular interest in the area of treatment assessment, particularly visual analog scales that measure current disturbances of psychological well being and lend themselves especially well to repeated measurement. These methods allow a very good description at the self-assessment level of response to a therapeutic intervention. Modern methods of statistical analysis, such as some of the procedures developed for time series analysis, allow satisfactory analysis of such data [9,24,45,46]. A full discussion of such approaches is presented by Morley [47].

IV. SOME FREQUENTLY USED OBSERVER RATING SCALES FOR DEPRESSION AND ANXIETY

A. Hamilton Depression Scale

The Hamilton Depression Scale (HAM-D) [48,49] became one of the first observer rating scales for depression to gain worldwide acceptance, although its weaknesses are increasingly criticized (see below). The original version of this scale contains 17 items, later versions 21 or even 24. The formulation of the items is not always precise enough, and is considerably worse than in the Montgomery-Asberg Depression Rating Scale [50], for example. Additional information from relatives and friends, etc., can be considered in the rating. In addition to the possibility of calculating a total score, it is also possible to calculate factor scores during the final analysis [48]. However, there is no uniform solution since the results of factor analytical evaluations resulted in solutions of two to six factors

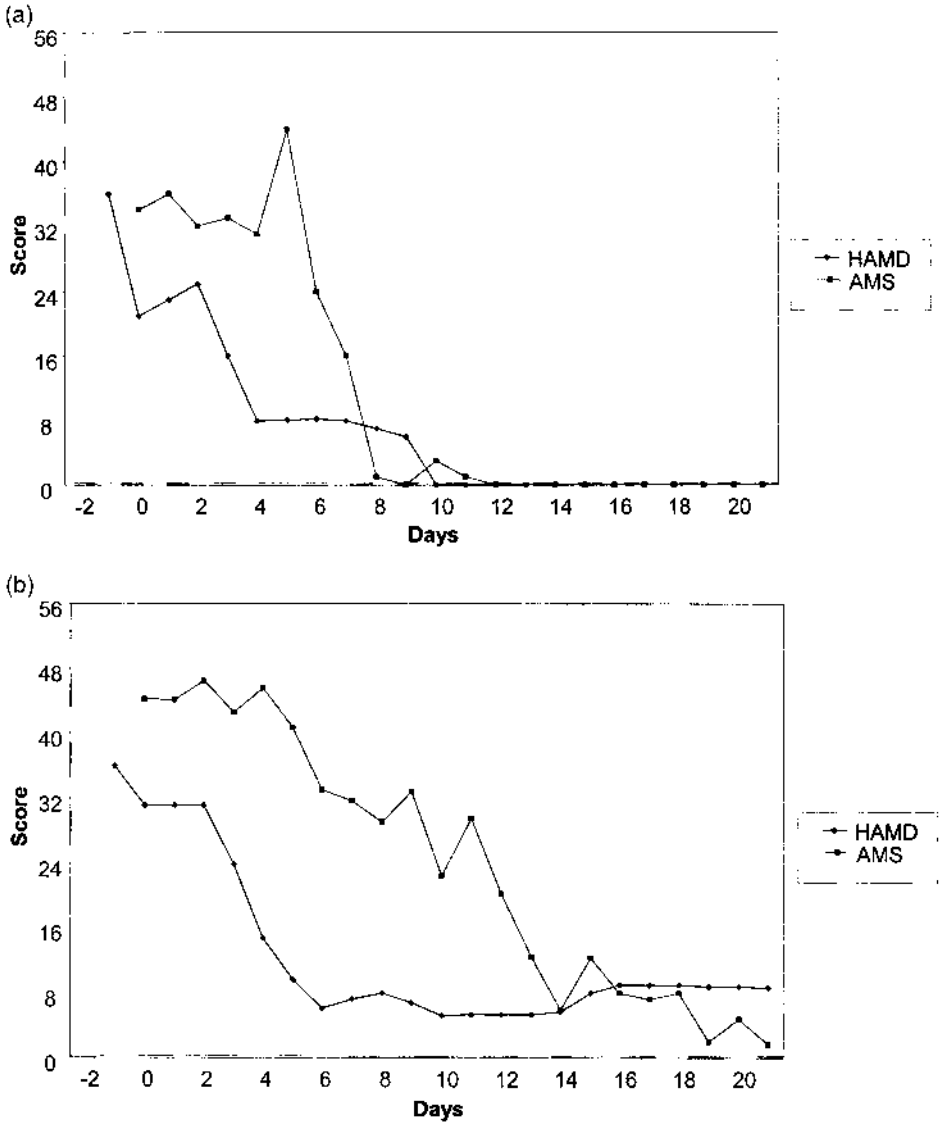

Figure 4 Two responders (a, b) and two nonresponders (c, d) under antidepressant therapy as assessed by psychiatrist ratings (HAM-D) and self-ratings (AMS). (From Ref. 9.)

[48,49,51]. The inter-rater reliability can be seen as very high, at least on the level of the total score [48,52]. The correlation with the Clinical Global Impression of the depressivity indicates the validity of the scale [53], as does the sensitivity for the recording of antidepressant-induced changes, which has been demonstrated in numerous antidepressant studies. Reference values for various clinical samples are available.

However, some further problems of the scale still remain unsolved, for example, concerning the content. The scale does not record certain diagnostically specific areas that are partially depicted in other depression scales and therefore proves to be unsatisfactory

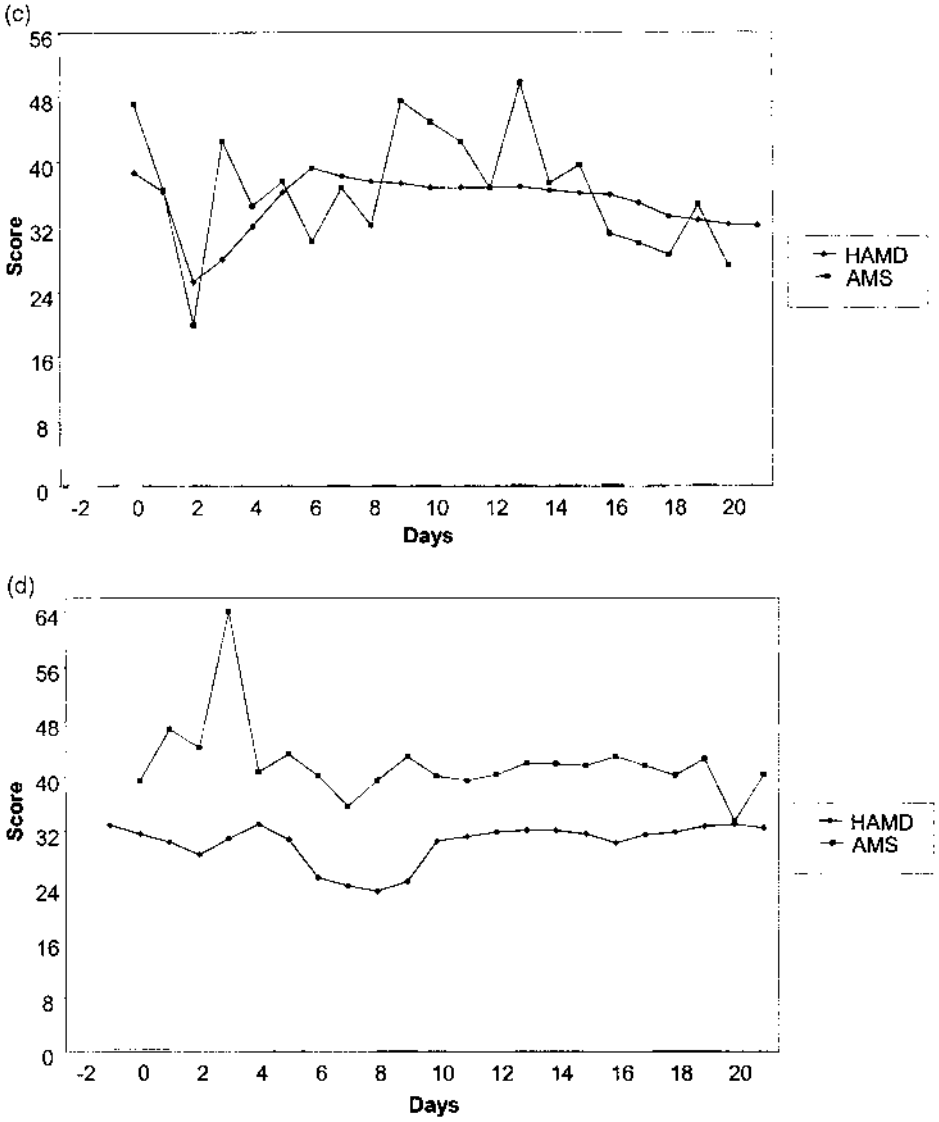

Figure 4 Continued

for a differentiated diagnosis of depressive disorders, particularly under the aspect of a differential diagnosis. As concerns the content, it is questionable whether the characteristic "daytime fluctuations" makes a difference in the sense of a higher depression score. This can lead to contradictions in the diagnosis of the course of the disease in view of the clinical experience that the most severe endogenous depressions often show no daytime fluctuations at first and that these only occur upon improvement of the severe depressive mood. The fact that sleep disorders are depicted with eight items leads to an efficacy bias in favor of sedating/sleep-inducing antidepressants in antidepressant studies.

The scale was subjected to a critical test-theoretical analysis in order to investigate

its homogeneity and the stability of the factor structure in repeated measurements during treatment [20,54,55]. On the basis of this analysis, it was modified to the Bech-Rafaelsen Melancholia Scale (BRMES) [54,56], which consists of 11 items, six of which are from the original scale.

B. Bech-Rafaelsen Melancholia Scale

As mentioned above, the Bech-Rafaelsen Melancholia Scale was constructed on the basis of an analysis of the HAM-D. The analysis used Rasch's Probabilistic Test Theory [54], which showed six items of the HAM-D to be suitable. In addition, another five items from the Cronholm-Ottosan Depression Scale [57], which also cover the aspect of "depressive retardation," were incorporated. The BRMAS Mania Scale by the same authors was similarly constructed for use in mania.

The scale consists of the following 11 items: decreased motor activity; decreased verbal activity; retardation (intellectual); anxiety (psychic); suicidal impulses; lowered mood; self-deprecation; retardation (emotional); sleep disturbances; tiredness and pains; work and interests. Unrotated factor analysis performed by Chambon et al. [58] showed that the first factor was a general factor. After varimax rotation, the following structure emerged: aspects of retardation, asthenia, anxiety, depression, and suicidal thoughts. Using Rasch models for testing whether the BRMES measures a dimension of severity of depressive states, it was found [59–62] that the sum total of the 11 items is a sufficient statistic. A BRMES score of 0–5 means no depression; 6–14 minor; and of 15 or more major depression [63]. A score of 15 or more on BRMES corresponded to DSM-III major depression [64]. The comparison to the HAM-D has shown correlation coefficients around 0.90 [65,66]. When comparing the HAM-D, the Montgomery-Asberg Depression Rating Scale (see below), and the BRMES, it was found that the BRMES was superior to the other scales in measuring sensitivity to change during antidepressive therapy [60,61]. In terms of Spearman correlation coefficients, the range of inter-observer reliability of the BRMES total score varied from 0.80 to 0.90 when several Scandinavian raters participated in joint rating sessions [65].

A substantial advantage that is offered by the BRMES in comparison to other depression scales is that it can be employed in conjunction with the BRMAS in long-term studies of bipolar disorder, and thus permits comparable evaluation of both manic and depressive episodes.

C. Montgomery-Asberg Depression Rating Scale

Although the HAM-D is still widely accepted, the Montgomery-Asberg Depression Rating Scale (MADRS) [67] is becoming increasingly important thanks to its conciseness and particularly to its better definition of characteristics. The aspect that the scale was constructed according to the principle "sensitivity to change" is of advantage for treatment-related studies.

The scale includes the following 10 items: apparent sadness; reported sadness; inner tension; reduced sleep; reduced appetite; concentration difficulties; lassitude; inability to feel; pessimistic thoughts; suicidal thoughts. The scale is supposed to include the main symptoms of depressive illnesses, even if certain important areas (e.g., psychomotor retardation, tendency to somatize) have been omitted as a result of the method of item selection [68]. Overall, the factor analyses and correlations with the Hamilton scale (in particular

with the various subscales) show that the MADRS covers more purely psychological symptoms than the HAM-D (see also Refs. 67,68).

Schmidtke et al. [50] used a heuristic procedure in which they subjected the correlations between individual item scores (calculated from the raw scores after dichotomization of the items according to various criteria such as less than the mean = 0/ = > mean = 1; 0 and 1 = 0, 2 and 2 = 1; (see Ref. 59) for 57 different patient ratings by MADRS and the HAM-D conducted by the same physician to a main axis factor analysis with varimax rotation. The analyses all show that, despite the methodological limitation that still pertains because of the similar calculations of the various factor analyses, the MADRS items do not represent a unidimensional scale. Four-factor solutions accounting for 51 to 54% of the total variance in all analyses proved relatively stable. In these analyses, the aspects covered by the MADRS items were classified under the headings sadness/pessimistic thoughts, inner tension, inability to feel, and reduced appetite.

In the construction studies, the sensitivity to change was claimed to be better than that of other procedures used simultaneously (e.g., Refs. 69,70). In later studies, the sensitivity of the MADRS for differences in the severity of depression [68] and changes in the symptoms of depression was again shown to be good [50,71,72].

Reference values of several clinical samples are available [50,59]. The inter-rater reliability has been given for different samples as 0.89 to 0.97 [67,69].

D. Hamilton Anxiety Scale

A number of symptoms that can be observed in association with anxiety states have been collected for the Hamilton Anxiety Scale (HAM-A) [73–76]. Thirteen symptom groups have been put together, and complemented by a fourteenth variable, namely the patient's observable behavior during the interview. The 14 groups of symptoms measure either psychic or somatic effects of anxiety and are as follows: anxious mood; tension; fears; insomnia; intellectual; depressed mood; somatic (muscular); somatic (sensory); cardiovascular symptoms; respiratory symptoms; gastrointestinal symptoms; genitourinary symptoms; autonomic symptoms; behavior at interview.

A factor score is calculated for both the symptom groups assessing somatic anxiety and the symptom groups assessing psychic anxiety (scores 1 and 2, respectively). The total raw score (score 3) can be taken as a measure of the patient's anxiety and makes it possible to compare groups receiving different treatments. Hamilton gave a very high coefficient of correlation for the inter-rater reliability, namely, 0.89 (following z transformation of the mean of the correlations between three raters).

E. Anxiety Status Inventory

The Anxiety Status Inventory (ASI) [77–79] records anxiety as a common symptom of various psychiatric disorders. The inventory includes 20 items, whereby items 1 to 5 and 20 refer to affective and items 6 to 19 to somatic symptoms of anxiety. The observer rating scale ASI was developed to supplement the Self-Rating Anxiety Scale (SAS) to assist the clinical evaluation of pathological anxiety conditions.

In a study by Zung [78], 22 patients with a diagnosis of anxiety disorder demonstrated a significantly higher ($p < 0.05$) average ASI score than 187 patients with other psychiatric diagnoses (schizophrenia, depressive disorders, personality disorders, transient situational disturbances), whereas the Taylor Anxiety Scale [77] could not show any significant difference.

F. Liebowitz Social Anxiety Scale

The first clinician-administered scale developed for the assessment of fear and avoidance associated with social phobia was the Liebowitz Social Anxiety Scale (LSAS) [80]. The LSAS assesses a wide range of both social interaction and performance/observation situations that are rated for degree of fear/anxiety and frequency of avoidance. It has been widely used in studies of the pharmacotherapy of social phobia. The LSAS shows a high degree of convergent validity with other measures of social phobia, such as the Social Interaction Anxiety Scale [81], the Social Phobia Scale [81], etc. Also other psychometric variables related to validity and reliability are satisfactory.

G. The Panic and Agoraphobia Scale

The Panic and Agoraphobia Scale (PAS) [82] was developed to determine the severity of panic disorder with or without agoraphobia and to monitor treatment efficacy in psychotherapy and drug treatments. The scale has 13 items and is divided into five subscores (panic attacks, agoraphobic avoidance, anticipatory anxiety, restriction of activities and quality of life, and worries about health). The scale is also available as a self-rating version, which consists of identical questions. The test–retest reliability of the observer-rated version is $r = 0.73$. Both the observer-rated and self-rated version have satisfactory validity.

V. SOME FREQUENTLY USED SELF-RATING SCALES FOR ANXIETY AND DEPRESSION

A. Paranoid Depression Scale

The Paranoid Depression Scale [26,83,84], which is available in two parallel forms, is composed of 43 items. It records the degree of subjective impairment by emotional reduction of the type anxious-depressive mood—these items are also on a separate depression scale—as well as a distinct cognitive dimension to determine a distrusting attitude and whether the subject is out of touch with reality. In addition, there are eight control items to measure disease denial and three items to assess motivation.

The values of the individual items are summarized as factor values. Among others, correlations of the paranoid scale with the criterion of the affiliation to a group of schizophrenic patients, correlations of the depression scale with the criterion of affiliation to a group of patients with depressive mood, correlations with relevant factors of other scales, and sensitivity in the recording of therapy-induced changes indicate the validity. The depression scale is also available as a separate scale, with 16 items, without the items of the paranoid scale. Norm values are available for a representative sample of the general population in the former West Germany, as well as reference values for various clinical groups (physically ill, mixed psychiatric groups, individual psychiatric diagnosis groups).

B. Beck Depression Inventory

Another frequently used self-rating scale is the Beck Depression Inventory [27,85], which was originally developed as an observer rating scale. It has 21 items and is still widely used. It has a special focus on cognitive aspects of depression, which might explain the fact that it is preferentially used in the context of psychological treatments, while the application in psychopharmacological studies is seldom. The psychometric criteria with respect to reliability and validity are satisfactory [86].

C. State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) is an inventory for evaluating two different components of anxiety [34,87,88]. One scale (scale 1) is designed to measure state anxiety and the other (scale 2) to measure generalized (trait) anxiety. Depending on the purpose to which they are being put, both scales can be used on their own or together. Scale 1 assesses a relatively stable personality trait and is thus neither time nor situation dependent. It provides an evaluation of the person's state at the time of testing. However, if the instructions are altered, scale 1 can also be used to assess state anxiety in any specific situation provided that the subject can bring to mind the situation in question and is willing to cooperate with the tester.

The total scores for scales 1 and 2 are calculated for each subject as measures of state and trait anxiety, respectively. The correlations with the IPAT anxiety scale, the Taylor Manifest Anxiety Scale (TMAS), and the Zuckerman Affect Adjective Checklist (AACL) are high (between 0.75 and 0.84). Studies on construct validity in which the scale was administered repeatedly under different conditions have shown point-biserial correlations of $r = 0.60$ and $r = 0.73$. Test–retest reliability is reported for trait as $r = 0.84$ and state $r = 0.34$ (men, interval 1 h). Alpha coefficients of $r = 0.83$ and 0.92 have been found for internal consistency. Norm values are available [83,84].

D. Adjective Mood Scale

The Adjective Mood Scale contains 28 items [26,83,84] and is available in two parallel versions. It records the degree of current impairment of subjective well being. The scale is especially indicated for course descriptions when tests are frequently repeated. It is suitable for healthy subjects, and physically or psychically ill patients, particularly for psychically ill patients with affective disorders.

The values of the individual items are summed to give a total score, which gives the impairment of the subjective well being. High inter- and intra-individual correlations with global assessments of the depressive mood as well as the sensitivity for the recording of therapy-induced changes prove the validity. There are norm values for a representative sample of the general population of the former West Germany and reference values for various clinical groups.

E. Self-Rating Anxiety Scale

The Self-Rating Anxiety Scale (SAS) is the self-rating version of the observer rating scale Anxiety Status Inventory developed by the same author [78]. The SAS contains 20 anxiety symptoms, five of which are affective and 15 somatic. Some of the items are formulated positively with respect to symptoms, other negatively.

With respect to content validity, items were selected using psychiatric diagnosis criteria of different psychiatric disorders that are associated with anxiety. And with respect to criteria-related validity, patients with the diagnosis of "anxiety symptoms" have a significantly higher mean index ($p < 0.05$) than patients with the diagnoses of "schizophrenia," "depressive symptoms," "personality disorder," or "situative transitional symptoms." The Taylor Manifest Anxiety Scale [77] could not show this difference. A group of 100 controls (57 men, 43 women, aged 18–62 years, mean 34 years) had a significantly lower mean index than the diagnosis groups listed above. Correlations with other tests are as

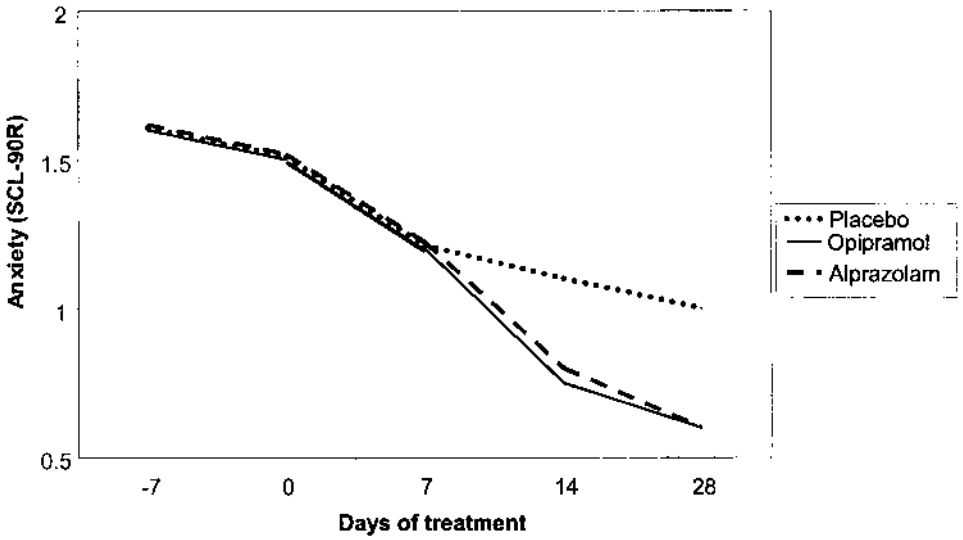

Figure 5 Course of the median values in the SCL-90-R anxiety subscale; median values dependent on drug and treatment duration ($n = 218$). (From Ref. 94.)

follows: $r = 0.66$ with the observer rating form ASI ($r = 0.74$ only in patients with a diagnosis of anxiety), $r = 0.30$ with the Taylor Manifest Anxiety Scale.

F. Self-Report Symptom Inventory

The Self-Report Symptom Inventory 90 items (SCL-90) [89–91] is the revised version of the Hopkins Symptom Check List. The scale is used for the self-rating of patients with respect to various burdening symptoms. It allows nine ranges of syndromes to be recorded and was specially constructed to register of effects of drug treatment (Fig. 5). It has already proved itself in this respect in various clinical studies with neuroleptics, tranquilizers, and antidepressants [92,93].

G. Complaint List

The Complaint List consists of 24 items [83,84] and is available in two parallel forms, the content of which can be supplemented with an additional form with 17 items.

The Complaint List records the degree of subjective impairment due to physical and general complaints. It is indicated to record those kinds of complaints in depression or anxiety disorders, among others.

For the analysis, the values of the individual items are summed to give a total score. Good correlations with the criterion of the affiliation to a corresponding clinical group, correlations with analog test scales, and the sensitivity for the recording of treatment-induced changes all speak for the validity. Norm values from a representative sample of the general population in the former West Germany are available, together with reference values for various clinical groups (physically ill, mixed psychiatric groups, individual psychiatric disorders).

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