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Brain Protection in Schizophrenia, Mood and Cognitive Disorders

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ISBN 978-90-481-8552-8 e-ISBN 978-90-481-8553-5
DOI 10.1007/978-90-481-8553-5
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2010921796

Foreword

Neuroprotection is a novel perspective for the treatment of Alzheimer's, Parkinson's, and Huntington's disorders that lead to neurodegeneration and disabilities as a result of deterioration of neurons due to apoptosis, oxidative stress, excitotoxicity, and other mechanisms. Since these mechanisms have implications not only for neurodegenerative disorders, but also for schizophrenia, mood and cognitive disorders, a synthesis of findings from neuroscience and clinical research across these disorders is warranted. We are now faced with the challenge to translate these new findings into therapeutic advantages. The purpose of this book is to provide an up-to-date overview of basic and clinical studies concerning the neuroprotective approach, mechanisms, several compounds with neuroprotective properties, nanotechnological scientific advances and neuromodulatory techniques that may contribute to more efficacious treatment of major mental health disorders.

The book is divided into two parts. The first part serves as an introduction and overview of conceptual issues of the neuroprotective approach, and some neurobiological advances. Chapters in this part review definitions, perspectives, and issues that provide a conceptual base for the rest of the book. In addition, this part includes chapters in which the authors present and discuss the findings from basic studies of neurodegenerative mechanisms that are associated with the pathogenesis of major mental health disorders. The second part focuses on findings obtained from clinical trials with neuroprotective compounds, and neuromodulatory techniques. The take-home message is that principles of the neuroprotective approach may be applied to treatment of schizophrenia, mood and cognitive disorders.

Contributors to this book are among the most active investigators and clinicians in the field who provide new perspectives not only clarifying ongoing controversies but also propose diverse aspects and new insights to neuroprotection. As often happens in publications composed of contributions by multiple authors from diverse

orientations and academic backgrounds, differences in approaches and opinions are inevitable, as is some overlap.

First of all, I thank Springer Science Business Media B.V. for the goodwill and publication of this book, particularly, to Mr. Peter Butler, and Ms. Melania Ruiz, publishing editors, who did their utmost to promote this project and provided valuable assistance that made the book possible. I would like to gratefully acknowledge the contributors for their excellent cooperation. I wish to acknowledge with thanks and appreciation the guidance, assistance and encouragement provided by Professor Evsei D. Krasik, Dr. Itzhak Levav, Professor Yigal Ginath, Professor Jean Endicott, Professor Abraham Weizman, and Professor Irving I. Gottesman. I also wish to take this opportunity to thank my close co-workers and colleagues Drs. Anatoly Gibel, Yael Ratner, Ehud Susser, and Professor Vladimir Lerner, as well as, *the nursing staff* of my clinical department in Shaar-Menashe Mental Health Center for their commitment, support, and excellent cooperation. Finally, for the support and patience of my family and friends I am truly thankful.

I sincerely hope that this book will further knowledge in the complex field of brain protection in psychiatry and will also be of interest to a broad spectrum of readers including neuroscientists, psychiatrists, neurologists, pharmacologists, psychologists, general practitioners, graduate students, and policy makers in the fields of mental health.

January 2010

Michael S. Ritsner
Editor

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Part I
Neuroprotective Strategies and Mechanisms

Chapter 1
Brain Protection in Neuropsychiatric Disorders: Past, Present and Future Challenges

Ehud Susser and Michael S. Ritsner

Abstract Brain- or neuroprotection is any therapy that prevents, retards, or reverses neuronal cell death resulting from neurodegenerative processes. In the past neuroprotection has been an active field of research in medical areas ranging diversely from neurology through cardiology to ophthalmology. However, it has only been in recent years that the psychiatric community began to "sit up and take notice" – be it due to improved technological imaging capabilities or because of the growing dissatisfaction with current pharmacological treatments (particularly in schizophrenia) regarding negative and cognitive symptom domains, second generation antipsychotics included. Mounting evidence indicates that neuropsychiatric disorders such as Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, epilepsy, schizophrenia, mood and cognitive disorders all have a neurodegenerative component proposed to be due to various mechanisms including oxidative stress, mitochondrial dysfunction, excitotoxicity, myelin dysfunction and apoptosis to name just a few. New and improved imaging techniques combined with advanced cognitive testing continue to help us assess this degenerative component. However, despite considerable research effort, clinical trials in search of an agent that can effectively protect the brain of patients with schizophrenia, mood and cognitive disorders have been largely disappointing as of yet. Thus, it would seem that the continuing search for neuroprotective factors in psychiatry would be especially pertinent for finding novel treatments and augmentation strategies – not only for treating active symptoms, but also for slowing down disease progression and possibly even providing primary prevention in recognized prodromal states. As of now several drugs, hormones, neurosteroids, vitamins as well as various other substances have been studied regarding their neuroprotective properties in psychiatric disorders, with variable amounts of success. Other neuroprotective approaches being studied include the utilization of nanotechnological scientific advances as well as

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_1,

neuromodulatory techniques (such as transcranial magnetic stimulation, vagal nerve stimulation and deep brain stimulation).

In this chapter we will review the trends in psychiatric neuroprotection in the past and the present in addition to raising questions for future directions and challenges. The present overview will cover the evidence concerning the different theoretical frameworks regarding the basis of psychiatric neuropathology and specifically their applicability to the field of neuroprotection. The different classification possibilities of neuroprotective candidates are also discussed. Finally, we will propose using a separate term in an attempt to differentiate psychiatric neuroprotection from other forms of neuroprotection – "psychoprotection".

Abbreviations

NMDA	N-methyl-D-aspartic acid
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
PKC	Protein kinase C
CNS	central nervous system
DHEA	dehydroepiandrosterone
EPO	erythropoietin
GABA	gamma-aminobutyric acid;
CRH	cortisol releasing hormone
HPA	hypothalamic- pituitary-adrenal axis
AChE	acetylcholinesterase
EPS	extrapyramidal symptoms
fMRI	functional magnetic resonance imaging
DTI	diffusion tensor imaging
VNS	vagal nerve stimulation
TMS	transcranial magnetic stimulation
DBS	deep brain stimulation
DCM	direct causal modeling
ALS	amyotrophic lateral sclerosis
MS	multiple sclerosis

1.1 Introduction

Neuroprotection is a term commonly used to refer to any type or therapeutic modality, usually pharmacological, that can prevent, delay or even reverse neuronal damage, whether it be neuronal death, axonal degeneration or any other form of neuronal injury [1], though the exact meaning of the term remains somewhat ambiguous as different researchers may mean different things – ranging from *cellular* protection (preventing apoptosis) to *intercellular* connectivity preservation, or as a general overall term denoting conservation of neural function. Additionally, the term may

be used to represent mechanisms of disease prophylaxis or conversely disease treatment aimed at minimizing further damage [2]. Although neuroprotection as such is a modern field of research, the search for neuroprotection in its most basic sense, i.e. the prevention of mental deterioration, has been an ongoing pursuit of scientists since the earliest of times. Indeed, the unremitting search for "fountains of youth" (see Fig. 1.1), "magical panaceas" and "cure-all elixirs" across the civilizations can be seen as early attempts of unearthing neuroprotective factors, thus seeking to avoid the mental decline towards dementia, madness or even aging itself. Today, the sale of reputed anti-aging products such as nutrition, cosmetics, hormone replacements, physical fitness, vitamins, supplements and herbs has become a lucrative industry, with the US market alone generating above $50 billion of revenue each year [3].

With this multitude of putative products purported to slow down aging and delay neural degeneration it is becoming increasingly difficult to sort out which of these has actual scientific basis. In fact, the very notion of neural regeneration and brain plasticity would have seemed preposterous in the not so distant past. However today there are literally hundreds of products being investigated within the scientific community specifically for their neuroprotective potential [4]. It is of interest to note that some chemicals recently shown to have neuroprotective properties have been in use for thousands of years – cannabis (specifically cannabinol), has been shown to have a protective effect in Alzheimer's disease, specifically through anti-oxidative and anti-apoptotic effects [5]. Similarly, green tea, which has been in use for over

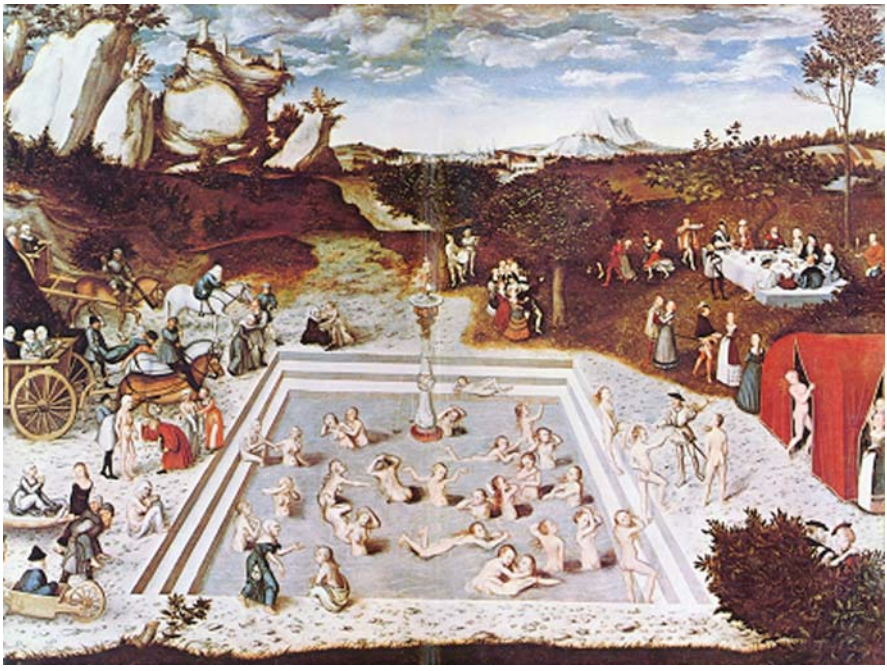

Fig. 1.1 The Fountain of Youth by Lucas Cranach the Elder, 1546 (public domain)

4000 years, has been shown to have neuroprotective properties through various complex mechanisms ranging from anti-oxidative properties to protein kinase C (PKC) modulation [6].

When approaching the subject of neuroprotection in psychiatry, we first need to address the issue of nosology. When Emil Kraepelin offered the categorical phenomenological construct of psychiatric diseases a century ago, he put the deteriorative degenerative nature of schizophrenia within its very name: "dementia praecox" [7]. However, today we know that neural degeneration, in one form or another, is not exclusive to schizophrenia. In fact, the categorical distinctions between the various mental diseases proposed by Emil Kraepelin (especially affective versus psychotic) have become blurry, sharing several genetic as well as anatomical and functional characteristics leading many to question the categorical construct and propose a dimensional construct in its place where the emphasis is placed on specific symptom dimensions (i.e. cognitive, affective, positive, negative etc.) in place of well defined disease entities [8]. It is thus of no surprise that previously defined categorical diseases also share neurodegenerative processes: ventricular dilation, reduced hyppocampal volume and reduced frontal volume are seen not only in schizophrenia but to a lesser extent in bipolar disorder and in major depressive disorder [9]. Additionally, reductions in dendritic spine density, neuronal size and synaptic proteins are seen along the entire affective − psychotic spectrum [10, 11]. Thus, the search for neuroprotection in psychiatry can have a potentially far more outreaching scope than previously thought in as much as the focus may shift from schizophrenia to the entire mental health spectrum.

1.2 A Brief History of Neuroprotection

Although the focus of this book is primarily about brain protection in psychiatric disorders, the evolving field of psychiatric neuroprotection is in essence situated on a continuum stemming from previous neuroprotective research of other various disciplines. Thus, it is of value to briefly summarize these first before we expand on psychiatric neuroprotection so as to gain a wider understanding of the mechanisms involved.

The origins of modern neuroprotective research date back to the late 1980's and initially focused on neurological disorders exhibiting extensive neuronal damage [12], whether be it ischemic (as in CVA), traumatic (as in cerebral or spinal cord injury) or neurodegenerative (via genetic, environmental, multifactorial or idiopathic pathways in diseases such as Parkinson's, Alzheimer's, ALS or multiple sclerosis). Research into neuroprotective candidates for ischemic stroke was, and is, particularly extensive with an exhaustive list of tested drugs including NMDA antagonists (the first of many was MK-801 in 1988) [13], radical scavengers, calcium antagonists, sodium channel blockers, potassium channel blockers, cell membrane stabilizers, anti-inflammatory agents, anti adhesive molecules, glycine receptor antagonists, AMPA receptor antagonists, serotonin receptor antagonists.

GABA agonists, phospholipid precursors, nitric oxide signal transcoding down regulators, leukocyte inhibitors, hemodilution and many more [14] with over 1000 experimental papers and 400 clinical articles appearing on the subject including some phase III randomized placebo controlled double blind studies such as the Stroke-Acute Ischemic NXY Treatment trials (SAINT I and II) [15], the Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke study [16] and the The Cervene Stroke Study [17] (an opoid antagonist) – all of which showed negligible clinical results. In fact, as of today most of the trials regarding post ischemic neuroprotection have been disappointing showing little success in human trials, despite having shown considerable promise in initial animal studies [18, 19]. The reasons for this discrepancy could be attributed to differences between experimental stroke conditions and the clinical situation, the clinical heterogeneous stroke population versus the homogenous (and healthy) animals and other reasons. Nonetheless, there is still much promise in this field (notwithstanding the need to reassess clinical strategies) including current ongoing efforts such as therapeutic hypothermia [20], high dose albumin therapy [the ALIAS (Albumin in Acute Stroke) trial] [21] and hyperacute magnesium therapy (the FAST-MAG protocol) [22] to name a few, the latter two currently undergoing phase III trials. Research regarding neuroprotection in other neurological disorders has also been largely unsatisfying. In Parkinson's disease there has been an abundance of laboratory research searching for new targets and candidate agents that might block the neurodegenerative cascade of the disease progression, however, currently there is no proven neuroprotective agent in Parkinson's disease [23], and a recent review by Olanow [24] suggests that for the time being we need to focus on currently available drugs (such as levodopa and dopamine agonists) and consider their own inherent neuroprotective effects. Similarly, in multiple sclerosis (MS) there is no currently available neuroprotective or regenerative therapy but only immunomodulatory or immunosuppressive agents [25]. Several (albeit small) studies targeting remyelination and neuroprotection in MS tested various candidates including IGF-1, riluzole, erythropoietin (EPO) and minocyline but have failed to exhibit significant neuroprotective effects. In ALS the research has focused primarily on antiglutamate agents such as riluzole which is one of the few neuroprotective agents today that have been approved by the FDA (along with memantine for Alzheimer's disease) and several reports show it to be have an ability to slow down the disease progression, albeit mildly, prolonging survival by 2–3 months according to the Cochrane review [26]. Current research in ALS is targeting the mutant superoxide dismutase 1 (SOD1) gene found in patients with familial ALS [27]. Among the neurodegenerative disorders, Alzheimer's disease is a prototypical example sharing some clinical similarities with psychiatric diseases thus serving as a common model for neuroprotective research. Indeed, shortly after the introduction of cholinesterase inhibitors and the NMDA antagonist memantine for the treatment of Alzheimer's disease they were studied for their neuroprotective potential in psychiatric (specifically schizophrenic) patients [28]. However, though these agents show some (albeit limited) benefit for the treatment of Alzheimer's dementia [29], they have been largely disappointing regarding schizophrenia (1.3). Another exciting new finding in

the field of neuroprotection is that of the effects of EPO on neurogenesis and apoptosis [30]. Consequently, EPO is currently undergoing extensive study in virtually all the areas of neuroprotection (see other chapters in this book for further detail). Specifically, there are initial promising results regarding the treatment of central and peripheral nerve trauma [31] where it has been found that one dose of EPO is effective for treating spinal cord injuries [32]. In ophthalmology neuroprotection is chiefly aimed at glaucoma in an attempt to slow down visual loss [33]. However, the only neuroprotection currently proven in glaucoma is intraocular pressure reduction. Initial studies involving cell culture models are showing potential (for example a study with the agent brimonidine, an alpha2 adrenergic agonist, showed a protective effect on rat retinal ganglion cells [34]) but no large clinical studies have been completed as of yet. A current large randomized trial still in progress (as of 2009) is the "Memantine in Open-Angle Glaucoma" study of more than 2000 patients followed for several years [4]. There is also much interest in neuroprotection in the field of cardiology, particularly involving ways to avoid neurological damage during heart surgery. Extensive research has been undertaken in an attempt to minimize the incidence of perioperative cerebral injury, and both pharmacological and nonpharmacological strategies have been investigated. Although many agents demonstrated promise in preclinical studies, there is currently insufficient evidence from clinical trials to recommend the routine administration of any pharmacological agents for neuroprotection during cardiac surgery [35].

The burgeoning field of neuroprotection is not restricted to conventional pharmacological trials alone. With the advancing sciences of nanotechnology [36] and neuromodulatory techniques such as transcranial magnetic stimulation (TMS), vagal nerve stimulation (VNS) and deep brain stimulation (DBS) [37] we are witnessing a current investigative surge showing promise regarding future neuroprotective possibilities that are not necessarily limited to pharmacological interventions. Nanotechnology is the science concerned with the design, synthesis and characterization of matter on an atomic and molecular scale [36] and is used in a wide variety of applications including medicine, aerospace, agriculture, optics, energy and information as well as many others. In recent years there have been major advances in nanomedicine, and specifically in nanoneuroscience, regarding neuroprotection and neuroregeneration [38]. These developments may prove to be of major significance in our understanding and treatment of neurodegenerative disorders and neural damage, both in the creation of monitoring and imaging tools (i.e. of neurotransmitter levels on a cellular level [39]) and by way of creation of novel neuroprotective treatments through various possibilities such as innovative techniques of drug delivery aimed at specific neural targets [40], gene therapy [41], tissue repair and neuroprosthesis [42] and nanosurgery [43] among others. Indeed, the field of nanoneuroscience has been growing rapidly in recent years and we expect to see major advances regarding neuroprotection (and specifically psychiatric neuroprotection) in the future. As of June 2009 it even has its own journal – the newly created "Journal of Nanoneuroscience" [44]. The last two decades have also seen the introduction of neuromodulatory techniques such as VNS, DBS and TMS, initially in an attempt to treat neurological disorders such as epilepsy, Parkinson's

disease and chronic pain but in recent years these techniques have been studied in the context of psychiatric disorders such as major depressive disorder, obsessive compulsive disorder, posttraumatic stress disorder and eating disorders (among others) with various degrees of success. In conjunction with advances in nanotechnology (such as the creation of nanotubes), neuromodulatory techniques such as DBS can be further refined, thus enabling improved precision and subsequent neuroprotecion [39]. For an extensive review of the neuromodulatory techniques and their suggested neuroprotective effects Chapter 21.

Considering the extent of research conducted in the fields of neuroprotection and neuroregeneration there is a clear need for organizations aiming at providing researchers with information, sponsorship and advice, as well as offering a gateway for collaborative ventures. Currently there are at least two such organizations: The Global College of Neuroprotection and Neuroregeneration, founded by Russell Pendleton, London, UK and Professor Hari S. Sharma, Uppsala, Sweden in 2004 [45] (GCNN; http://www.gcnpnr.org/index.html) and the Society for the Study of Neuroprotection and Neuroplasticity [46] (SSNN; http://www.ssnn.ro), which became an affiliated organ of the GCNN.

In conclusion, the flurry of neuroprotection research in the last 20 years has been at the same time promising and disappointing though it should be said that most of the trials have been small, early phase trials, many of which using animal models in laboratory settings. Even though many of the clinical trials have been disappointing, we cannot ignore the wealth of theoretical knowledge gained in the past years regarding neuroprotective agents and their projected mechanisms of action. Thus, we are still in need of large scale, multicenter clinically oriented phase III trials if we aspire to attain clinical applicability in neuroprotection, including in the field of psychiatry, as detailed below.

1.3 Psychiatric Neuroprotection

As early as 1801, French psychiatrist Philippe Pinel posed the following question: "*Does insanity depend upon organic lesion of the brain?*" [47]. Pinel proceeded to perform numerous autopsies and eventually concluded: *No facts, yet clearly established, relative to the influence of the size and configuration of the cranium upon the faculties of the mind*. Indeed, this view was a prevalent one for many years, and not without reason. There are minimal gross anatomical findings in mental diseases when compared to the degenerative neurological disorders such as Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis. Additionally, mental disorders differ from progressive neurological disorders in as much as they may remit or even recede. Consequently, mental diseases were seen by many as *functional* as opposed to *organic* (a viewpoint still held by many to this day), and treatment options were developed accordingly. It was only in the late 20th century, when advanced functional brain mapping technologies have become widely available, that the so called "organic lesions" (anatomical as well as functional) that

Pinel failed to locate in the brains of the mentally ill have been finally delineated so that we can now state with some certainty that psychiatric illness indeed has a component of neural damage and we can characterize the various mechanisms of the neuropathology involved [48]. These mechanisms can be divided into three main types: Neurodegenerative, neurodevelopmental and dysconnective. An understanding of these mechanisms is essential in order to tackle the field of neuroprotection since each of these mechanisms provides different pathophysiological as well as pharmacological angles of understanding in the path of neuroprotective research. Thus, a brief summary follows.

1.3.1 Neurodegenerative Mechanisms

The most consistent postmortem morphometric findings in the brains of schizophrenics are enlarged ventricles with focal decreases in the size of mesial temporal lobe structures, including the amygdala, hippocampus, parahippocampal gyrus, and entorhinal cortex when compared to normal controlls [49]. Additionally, prospective studies following schizophrenic patients from the first episode onwards show that many of these morphometric findings develop over time and are only minimally present in early stages [50], thus reinforcing the neurodegenerative hypothesis. For instance, we know that schizophrenic patients lose around 0.5% of their brain volume per year (as opposed to around 0.2% in normal controls), especially (but not only) in the frontal and temporal areas [51]. Thus, many have begun to see schizophrenia, as well as bipolar disorder [52] to a lesser extent and even unipolar disorder [53] as diseases with a neurodegenerative component, thus sharing similarities with known neurological neurodegenerative disorders such as Alzheimer's or Parkinson's, consequently sparking a hunt for similar pathophysiological pathways and ultimately similar neuroprotective treatments. However, as of today these attempts have shown mixed (and modest) results.

Studies investigating the neuroprotective properties of Alzheimer's drugs such as the central acetylcholinesterase (AChE) inhibitors and glutamatergic agents in schizophrenia have been inconclusive. Friedman et al. [54] showed that the AChE inhibitor donepezil produced no significant improvements in cognitive measures of schizophrenic patients when compared with placebo. However, Chung et al. [55] and Schubert et al. [56] showed some beneficial cognitive effects in schizophrenic patients with the AChE inhibitors donepezil and galantamine respectively. The research regarding glutamatergic agents in schizophrenia has been disappointing as well. Despite initial favorable reports in small samples regarding effects of glutamatergic agents such as glycine and d-cycloserine, a recent multicenter study by Buchanan et al. [57] showed no therapeutic effect regarding negative or cognitive symptoms. Similarly, a recent study by Lieberman et al. [58] showed no beneficial effect with memantine (an NMDA antagonist) augmentation in residual schizophrenic patients. This seems true regarding other psychiatric disorders as well – memantine has showed mixed and inconclusive results in depression, bipolar

disorder and obsessive compulsive disorder [59]. Further discussion of the role of glutamate in neuroprotection is provided elsewhere in this book. Consequently, even though there is robust evidence regarding the neurodegenerative nature of mental disorders, there is still clearly a need for other viewpoints that can complement and expand our understanding.

1.3.2 Neurodevelopmental Mechanisms

The neurodevelopmental theory of schizophrenia posits that pathogenetic biological events or characteristics are present much earlier in life than the onset of the full blown disease [60]. This idea has a long history dating back a century when Emil Kraepelin observed that premorbid signs could be detected in early childhood [7]. Prenatal and perinatal factors supporting the neurodevelopmental viewpoint in schizophrenia are well established including factors such as season of birth [61], maternal infection [62] and obstetrical complications [63] among others. Thus, different etiological theories have been proposed over the years in an attempt to explain these findings including "excessive neuronal pruning" [64] as well as developmental alterations in brain symmetry and subsequent language function [65]. However, the debate remained largely in the theoretical realm due to the limitations of neural research. Since the 1980's there has been renewed interest in this viewpoint, and in 1987 Weinberger proposed what became later known as the "two hit model", i.e. that schizophrenia is a neurodevelopmental disease in which a fixed lesion from early life (which he could not specify) interacts with maturational events later in life to produce the full blown disease symptomatology [66, 67] (see also Chapter 12 in this book). It is only recently, again due mostly to improved imaging technologies that the neurodevelopmental model has benefited from a wealth of tangible evidence. Through fMRI studies we now know that the reduced brain symmetry in schizophrenia is not only anatomical, but functional as well affecting language related activiation [68]. Additionally, studies of childhood onset schizophrenia show early loss of grey matter in temporal and frontal regions, again supporting a neurodevelopmental theory [69]. Other findings, such as disorganized and misplaced cytoarchitecture are difficult to explain in other than neurodevelopmental terms [70−72] as well as sulcal-gyral abnormalities found in schizophrenic brains when we know that gyrification occurs in utero [73]. Finally, we cannot ignore the recent deluge of knowledge regarding genetic research (particularly involving genetic markers and endophenotypes), since genetic predisposition would be the hallmark of the neurodevelopmental hypothesis, even though it should be said that the study of the genetics of schizophrenia is frustrating as few linkage studies have offered robust findings; study samples are small, few findings have been replicated and the underlying genetic structure is appearing to be more and more complex with passing time [8]. In conclusion, the neurodevelopmental model is particularly exciting regarding the field of neuroprotection − if we can further delineate

the nature of the proposed early neuronal injuries (genetic, environmental or epigenetic) that later predispose disease manifestation, we can develop neuroprotective agents that not only treat but can possibly prevent the full blown symptomatology, thus providing psychiatry with tools that exist today only in medical realms such as oncology, infectious diseases, cardiology etc. – namely primary prevention.

1.3.3 Dysconnective Mechanisms

The theory of neural dysconnectivity, though too not strictly a new one, is today among the most active fields of research in psychiatric disorders. Buzz words like "circuitry", "wiring", "synaptic plasticity" etc., coupled with newer imaging technologies such as diffusion tension imaging (DTI), magnetoencephalography (MEG) as well as continually advancing forms of FMRI pervade conferences and research meetings rapidly supplanting previous anatomical viewpoints of focal specified functionality. As is common with emerging concepts, the very definition of the term "dysconnectivity" is not clear and can mean different things to different people. Indeed, even the spelling of the term is uncertain [74] – do we mean *dis*connectivity, i.e. *decreased* connectivity leading to disintegration of cognitive function, or *dys*connectivity, i.e. *abnormal* (as opposed to decreased) connectivity? Current research, mainly aimed at the study of abnormal NMDA receptor modulation, seems to suggest the latter [75, 76]. Another phenomenological issue regarding the dysconnectivity theory is whether it can be seen as a discrete theoretical framework for the understanding of mental disease (and more specifically but not exclusively schizophrenia) as advocated by some [77, 78] or conversely as an elaboration of the previous two theoretical frameworks described above (neurodegenerative and neurodevelopmental). Indeed, we still don't know if the documented dysconnectivity in mental diseases is an outcome (therefore degenerative) or a cause (and thus developmental). This is no minor issue since once we establish the etiological nature of the dysconnectivity we may be able to utilize this knowledge in order to provide neuroprotection in the most basic and primary sense. So what do we know about the causes of dysconnectivity? Recent findings suggest that the cause may be due to abnormal NMDA receptor modulation, leading to abnormal synaptic plasticity [78]. This can be good news regarding neuroprotective possibilities since these findings may rule out previous notions of structural anatomical defects formed in utero (which would be virtually impossible to prevent) and shifts the focus to a developmental process in which we may be able to intervene pharmacologically. This said, as stated earlier in this chapter, trials studying the neuroprotective properties of glutamatergic agents have not had the expected results as of yet, though it needs to be said that we may be looking in the wrong place – the established neural damage in existing psychopathology may to some extent be irreversible and thus unresponsive to neuroprotective treatment strategies. Thus, we need to shift our focus to prodromal or even healthy subjects at risk if we indeed want to establish the neuroprotectivity of the studied agents (1.5). So what do we actually

know about dysconnectivity today now that we have access to new and improved imaging capabilities? FMRI and DTI studies have provided us with a wealth of information we couldn't have foreseen some years ago. Recent DTI findings indicate that language tasks rely on effective interhemispheric connectivity [79] and that in schizophrenia there are widespread abnormalities affecting most white matter tracts, including interhemispheric ones [80]. Connectivity can be directly measured by fMRI as it is possible to examine activation coupling between regions and hemispheres. A recent study by Bleich-Cohen et al. [81] demonstrated a decreased time course between visual areas among schizophrenics when exposed to different facial expressions, thus indicating reduced interhemispheric connectivity. Another fMRI study by Radulescu et al. [82] showed disturbed connectivity between the amygdala and the prefrontal cortex in schizophrenics. New directions include integrating DTI and fMRI thus facilitating a view of both resting and active states, consequently expanding our understanding of dysconnectivity even further. Zhou et al. [83] did just this and demonstrated reduced functional connectivity of the bilateral hippocampus to the medial prefrontal cortex and the cingulate cortex.

In conclusion, dysconnectivity theories provide us with new insights regarding our understanding of the pathophysiological mechanisms of mental disease, replacing former notions of focal functionality and thus posing us with new clinical challenges pertaining to our formulation of neuroprotective approaches. Studies of neural connectivity are particularly challenging, as interpreting neuroimaging data in terms of connectivity is an exceedingly complex and daunting task, sometimes requiring advanced and novel mathematical models to asses and predict the various degrees of dysconnectivity found. On such model in current investigation is "Direct Causal Modeling" (DCM) [84], a method used to establish effective connectivity using Bayesian mathematical methods. By using DCM we may be able to detect, as well as predict, the functional status of particular neurotransmitter receptors causing dysconnectivity in specific subjects [85] thus facilitating a form of "tailor made" neuroprotective strategy depending on the patient's specific receptor functionality. So while DCM research is still in early stages regarding validation issues, it may yet prove to be an important step towards neuroprotective study.

1.4 Neuroprotective Candidates in Psychiatry

As stated earlier in the chapter, there are literally hundreds of pharmacological agents being studied for their neuroprotective properties. These agents, while having in common an anticipated potential to prevent, delay or reverse neuronal damage, differ widely regarding numerous parameters such as structure, proposed mechanism of action and symptom domain specificity to name just a few. This vast heterogeneity can be confusing, and since neuroprotection in psychiatry is an emerging field of research there is no organized classification as of yet to help make "heads and tails" of things. In the following section we will discuss the different classification possibilities of the various neuroprotective candidates.

1.4.1 Classification by Mechanism of Action

There are several proposed mechanisms via which different neuroprotective candidates are hypothesized to work:

- by prevention of apoptosis [86]
- by prevention of oxidative stress [87]
- by prevention of glutamate toxicity [88]
- by modulating stress sensitization [89]
- by direct neurotrophic properties [90]

(see elsewhere in this book for in depth discussion of the above mechanisms). It's tempting to classify neuroprotective agents by their mechanism of action: It's etiological, it's self explanatory and it's a neat solution. However, at second glance it may not be a practical solution for several reasons: The mechanism of action of many pharmacological agents remains elusive and a hypothesized method of action is just that; some agents can potentially work on several of the above mechanisms; and the clinical meaning of such a classification wouldn't mean much in a treatment setting where the psychiatrist needs to tailor a treatment for a specific patient. Thus, the long term validity of such a construct may prove rather fragile. Hence, we need to integrate other factors in the classification process.

1.4.2 Classification by Symptom Domain Specificity

In an attempt to bring the classification down to the clinical setting, we may classify neuroprotective candidates by the different symptom domains that they target via the delay or prevention of neuronal damage:

- Agents that primarily affect cognition
- Agents that primarily affect negative symptoms
- Agents that augment antipsychotic effect on positive symptoms
- Agents that target affective symptomatology
- Agents that lead to improving general functioning
- Agents that lead to improvement on quality of life
- Agents that lead to improving side effects of antipsychotics

(For further reading regarding symptom domains in mental disease see elsewhere in this book as well as a review written by the authors previously [8]). Though this classification has clinical merit, it lacks a scientific basis and thus is a somewhat empirical approach. Additionally, many agents affect the symptomatology of several domains while others may affect domains that we still don't know about. For instance, the neuroprotective qualities of some second generation antipsychotics (such as clozapine and olanzapine) are only recently being understood via prospective imaging studies with schizophrenic patiens [50].

1.4.3 Classification by Type of Agent

This classification is the most straightforward – we use previously accepted categorized terminologies to outline the nature of the potential neuroprotective candidates[1]:

- Antipsychotic agents (especially olanzapine and clozapine [50])
- Anti-epileptic drugs and mood stabilizers (especially valproate [91] and lithium [92])
- Antidepressants (shown to prevent apoptosis [93])
- Cognitive enhancers in Alzheimer's (AChE inhibitors such as donepezil [55] and galantamine [56])
- Glutamatergic agents (such as glycine and d-cycloserine [57])
- Neurosteroids (such as DHEA and pregnenolone [94])
- Other hormones (such as estrogen [95] and erythropoietin [EPO] [96])
- Vitamins (reviewed in chapter 17)
- Stimulants (especially modafinil [97])
- HPA modifiers (i.e. CRH mediators such as the newly studied connexin43 [98])
- Neurotrophic agents (BDNF [99], neurotrophins [100])
- Miscellaneous agents (green tea [101], omega 3 fatty acids [102], cannabinoids [103], S-adenosylmethionine [SAMe] [104], piracetam [105])

While classification by type of agent is the most comprehensive it is at the same time ultimately meaningless: It lacks both a theoretical framework and a clinical applicability. However, it is a classification that uses categorical terminology that we are familiar with and thus minimizes confusion when relaying research information.

Thus, it would seem that an attempt to integrate and condense aspects of the different classification methods detailed above could combine clinical applicability with the proposed mechanisms of action while using familiar agent type terminology as follows:

- Established psychiatric medications already proven to have neuroprotective qualities (such as lithium, olanzapine, clozapine, antidepressants etc.)
- Cognitive enhancers (such as donepezil, galantamine, memantine, glycine, D-cycloserine etc.)
- Hormones (such as DHEA, pregnenolone, EPO, estrogen etc.)
- Stress modulators (such as CRH modulators, connexin43 etc.)
- Neurotrophic agents (such as BDNF, neurotrophin-1 etc.)
- Others (such as vitamins, green tea, cannabinoids, SAMe, stimulants, omega3 fatty acids, piracetam etc.)

[1] It is not in the scope of this introductory chapter to go into detailed discussion about each and every agent – that's what the rest of this book is for. The reader is advised to refer to the other relevant chapters as well as the references provided with each agent.

However, this classification is also problematic as it lacks conventional taxonomic guidelines and is also far too simplistic considering the diversity as well as sheer magnitude of the current neuroprotective research data available. In addition, as stated above regarding the other classification methods, there is considerable overlap between the proposed categories eventually defying the primary classification purpose.

Ultimately, it appears that at this point in time it is still too early to offer a comprehensive and yet clinically meaningful classification of psychiatric neuroprotective agents considering that much of the research being conducted is still set in the laboratory setting while much needed large clinical phase III studies are few and far between. We leave the reader with a basic classification blueprint (see Fig. 1.2), albeit simplistic and overlapping, in an attempt to sum up our current knowledge in the hope that future clinical trials will pave the way for the formation of a proper etiological as well as clinical classification of neuroprotective agents in psychiatry.

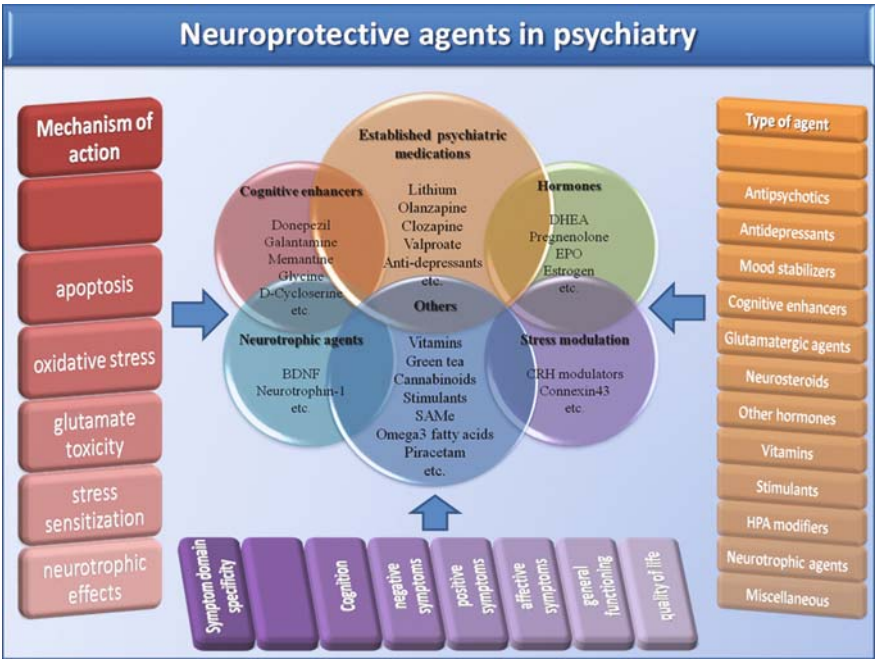

Fig. 1.2 An attempt at classification of neuroprotective agents in psychiatry. It seems that at this point in time it is still too early to attempt a meaningful, comprehensive and yet clinically applicable classification owing to the fact that we simply don't know enough regarding mechanisms of action and potential reductions in symptomatology within the various symptom domains delineated above taking into account the multitude of proposed (and mostly clinically unproven) neuroprotective agents in psychiatry. Consequently we offer the reader with a basic classification blueprint, albeit simplistic (and overlapping), thus summing up what we currently know in the hope that future clinical trials will pave the way for the formation of a proper etiological as well as clinical classification of neuroprotective agents in psychiatry

1.5 Current and Future Challenges

The study of neuroprotective agents is difficult and can be frustrating. Study samples are small due to expensive imaging machinery and the technology is not readily available to many research centers; few findings have been replicated, and the interpretation of elaborate advanced three dimensional imaging is an extraordinarily complex procedure requiring new mathematical models as stated earlier in the chapter. Specific challenges need to be addressed in the future if we hope to move forward in our goal to reach meaningful and applicable clinical results:

- *We need long-term trials*. Most trials are within the 6–12 week range and when studying neuroprotective effects of agents (specifically changes in connectivity or even reversal of neural damage) we need to follow study samples for months and even years (as was done by Cahn et al. [50]).
- *We need multicenter trials*. The logistics and expense of neuroprotective study means that inclusion criteria need to be strict (gearing towards early stages of disease) and the equipment is expensive. The result is that single center studies tend to be unreliable. The case in point is the study of glutamatergic agents in recent years. Single center studies showed promise, but when a large scale multisite trial was performed the results were less favorable [57].
- *We need to use a wider range of doses in our trials*. Most studies use low doses of neuroprotective candidates even though most of these candidates are readily available OTC and generally have only mild side effects.
- *We need to consider conducting trials using two or more neuroprotective candidates* (a form of "neuroprotective polypharmacology" if you will) utilizing the same strategies of augmentation and combination therapy that are well established in numerous psychiatric disorders.
- *We need to target young populations – before* irreversible neurodegeneration has taken place, possibly even premorbid and prodromal populations. This rationale is less extreme than it sounds – as stated above, most neuroprotective candidates aren't high potent drugs with a slew of side effects. Most are in fact fairly innocent compounds (such as green tea, omega 3 fatty acids and many others).
- *We need new and improved clinical assessment tools*. Most tools today are aimed at assessing active symptomatology, but the new challenges should be in halting the disease progression (and the ensuing neurodegeneration) *before* these occur. Some new tools have been devised (McGlashan et al. [106] developed a scale of prodromal symptoms for his landmark Prevention Through Risk Identification, Management, and Education [PRIME] study) but these are few and far between.
- *We need tools that can directly measure neuroprotective effects within the constraints of clinical psychiatric research*. Currently, most psychiatric clinical studies investigating a neuroprotective compound examine changes by psychopathological, neurocognitive, behavioral, functional and quality of life measures, but not by those that can indicate direct neuroprotective effects on the patient's brain.

- *We need to utilize new approaches* in assessing the neurodegenerative nature of mental diseases beyond the various imaging technologies used today, as promising as they may be. A new emerging approach may be that of proteomics (the study of proteins, their structures and functions). A recent study by Clark et al. [107] identified altered levels of 39 different proteins within the anterior cingulate cortex in post mortem brains of schizophrenic patients, one of these being phosphatidylethanolamine-binding protein (PEBP) – a protein implicated with increased neuronal proliferation and neuroprotection. Further study within the field of proteomics may provide exciting new vistas in the study of neurodegenerative processes in mental diseases, not only by identifying proteins and their function but also by giving us new unique genetic information by way of chromosomal loci identification.
- We need to utilize recent advancements in *nanotechnology and nanoneuroscience* in the aid of neuroprotection and neuroregeneration as detailed earlier in this chapter, specifically regarding the creation of novel imaging tools as well as in the development of new treatment options such as innovative drug delivery systems, gene therapy, tissue repair, neuroprosthesis and nanosurgery among others.
- *We need to start shifting our focus* from the largely exclusive study of schizophrenia regarding neuroprotective approaches and start studying affective and anxiety disorders in earnest in this regard. Indeed the few studies done regarding bipolar disorder, depression and to a lesser extent anxiety disorders show evidence of neurodegenerative, neurodevelopmental and dysconnective components [52, 53, 93] that only reinforce our belief that neuroprotection is a major issue regarding the whole gamut of psychopathology (in accordance with the dimensional approach described earlier in this chapter).

Several issues are still in need of academic and ethical deliberation:

- Do we prescribe the neuroprotective candidates as augmentation to the traditional medications or as a sole treatment?
- Do we dispense these drugs as preventive medicine? If we do, do we prescribe them to healthy family members of patients with severe psychopathology or only to patients with obvious premorbid signs?
- Similarly, do we offer potentially harmful neuromodulatory interventions such as DBS or VNS as neuroprotective preventive modalities as opposed to treating active (and generally severe) psychopathology?
- Do we combine our knowledge of neuroprotective factors with pharmacogenetic information such as polymorphisms in the cytochrome p450 complex to elicit tailor made neuroprotective treatments?
- How do we combine our knowledge of neurodegenerative mechanisms with metabolic and enzymatic pathways as well as proteomics and other fields in order to discover novel treatment options?

We leave these various reflections unanswered for now as we await future study and deliberation.

1.6 Psychoprotection

Neuroprotection has become a term synonymous with many fields as stated above ranging from ophthalmology to cardiology. The term is thus traditionally used for diseases that have a beginning, middle and end (i.e. CVA, Alzheimer's, Huntington's, ALS, etc.) and refers to specific mechanisms which protect neurons from apoptosis or degeneration. In psychiatry however the diseases are not as delineated on the temporal plane in as much as there isn't an ultimate endpoint (i.e. the diseases are not fatal, nor is there massive cell death) and the neuroprotection aim is not necessarily about preventing apoptosis but also about promoting connectivity, altering neurodevelopmental trends and thus delaying or even preventing full blown psychiatric symptomatology. Consequently, when compared to traditional uses of the term "neuroprotection" in other fields we find that there are more differences than similarities. Thus, we propose using a different term regarding the psychiatric field, aptly named "psychoprotection". Our reasoning for this is as follows:

(1) *Differences in pathophysiological formulation.* Primarily, some of the proposed mechanisms of neural damage are different in psychiatry. In other fields the proposed mechanism is almost invariably neurodegenerative in nature (whether be it due to infarction, autoimmune mechanisms, genetic, idiopathic or otherwise) while in psychiatric disease we are witnessing a shift from the strict neurodegenerative viewpoint towards a model of dysconnectivity and neurodevelopmental pathology as described above. Thus, when speaking of neuroprotection in the psychiatric domain, we are less about "saving" cells from dying and more about restoring connectivity or preventing its loss. Consequently, it would make sense that the candidates for neuroprotection in psychiatry would be different from those used in other fields (see below). We think that this difference is of enough magnitude to warrant a different mode of thinking – and a different term.
(2) *Different clinical presentation.* As stated above, traditional neurodegenerative diseases are well delineated in time and have an obvious endpoint while psychiatric diseases do not, supporting the viewpoint that a different type of neuroprotection is needed, thus justifying a discrete terminology.
(3) *Different neuroprotective goals*. Simply put, the neuroprotective goals in psychiatry diverge from those in other medical fields, chiefly regarding the restoration of connectivity versus a primary emphasis on apoptosis.
(4) *Different pharmacological mechanisms*. In light of the different etiological framework and different treatment goals, we have also begun to look for neuroprotective agents with differing modes of action, leading to:
(5) *Different neuroprotective candidates.* As opposed to initial studies, we are now witnessing a shift from using the known agents of neurodegenerative neurological disorders towards agents unique for psychiatric neuroprotection (e.g. atypical antipsychotics, antidepressants, neurosteroids, etc.).

(6) *A need for a more focused term*. The traditional term has become too broad over the years, including just about any and every form of neuronal damage prevention from all medical fields, and when a term encompasses everything it ultimately says little. Thus, we are in need of a distinct term in psychiatry.

In conclusion, we think that by using the term "psychoprotection" we not only relay our intent more clearly but also make an important statement regarding our differing etiological, methodological and pharmacological viewpoints as compared to other medical fields. That said, we are aware that any new term is not without its drawbacks. Firstly, the very reasoning for our introduction of the term can also be a source of contention by seemingly inferring that psychiatry is a distinct discipline rather than part of the other neurosciences. However, that is not our intention. We do see psychoprotection as a direct continuation of neuroprotection research, firmly rooted within the neurosciences, only that it is distinct enough to warrant a different terminology for the various reasons detailed above. Secondly, the introduction of a new term can seem imposing, "obliging" researchers to change the terminology they have been using for over two decades. Again, that is not our intention, rather we mean to enrich the research vocabulary (not change it) in order to enable a more specific common language between psychiatric researchers.

1.7 Concluding Remarks

In this chapter we reviewed the various trends of psychiatric neuroprotective research detailing its origins stemming from various medical fields while steadily growing into a distinctive realm of its own, differentiating itself from other disciplines regarding pathophysiological, pharmacological and clinical aspects as well as having some unique treatment goals. Future challenges include a need for large (and long) multisite clinical trials focusing on early stages of disease while searching for new and improved technological imaging capabilities as well as new assessment tools targeted at early prodromal states. There is also a need to develop new research strategies and approaches (such as proteomics) and make use of new technologies such as nanoneuroscience and refined neuromodulatory techniques in our continuing effort to understand neuroprotective mechanisms. Use of multiple treatments that target different cell death cascades or single agents that moderate multiple cell death pathways are likely to lead to more effective neuroprotective results in the future. As in any emerging field of research, there is a need for classification of psychiatric neuroprotective agents for future clinical applicability as well as attaining a conceptual understanding. However, though we provided a basic classification blueprint, it is of our opinion that it is not yet possible to formulate a comprehensive classification at this point due to too much still being unknown. Ethical issues regarding future neuroprotective treatments focus primarily on the possibilities of preventive treatment. Finally, taking into consideration all the above, we proposed using a distinct term for psychiatric neuroprotection, namely "psychoprotection".

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Chapter 2
Towards a New Paradigm in Neuroprotection and Neuroplasticity

Dafin F. Muresanu

Abstract Neurologists are confronted with more and more new information regarding the intimate processes taking place in both the normal and pathological brain. This chapter briefly reviews some of the mechanisms involved in the pathogenesis of neurological diseases and the ways to bind them with therapy principles. It is becoming increasingly clear that using neuroprotective molecules with only one mechanism of action is a utopian idea. It is still not easy to find the correct therapeutic approach for neurological disorders, especially because we do not deeply understand all endogenous basic biological processes, the complete nature of pathophysiological processes and the links between these two categories. In particular, concepts like neurotrophicity, neuroprotection, neuroplasticity, neurogenesis and anoikis, as well as their clinical utility, may be daunting. Even more specifically, there are many points in the pathophysiological cascade where pharmacological intervention might be beneficial. Comprehensive research of multimodal drugs and combination therapy, followed by appropriate clinical use, should be encouraged. Although the majority of scientific data available in the present chapter are referring mainly to vascular and neurodegenerative disorders, they can be easily extrapolated to other different pathologies.

Abbreviations

ATP	Adenosine-5′-triphosphate
DNA	Deoxyribonucleic acid
ACD	active cell death
PCD	passive cell death
EDA	endogenous defense activity
PI3K	(phosphoinositide 3-kinase) Akt
Ras–ERK1/2	extra cellular-signal-regulated kinase 1/2
CREB	CRE (cAMP-response element)-binding protein

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_2,

NMDA	N-methyl-D-aspartic acid
NMDAR	NMDA receptor
TRPM	transient receptor potential melastatin channel
CaMKIV	Calcium/calmodulin-dependent protein kinase type IV
ERK1/2	Extracellular Signal-Regulated Kinases 1 and 2
PI3K	Phosphoinositide 3-kinases
nNOS	neuronal nitric oxide synthase
TRPM	transient receptor potential melastatin channel
BBB	blood–brain barrier
CNS	central nervous system

2.1 Cell Death Pathways

Two main manners of cell death, a passive and an active one, have been described. *Necrosis* is a process caused by almost any pathological insult (including physical, chemical and biological agents). The sequence is the same every time: osmolysis caused by the cellular edema leads to passive death of the damaged cell. Necrosis affects not only the dying cell itself but also one of the secondary effects of necrosis is inflammation triggered by the release of the cell's contents, which is accompanied by cytokine discharge. The other mechanism of cell death is *apoptosis*, which, in contrast to necrosis, requires ATP. Derived from an ancient Greek word, the term "apoptosis" designates in modern terminology a form of cell death with specific morphological characteristics used by the organism to control the number and quality of cells, in order to keep organs functioning. The nervous system is one of the best examples where developmental cell death shapes final structure and function. Neurotrophic factors, apart from giving support to neurons throughout their lifetime, also regulate this kind of cell death, thus forming an important protection mechanism. Events similar to this form of cell death (including cellular signaling) were noticed not only in neurodegenerative diseases but also in acute processes (e.g., trauma or stroke) in which neurons degenerate and die too. There are clear differences between the two processes, the most evident being the time span involved: apoptosis-like cell death takes longer, whereas necrosis is usually faster. Sometimes, however, dying cells display characteristics of both necrosis and apoptosis. In neurons, membrane rupture and DNA fragmentation, indicating necrosis and apoptosis, respectively, have been described to affect the same cell sometimes.

It is clear that the current vocabulary of cell death is quite inadequate. Sloviter [1] states that a term like active cell death (ACD) was suggested to designate cell death involving activation of intracellular mechanisms, regardless of its morphology; in contrast, passive cell death (PCD) was to replace the "old-fashioned" term necrosis. The relationship between pathophysiological mechanisms and types of cell death can be briefly summarized as follows: excitotoxicity can lead to both necrosis and apoptosis-like death. Inflammation can also result in necrosis and apoptosis-like death, whereas protein misfolding induces apoptosis-like death.

2.2 Basic Biological Endogenous Processes and Endogenous Defense Activity (EDA)

Neurotrophicity, neuroprotection, neuroplasticity and neurogenesis are the most important neurobiological processes that act together under genetic control to generate endogenous defense activity (EDA), which attempts to counteract pathophysiological processes.

2.2.1 Definitions

(1) Neurotrophicity denotes a natural biological process by which the continuous effort of the cell maintains correct DNA expression, thus maintaining a normal phenotype.
(2) Neuroprotection represents the sum of all mechanisms directed against harmful factors.
(3) Neuroplasticity is the permanent adaptation to new functional horizons and responsibilities. Kalivas and Volkow [2] and Muresanu [3] state that the concept describes the brain's ability to change *already-existing structures* in response to environmental stimuli, such as learning, experiencing something new or injury.
(4) Neurogenesis is the process by which new nervous tissue cells (such as neurons, astrocytes and oligodendrocytes) are created from stem cells. In a strict sense, neurogenesis is defined as the creation of new neurons.

These fundamental biological processes lack absolute boundaries; they overlap and share common mechanisms.

When studying neuroprotection, we have to distinguish between two different aspects: the so-called absolute and relative mechanisms. The **absolute** aspect refers to all mechanisms that determine the activation of DNA expression followed by protein synthesis induction. The **relative** aspect refers to all mechanisms that finally determine neuroprotective activities with preponderant expression in the membrane, cytosol and organelles. Muresanu [4] states that the absolute mechanisms are predominantly controlled by neurotrophic factors and neurotrophic-like molecules, whereas the relative mechanisms mainly utilize ion channel blockers, agonists and antagonists of certain receptors, scavenger antioxidants, chelators of certain metals and many others.

All of these biological mechanisms can be naturally or pharmacologically activated. If we want to fight successfully against pathophysiological processes, we have to pharmacologically enhance the effect of EDA. Sometimes basic biological processes of EDAs share common mechanisms with pathophysiological processes. For example, excitotoxicity and neurotrophicity together with neuroplasticity have NMDAR activity as their common important driver. Inflammation is an important contributor to neuroregeneration, stimulating neuroplasticity via trophic factors.

Regulation disturbances in each of the four major players of EDA are themselves causes of some pathological conditions. A deficit of neurotrophicity will always increase the susceptibility for a lesion. So far, no pathologies have been discovered that arise because of an excess of neurotrophicity or neuroprotection. For neuroplasticity, both up-regulation and down-regulation generate pathologies. Down-regulation generates a deficit of recovery, whereas up-regulation generates hundreds of neuropathological patterns of plasticities like in: neuropathic pain, multiple sclerosis, movement disorders, tinnitus, and more. With regard to neurogenesis, both up-regulation and down-regulation generate pathological conditions (e.g., down-regulation in Alzheimer's disease). Up-regulation of oligodendrogenesis and astrogenesis beyond normal regeneration is responsible for neuroproliferative disorders.

2.3 Neuroprotection

Different pathophysiological mechanisms are triggered by different etiologic agents or biological events many neurological disorders with different evolutions (acute or chronic) being generated. Blocking these pathogenic pathways is the aim of neuroprotective treatments.

These pathological cascades contain a limited number of pathophysiological processes (see Table 2.1) and have many similarities to various CNS diseases. Thus, in stroke, there are excitotoxicity, oxidative stress, inflammation and apoptotic-like processes. In neurodegenerative disorders, we have excitotoxicity, inflammation, apoptotic-like processes and protein misfolding. Excitotoxicity, inflammation and apoptosis-like processes represent the backbone common to most neuropathologies. Controlling these processes is the key to efficient neuroprotection in all neurological disorders. *The classic strategy in neuroprotection is to suppress pathophysiological processes*. For this, we are using drugs with well defined single mechanism acting against one pathophysiological process.

Table 2.1 Endogenous fundamental biological processes and pathophysiological mechanisms

Fundamental biological processes	Pathophysiological mechanisms
Neurotrophicity	excitotoxicity free radicals
Neuroprotection	metabolic dysfunction inflammation
Neuroplasticity	apoptosis-like processes protein misfolding
Neurogenesis ↓	↓
Endogenous Defense Activity (EDA)	Genetic characteristics of the individual

The rationality of this strategy is based on the assumption that every pathophysiological process is deleterious. The clinical results in neuroprotection, based on this paradigm, and using this single mechanism molecule, are very poor. As we can see further on, the main pathophysiological processes, have a *dual* character.

Hawkins and Davis [5] describe a relatively new concept on the horizon of neurophysiology known as the *neurovascular unit* (comprised of endothelial cells and brain cells and matrix that function together using biochemical signaling). This unit exists everywhere in the brain, in both grey and white matter. Its malfunction can explain the occurrence and evolution of several brain diseases, such as stroke, vascular dementia, migraine, trauma, all neurodegenerative disorders and even normal aging. Because the neurovascular unit is unique in the human body, the way cells die here is also unique. Frisch and Screaton [6] state that the lack of support coming from any component of the unit cell or matrix causes a particular apoptosis-like phenomenon known as anoikis.

2.4 The Dual Character of Pathophysiological Processes

2.4.1 Excitotoxicity

Excitotoxicity is the pathological process by which nerve cells are damaged by excess glutamate and similar substances. NMDA receptor activation is one of the key features of excitotoxicity. Wu et al. [7] state that continuous activity of NMDAR is crucial for cell survival, and this is achieved through regulation of neurotrophicity and neuroplasticity via calcium-controlled proteolytic systems (e.g., calpain system). Physiological patterns of synaptic NMDAR activity actually promote neuronal survival by controlling the minimum calcium influx into the neuron. These very small quantities of calcium activate "high affinity calcium molecules" like μ calpain. These have the physiological roles of conducting proteolytic activity, an important factor in neurotrophicity and neuroplasticity. Hutter et al. [8] state that this process is highly regulated by neurotrophic factors.

Hetman and Kharebava [9] show that key pro-survival pathways involving NMDA receptors are essential for neurotrophicity and neuroplasticity. Brunet el al. [10] proved that the PI3K (phosphoinositide 3-kinase)–Akt cascade is strongly activated by NMDARs in many but not all neuronal types. Hardingham et al. [11] affirm that synaptic NMDAR signaling also activates the Ras–ERK1/2 (extra cellular-signal-regulated kinase 1/2) cascade, with pro-survival consequences including CREB [CRE (cAMP-response element)-binding protein] activation, BAD inactivation and antagonism of GSK3 b – induced apoptosis. Hardingham et al. [12] demonstrated that synaptic NMDAR-dependent calcium transients trigger a number of transcriptional changes that mediate long-lasting neuroprotection (via CRE-dependent gene expression).

When NMDARs are over-activated by glutamate under pathological circumstances (such as stroke or trauma), large quantities of calcium enter the cells and

activate “low affinity calcium molecules” like m-calpain. These have non-selective and uncontrolled proteolytic activities that lead to cell death. There are several fundamental mechanisms implicated in NMDAR-dependent cell death [13–15]. Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger by the Ca^{2+}-dependent protease calpain leads to necrosis.

Mitochondrial dysfunction brought about by excessive Ca^{2+} uptake through the uniporter also leads to apoptosis-like processes. Finally, overactivation of the Ca^{2+}-dependent nNOS (neuronal nitric oxide synthase) by NMDAR activity has toxic downstream effects: p38 mitogen-activated protein kinase signaling, mitochondrial dysfunction and TRPM (transient receptor potential melastatin channel) activation leading to apoptosis-like processes.

Next, we will examine the drivers for pro-survival signals (neurotrophicity, neuroplasticity) and pro-death signals (excitotoxicity).

The first factor is the magnitude of activation of NMDAR (intensity or duration). Low levels are protective. Ca^{2+}effectors of survival have considerably lower requirements for Ca^{2+} than death effectors. Thus, the $[Ca^{2+}]$ threshold for activating pro-survival signaling by PI3K, ERK1/2 and CaMKIV–CREB must be lower than that which triggers toxic levels of calpain activation, mitochondrial uptake or NO production. Soriano et al. [16] highlighted that certain potential death effectors, such as m-calpain and the mitochondrial uniporter, have low Ca^{2+} affinity. *The second important factor* is NMDA receptor location. Extrasynaptic NMDAR activity promotes inactivation of CREB (by dephosphorylation) and early excitotoxic events (e.g., mitochondrial depolarization) concomitant with inactivation of the ERK1/2 pathway, causing necrosis and apoptosis-like processes. On the other hand, synaptic NMDAR activity promotes activation of CREB and activation of the ERK1/2 pathway. It does not disturb mitochondrial function, but it offers overall neuroprotective activity and promotes neurotrophicity and neuroplasticity.

2.4.2 Inflammation

The pathological role of inflammation has been recognized in almost all neurological conditions. This well-orchestrated process situated on the borderline between physiology and pathology tends to become highly destructive when prolonged or deregulated. However, inflammatory cells and mediators may also have beneficial functions and contribute to tissue repair processes.

There is evidence demonstrating that inflammation plays a positive role in neuroprotection and neuroplasticity [17, 18]. The major players in this process are neurotrophic factors. Masson et al. [19] state that the neurotrophic factors produced by activated immune cells seem to participate in neuronal protection as well as neuroplasticity. Neurotrophic factors either bind directly to their receptors or act by modulating the local immune response. Arnett et al. [20] and Marchetti et al. [21] state that even a very potent pro-inflammatory molecule, like TNF-α, has neuroprotective and neurotrophic effects (via trophic factors) when activating R2.

The very low permeability of the blood–brain barrier (BBB) extends to immune cells and molecules. Usually, resident cells in the central nervous system (CNS) (particularly astrocytes and microglia) are able to regulate immune reactivity within the CNS. Other alien immune entities enter the CNS only through highly regulated processes mediated by adhesion molecules, chemokines, cytokines and matrix metalloproteinases [22–24].

2.4.3 Apoptosis and Apoptosis-Like Processes

Apoptosis is a positive process maintaining the number and quality of cells. If a cell has a DNA lesion, it activates the p53 gene. Then, the cell will either halt in the G1 phase of its cycle (by bcl-2 activation) and repair its DNA and recommence division. Alternatively, altered DNA repair may cause the activation of "bax", leading to apoptotic death. If apoptosis is not effective, then a malignant clone formation will occur.

From the above highlights regarding the links between pathophysiological processes and endogenous defense activity, we can draw the following conclusions:

1. *NMDAR activity* might play a positive role during physiological activation generating neuroprotection and neuroplasticity, or a deleterious role, during overactivation, generating pathological processes (stroke, trauma, neurodegenerative disorders) by facilitating excitotoxicity.
2. *Inflammation* is generally a negative phenomenon, but it can positively influence neuroprotection and neuroplasticity via neurotrophic factors.
3. *Apoptosis* is a positive process, whereas *apoptotic-like processes* are always negative. Apoptotic-like processes have to be endogenously and therapeutically controlled.

Therefore, the best approach for clinical neuroprotection is pleiotropic drug administration, which would modulate (not suppress) pathological processes. Therefore these drugs down regulate excitotoxicity induced via extrasynaptic NMDARs, decrease the "bad" effects of inflammation, increase the "good" effects of inflammation and prevent apoptotic-like processes.

The search for clinical neuroprotection is complicated for several reasons. First, we are using synthetic translational molecules (which are not used by EDA) with only one mechanism of action against complex cascades.

Because they are blocking as well negative and positive biological mechanisms of pathophysiological processes, they are suppressive molecules that leaving very few opportunities for create pharmacological neuroprotection. The design of clinical neuroprotection studies must be more rigorous. Grotta [25] shows that despite a lack of consistent clinical results in neuroprotection, using these synthetic molecules with single mechanism, there are several trials that were successful (including erythropoietin, cerebrolysin, cyticholine) (Table 2.2).

Table 2.2 Past and current cytoprotective clinical trials

Drugs	Phase	Latest extent of time window (h)	Adeq. power ∞	Adeq dose	Dose-limiting AEs	Homogen patient population	Linked to TPA	Biologic imaging marker	Results
Calcium Antagonists									
Nimodipine	3	6–48	+		Hypotension				Neutral
Nicardipine	2	12			Hypotension				Neutral
Glutamate Antagonists									
Selfotel	3	6–12	+	No	Neuropsych				Negative
Dextrorphan	2	48		Yes	Neuropsych				Neutral
Cerestat	3	6–24	+	Yes	Hypertension				Negative
AR-R15696	2	12		Yes	Neuropsych				Neutral
Magnesium	3*	2–12	+	Yes	No	+		+	?
AMPA Antagonists									
YM872	2b	3–6	+	?	?	+	+	+	Neutral
ZK200775	2	24		?	Sedation			+	Negative
Indirect Glutamate Modulators									
Eliprodil	3	?	?	?	?	?	?	?	Negative
Gavestinel	3	6	+†	Yes	No	+			Neutral
Sipatrigine	2	12		?	Neuropsych	+			Negative
Fosphenytoin	2/3	4	+	?	No				Neutral
BMSS-204352	3	6	+	?	No	+		+	Neutral
Lifarizin	2	?		?	Hypotension				Neutral
Lubeluzole	3	4–8	+†	No	Cardiac	+	+		Neutral
Other Neurotrans Modulators									
Trazadone	2	?	?	?	?	?	?	?	Neutral
Repinotan	3*	6	+†	Yes	?	+			?
ONO-2506	2/3*	6	+	?	?	+			?

Table 2.2 (continued)

Drugs	Phase	Latest extent of time window (h)	Adeq. power ∞	Adeq dose	Dose-limiting AEs	Homogen patient population	Linked to TPA	Biologic imaging marker	Results
Opioid Antagonists									
Naloxone	2	8–60		?	No				Neutral
Nalmefene	3	6	+†	?	No	+			Neutral
GABA Agonist									
Clonethiazole	3	12	+†	Yes	Sedation	+			Neutral
Diazepam	3*	12	+	?	?				?
Free Radical Scavengers									
Tirilazad	3	6	+	?	No			+	Negative
Ebselen	3*	48	+	?	?	+			?
NXY-059	2b/3*	6	+†	?	?	+			Negative
Anti-inflammatory Agents									
Enlimomab	3	6	+	Yes	Fever	+			Negative
LeukArrest	3	12	?	?	?				Neutral
FK-506	2*	12		?	?	+			?
Steroids	2	48		?	Infection				Negative
Membrane Stabilizers/Trophic Factor									
GM1	3	72	+	?	No				Neutral
Cerebrolysin	2	12–24		?	No				Positive Trend
Citicoline	3	24	+†	?	No	+		+	Positive *Post hoc*
EPO	2a*								
bFGF	2/3	6	+	?	Hypotension	+			Negative
Hypothermia	2*	5–24		Yes	Pneumonia, arrhythmias, hypotension	+		+	?

Table 2.2 (continued)

Drugs	Phase	Latest extent of time window (h)	Adeq. power ∞	Adeq dose	Dose-limiting AEs	Homogen patient population	Linked to TPA	Biologic imaging marker	Results
Caffeinol	2*	4–6		Yes	No	+			?
Oxygen Delivery									
DCLHb	2	18		?	HTN				Negative
HBO	2/3*	24		?	?				Neutral

Only relevant to phase 2b or 3 efficacy trials
*Currently enrolling
†Not adequately powered for TPA subgroup
+ Positive, *HTN* Hypertension, *AE* Adverse effects, *Neuropsych* Neuropsychiatric side effects

All these successful molecules have a common pattern of action: they have *pleiotropic mechanisms* and are therefore able to control multiple pathophysiological processes in a biological cascade. From all these molecules only neurotrophic factors have the capacity to *modulate* (not suppress) pathophysiological processes, in order to maintain their positive effect.

The third strong point of neurotrophic factors is that there are no other molecules that simultaneously promote neuroprotection and retain the ability to switch to neuroplasticity except neurotrophic factors (Fig. 2.1). The capacity to switch to plasticity is crucial when considering the clinical effect on the patient. Drugs with this ability are called multimodal drugs. A multimodal drug is a drug that is able to regulate at the same time two or more basic EDA biological processes.

A multimodal drug available for clinical use, Cerebrolysin, is based on active fragments of different neurotrophic factors. Taking stroke as an example (Fig. 2.1), we see that glutamate is deleterious in the first minutes of exposure and for several hours after the event.

After 48–72 h, however, glutamate becomes the key player in controlling neurorecovery. Therefore, multimodal drugs must be able to control the switch from neuroprotection to neuroplasticity within the continuous EDA process.

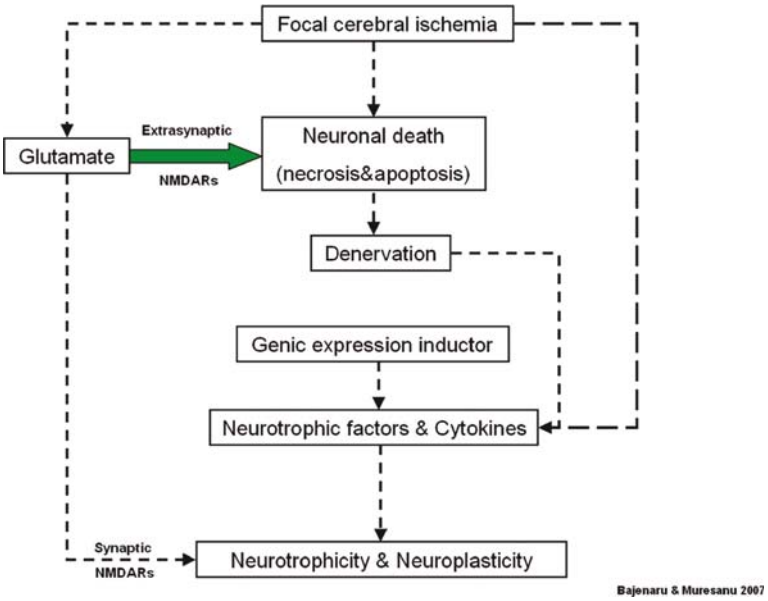

Fig. 2.1 The dual roles performed by glutamate makes it a potent and indispensable factor for neurotrophicity and neuroplasticity processes

2.5 Neuroplasticity and Neurorecovery in Acute and Chronic Neurological Disorders

Endogenous defense activity (EDA) of the nervous system is a polychronic continuous process that simultaneously performs and integrates neurobiological processes of neurotrophicity, neuroprotection, neuroplasticity and neurogenesis. *Neuroregeneration* is the morphological outcome of the interactions between these basic neurobiological processes developed in a particular biological individual context. *Neurorecovery* is the positive outcome producing clinically relevant results, with immediate functional and late structural effects. Immediate and late effects generate two types of changes: restitution and substitution. *Restitution* is an intrinsic process involving biochemically and genetically induced events, such as reduction of edema, absorption of heme and restoration of axonal transport and ionic currents. Barnes et al. [26] state that *substitution* depends on external stimuli like practice, which drives activity-dependent plasticity through learning. *Compensation* is targeted to improve the mismatch between patients' impaired skills and the demands of the patient or environment.

2.6 Neurorecovery – Biological Background

All basic biological processes can be naturally endogenously or exogenously activated. Altered regulation of any of the four components of EDA may generate pathological conditions. Read et al. [27] state that altered neuroplasticity in the form of both up- and down-regulation generates pathologies. Naturally, these processes are regulated via key endogenous players like neurotrophic factors and neurotrophin-like molecules. In order to successfully compete with pathological processes and support neurorecovery, EDA might be exogenously enhanced by pharmacological intervention, physical means, electromagnetic stimulation, psychological support, environmental stimulation, stem cell transplantation or any demonstrated combinations of these factors capable of improving a patient's condition.

From the pharmacological perspective, it is clear that targeting molecules capable of mimicking the structure and function of endogenous molecules with pleiotropic and multimodal activities is the best approach in neuroprotection and neuroplasticity; these two processes occur in a particular sequence in the continuous process of EDA.

The brain uses the same neurotrophic factors for both neuroprotection and neuroplasticity, although in different combinations. They are activated during altered gene expression induced by lesioning. Danton and Dietrich [28] and Ginsberg [29] state that brain ischemia regulates the expression of more genes than any other condition. However, many activated genes are not translated into proteins after injury. Endogenous neuroprotection is maximally effective in the ischemic area 72 h following an insult. Any further positive clinical outcome is driven by processes of neuroplasticity and neurogenesis. A brief overview of post-lesion regulation is presented in Table 2.3.

Table 2.3 Post-lesional regulation (J Neuropathol Exp Neurol, Vol 61, October, 2002)

Early regulation ≤ 3 days post-lesion			Late regulation > 3 days post-lesion		
Molecule	Ipsi-lesional	Contra-lesional	Ipsi-lesional	Contra-lesional	Involvement in experience – induced plasticity
Immediate early gene/transcription factor					
c-Fos	↑	–	↑	↑	Environmental enrichment
c-Jun	↑	–	↑	–	Learning
JunB	↑	–	–	–	Environmental enrichment
NGFI-A	↑	–	↑	–	Environmental enrichment
NGFI-B	↑	–	–	↑	Learning
NGFI-C	↑	–	–	–	Used-induced
Krox-20	↑	–	–	–	–
Arc	↑	–	–	↑	Environmental enrichment
CREB (increased phosphorylation	↑	–	↑ in c. callosum	↑	Used-induced
NF-kB	Controversial results				Learning
Kinase network molecules					
MAP kinase	↑	–	–	↑	Learning
CaM kinase	↓	–	↑	–	Physical exercise
Neurotransmitter receptors					
GluR1	↓	–	↓	–	Environmental enrichment
GluR2	↓	–	↓	–	–
GluR3	↓	–	↓	–	–

Table 2.3 (continued)

Early regulation ≤ 3 days post-lesion			Late regulation > 3 days post-lesion		
Molecule	Ipsi-lesional	Contra-lesional	Ipsi-lesional	Contra-lesional	Involvement in experience – induced plasticity
NMDAR (receptor binding)	–	↑	↑	↑	Environmental enrichment
mGluR3	↓	–	–	–	Learning
mGluR2	↓	–	↓	↓	Learning
GABAR (receptor binding)	↓	–	↓	↓	Learning
Growth factors/receptors					
NGF	↑	–	↑	↑	Environmental enrichment
BDNF	↑	↑	–	–	Environmental enrichment
NT3	↓	–	↓	–	Environmental enrichment
BFGF	↑	–	↑	↑	Learning/physical exercise
GDNF	↑	–	–	–	Environmental enrichment
PDGF	↑	–	↑	–	–
IGF	–	–	↑	↑	–
TGF-β1	↑	–	↑	–	–
Trk B	↑	↑	–	–	Learning
Neuropilin -1, -2	↑	–	↑	–	–
TNF-α	↑	↑	–	–	–
APP	–	–	↑	–	Learning

Table 2.3 (continued)

Early regulation ≤ 3 days post-lesion			Late regulation > 3 days post-lesion		
Molecule	Ipsi-lesional	Contra-lesional	Ipsi-lesional	Contra-lesional	Involvement in experience – induced plasticity
Growth – associated/cytoskeletal molecules					
GAP – 43	↑	–	↑	↑	Learning
SCG – 10	–	↑	–	↑	–
α – tubulin	–	–	↑	–	Learning
MAP – 2	↑	–	↑	–	Learning
apoE	↓	–	↑	–	Learning
apoD	↑	–	↑	↑	–
Synapse-related molecules					
Synaptophysin	–	–	↑	↑	Environmental enrichment
synapsin-I	↑	–	↑	–	Learning
SNAP-25	–	–	↑	–	–
Adhesion molecules					
PSA-NCAM	↑	–	↑	–	Learning
L1	↓	–	↑	–	Learning
F3	↓	–	↓ up to 1 week then ↑	↑	–
Tenascin-C	↑	–	↑	–	–

– = no report, ↑ = upregulation, ↓ = downregulation

Identification of when particular changes occur (early or late) and interpretation of their influence is often difficult. *Early changes* seem to reflect neuroprotective efforts induced by cell damage and have little relevance to recovery potential. *Late changes* generally suggest recovery processes, but simultaneous overlapping events add complexity to the identification of individual changes. With regard to gene expression, patterns of gene expression changes – and not simply individual genes – need to be considered. Ginsberg [29] states that it is important to acknowledge the remarkable flexibility of endogenous programs. For simplicity, 72 h is considered as a distinguishing time point: the first 72 h after insult represents the early time window, whereas anything after 72 h represents the late time window.

2.7 The Need for Better Management of Early and Late Patterns – A New Paradigm in Pharmacological Support for Neurorecovery

Neurotrophic factors are important molecules in the recovery process. More than 100 neurotrophic factor-related molecules are organized into different families. Neurotrophic factors act on specific receptors. Usually, they target two or more receptors.

Some receptors are high-affinity receptors, whereas others are low-affinity receptors. Each receptor initiates an intracellular cascade that finally activates a transcription factor. Transcription factors are DNA-binding proteins that control gene expression. Millán and Arenillas [30] state that cellular stimulation induces biochemical modifications of these molecules that allow nuclear entry, binding to essential co-factors and other processes.

With the exception of neurotrophic factors, no synthetic or biological drugs are able to provide simultaneous pharmacological neuroprotection and neuroplasticity.

2.8 Conclusions and Future Directions

This chapter briefly reviews some of the mechanisms involved in the pathogenesis of neurological diseases and the ways to bind them with therapy principles. Because it is becoming increasingly clear that using neuroprotective molecules with only one mechanism of action is a utopian idea. It is still not easy to find the correct therapeutic approach for neurological disorders, especially because we do not deeply understand all endogenous basic biological processes, the complete nature of pathophysiological processes and the links between these two categories. In particular, concepts like neurotrophicity, neuroprotection, neuroplasticity, neurogenesis and anoikis, as well as their clinical utility, may be daunting. For a better understanding of the complex interaction between plasticity and recovery, we must acknowledge several findings:

(1) Resulting changes in post-injury behavior are highly individualistic. Xerri et al. [31] states that early experience and possibly genetic factors play a role in the extent of cortical reorganization and recovery.
(2) Recent studies using fMRI and PET scanning have shown that functional compensation is due to extensive reorganization of activity in the damaged brain.
(3) The "serial lesion" effect is an eloquent example for animals and humans. In this circumstance, lesions are inflected in different stages (e.g. one month after a left frontal cortex extirpation the homologous right cortex ablation will follow; in this scenario both animals and humans show better and robust post injury sparing of function compare to counterparts with the same lesion inflicted in one stage).
(4) Slow growing lesions, even if they are large, often have minimum clinically visibility, sometimes being even silent. This is never the case in acute lesions like stroke or acute traumatic brain injury. Kolb et al. [32] and Beaulieu [33] concluded that the significant differences in functional and clinical outcome between acute and chronic lesions raised the possibility that functional reorganization is not restricted in a certain type of cortex. In reality we have an equipotentiality of vicarious function in CNS.
(5) Kolb et al. [32] state that it is important to apply the appropriate rehabilitation strategy at the appropriate time for a particular lesion.

Large clinical trials with a better design are needed to confirm the clinical benefit of this new approach in modulation of EDA.

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Chapter 3
What Protects Patients with Schizophrenia from Developing Alzheimer Pathology?

Hans-Gert Bernstein, Theresia S. Ernst, Uwe Lendeckel, Henrik Dobrowolny, Johann Steiner, and Bernhard Bogerts

Abstract Disturbed insulin signal transduction and increased glycogen synthase kinase-3beta expression/activity are core features of Alzheimer's disease (AD). Moreover, compromised insulin signalling (including reduced insulin-degrading enzyme activity) is blamed to significantly contribute to the development of typical hallmarks of AD: amyloid deposits and hyperphosphorylation of tau protein. Interestingly, patients with schizophrenia often suffer from the metabolic syndrome. Treatment with typical and atypical neuroleptics either initiates or further increases the metabolic problems of many schizophrenics. In post-mortem brains of schizophrenics considerable functional decrease of insulin receptors, disruption of the Akt-dependent insulin signalling system, and increased glycogen synthase kinase-3beta expression/activity have been found. The striking similarities of pathologic changes in brain insulin metabolism of schizophrenics and AD patients should lead to an increased incidence of AD in aged schizophrenics. Remarkably, this is not the case. We try to identify possible protective mechanisms that prevent AD pathology in patients with schizophrenia.

Abbreviations

Abeta	amyloid beta protein
AD	Alzheimer's disease
AGE	advanced glycation end products
APP	amyloid precursor protein
ATP	Adenosine-5′-triphosphate
BACE1	Beta-site amyloid precursor protein cleaving enzyme 1
Cat K	cathepsin K CSF, cerebrospinal fluid
CNS	central nervous system
DNA	Deoxyribonucleic acid
GSK-3alpha/beta	glycogen synthase kinase-3alpha/beta
IDE	insulin-degrading enzyme

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_3,

IGF-1	Insulin-like growth factor-1
IR	Insulin receptors
IRS	insulin receptor substrate
MAPK	mitogen-activated protein kinase
NFT	neurofibrillary tangles
PDK	phosphatidylinositol 3-kinase
PI3K	phosphoinositide 3-kinase
SP	senile plaques
sRAGE	soluble receptor for advances glycation end products
Wnt	Wingless (gene) and Int.
Amino acids: Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Glu	glutamine
Gly	glycine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
Phe	phenylalanine
Ser	serine
Try	tryptophan
Val	valine

3.1 Introductory Remarks

New insights into mechanisms protecting our brain from harmful influences are always very welcome. Understanding why elderly patients with schizophrenia do not show an increased incidence of Alzheimer-like neuropathology, is a potential chance to get answers that help to better understand some basic mechanisms leading to AD pathology. Schizophrenia may in many respects be regarded a disease of accelerated aging. Notably, patients with schizophrenia often seriously suffer from the metabolic syndrome, which is characterized by visceral obesity, type 2 diabetes, elevated lipid levels, hypertension, and decreased sensitivity to insulin. Overweight is a major reason for non-compliance of patients. Interestingly, drug-naïve schizophrenics may already show symptoms of the metabolic syndrome. Treatment with typical and atypical neuroleptics either initiates or further increases the metabolic problems of many schizophrenics. Indicative of such metabolic dysregulation, in post-mortem brains of schizophrenics considerable functional decrease of insulin receptors, disruption of the Akt-dependent insulin signalling system, and increased glycogen synthase kinase (GSK)-3beta expression/activity have

been found. Remarkably, the disturbed insulin signal transduction and increased GSK-3beta expression/activity are also core features of Alzheimer's disease (AD). Moreover, compromised insulin signalling (including reduced insulin-degrading enzyme activity) is blamed to significantly contribute to the development of typical hallmarks of AD: amyloid deposits and hyperphosphorylation of tau protein. From a logical point of view the striking similarities of pathologic changes in brain insulin metabolism of schizophrenics and AD patients should lead to an increased incidence of AD in aged schizophrenics. However, this is not the case. We herein try to find out if there are protective mechanisms that prevent AD pathology in patients with schizophrenia.

3.2 Alzheimer's Disease as a Disease of Aging, Cognitive Decline, and Metabolic Abnormalities

A century ago, Alois Alzheimer published a short note about the case of Auguste D., a 56 years old woman who had suffered from severe memory impairment and delusions [1]. When morphologically analyzing her brain after death Alzheimer observed some of the characteristic structural alterations (compromised neurons, extracellular senile plaques, intraneuronal tangles), which now are commonly accepted as neuropathological hallmarks of the disease named after him. Alzheimer disease (AD) is currently the fourth leading cause of death and most common cause of dementia. Nosologically, AD is not a single disorder in spite of a common clinical phenotype. Etiologically, at least two different types exist. In a minority of 5% of cases or even less, AD is due to mutations in certain genes, resulting in the permanent generation of Abeta fragments [2]. The majority of cases of AD, however, are sporadic, with old age being a major risk factor [3–6]. Accordingly, the incidence of sporadic AD is approximately 14 times higher among persons older than 85 years compared to those between 65 and 69 years [7], with the consequence that about 50% of people aged 85 years are affected by sporadic AD [5]. With age being an important risk factor for the development of AD, and with the population aging rapidly in developed countries, the number of persons suffering from the disease will dramatically rise within the next decades.

Cognitive dysfunction in AD mainly includes memory loss, language difficulties, and executive dysfunction (i.e. loss of higher level planning and intellectual coordination skills). These symptoms progress from mild symptoms of memory loss to very severe dementia. Interestingly, psychiatric symptoms and behavioural disturbances such as depression, hallucinations, delusions, and agitation often accompany the cognitive decline (for recent review, see Ref. [8]).

Considerable efforts have been directed towards identifying reliable pathological markers which are the best predictors of dementia and cognitive profile. Markers of neuropathology that have been investigated include senile plaques (SP), neurofibrillary tangles (NFT), synaptic loss, and neurochemical changes. In addition, many clinical measures have been evaluated as possible ways of both predicting

the presence of pathological changes of AD as well as correlating with the specific measures of pathology. They included Clinical Dementia Rating, Mini Mental State Examination, and Functional Assessment Staging. Importantly, accumulation of neuropathology appears to correlate with functional, global, and cognitive decline as people progress through AD, whereby NFT and synaptic loss are better correlates of cognitive decline (reviewed in [9]).

Although the most widely accepted hypothesis for AD aetiology is based on the assumption that pathological aggregations of the Abeta peptide are the cause of all forms of AD, a growing body of evidence implicates impairment in brain insulin signalling in early sporadic AD pathology [10]. Nowadays, there is no doubt that insulin and insulin receptors are present in the brain [11]. While demonstrating a certain local neuronal synthesis of the hormone [12], it is obvious that most cerebral insulin comes from outside the central nervous system. It has been shown that peripheral insulin at considerable amounts enters the brain by crossing the blood brain barrier, where it exerts powerful actions via binding to its receptors [13]. Insulin receptors (IR), located in astrocytes and neuronal synapses, are highly concentrated in the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala, and septum. Localization of IR in the hippocampus and medial temporal cortex is consistent with the evident effects of insulin on memory [14, 15]. It is known for more than a decade that in AD patients brain glucose utilisation is already reduced in the early stages of disease and the regulatory enzymes important for glucose metabolism are reduced in their expression. In post-mortem studies it was shown that in brains of AD patients brain IR densities were decreased compared to middle-aged controls, but increased in comparison to age-matched controls, whereas IGF-I receptor densities remained unchanged during aging and in AD. Tyrosine kinase activity, a signal transduction mechanism common to both receptor systems, was reduced in AD in comparison to middle-aged and age-matched control groups. These data demonstrate a disturbance of insulin signal transduction in AD brain and favour the hypothesis that insulin-dependent functions may well be of relevance in sporadic AD [16]. Despite the increased insulin binding, IR activity is reduced in the AD brain [17], which is consistent with resistance to insulin. Insulin resistance in the brain has even been proposed to be a primary event clearly preceding the Abeta pathology and triggering its development [10]. In addition to IR impairment, reduced mRNA levels of insulin receptor substrate (IRS) and tau protein, reduced amounts of IRS-associated phosphatidylinositol-3-kinase (PI3K) and of activated (phosphorylated)-Akt, and increased activity of glycogen synthase kinase-3beta (GSK-3beta), elevated mRNA levels of amyloid precursor protein (APP) are characteristics of sporadic AD [18, 19]. Moreover, expression of the insulin-degrading enzyme (IDE), a downstream target of IR signalling cascade [20], was found to be reduced in brains of individuals with AD. This is of crucial pathophysiological importance, because of the dual function of this enzyme in the brain: on the one hand it cleaves insulin (and some other naturally occurring peptides), and it is prominently involved in Abeta protein clearance on the other hand. Reduced expression and decreased catalytic activity of IDE in AD brains may thus significantly contribute to amyloid formation in AD [21–23].

Interestingly, epidemiological studies have shown that patients with AD are more vulnerable to type 2 diabetes [24, 25]. AD and type 2 diabetes are characterized by an increased prevalence with older age, a genetic predisposition, and, most noteworthy, by the existence of comparable pathological features in pancreatic islet and brain (amyloid derived from Abeta protein in the brain [24]). Similar to AD, diabetes type 2 is a disorder of glucose regulation, being characterized by hyperinsulinemia, hyperglycemia, and hypo-responsiveness of the IR. Impaired memory performance becomes particularly evident in elderly patients suffering from the disease [26]. The striking similarities between diabetes type 2 and AD with regard to reduced glucose utilisation, abnormalities in insulin expression, impaired insulin signalling and other metabolic abnormalities prompted Steen and colleagues to call AD the type 3 (brain specific) diabetes [18]. Remarkably, insulin in combination with other antidiabetic drugs has recently been found to be associated with less AD neuropathology [27].

3.3 Schizophrenia as a Disease of Accelerated Aging, Cognitive Deficits, and Metabolic Abnormalities

Schizophrenia is the most common psychotic illness, affecting approximately 1% of the population. It is a life-long disorder characterized by illogical, delusional, or paranoid thoughts (positive symptoms), and cognitive deficits, including impairments in working memory, attention, and executive function. The positive symptoms constitute the more overt manifestations of psychosis and are typically the first to draw attention to the disorder. Negative symptoms including social withdrawal, flattened affect, and decreased initiative characterize most schizophrenic patients to some extent, and constitute the majority of symptoms in a subset of patients with schizophrenia. Among the many symptoms, the severe deficits in cognition are perhaps the most disabling components of the illness. Historically, in 1860 psychoses were even first named “dementia praecox” by the French Psychiatrist Morel [28]. Although it became subsequently clear that this term is too restrictive to describe the many facets of schizophrenia, Kraepelin [29] conceptualized the disease as a disorder of progressive cognitive decline, and the cognitive changes with aging in schizophrenia are also consistent with the hypothesis that schizophrenia is associated with accelerated aging [30]. Indeed, numerous findings support the idea that a core feature of schizophrenia is accelerated aging, in that many physiological changes throughout the body which are associated with normal aging occur at an earlier age in individuals with schizophrenia than in the general population (as recently reviewed in [30]).

1. Schizophrenia is associated with a striking increase in mortality, resulting in a decrease in average life span of 20%. In addition to the well-known factors (such as suicidal behavior, accidents, poor health care, impropriate nutrition and

medication-induced effects), physiological abnormalities found in schizophrenia make a significant contribution.
2. Patients with schizophrenia show often a further rapid cognitive decline beyond the age of 65 years in addition to the already existing cognitive deficits [31].
3. Patients with schizophrenia exhibit an increased prevalence in several aging-related conditions (increased insulin resistance, hyperlipidemia and other severe metabolic problems, hypertension, decreased bone density, thickening of the eye lens, thinning of the skin, thinning of the hair, decrease in muscle mass [30]).

Although the cognitive deficits are a defining feature of schizophrenia, and the cognitive decline after the age of 65 years shows many parallels to what is observed in AD, we will in this book chapter mainly focus on certain metabolic aspects of the illness, especially on abnormalities in the glucose metabolism and insulin signalling pathway. It is a well-documented fact that patients with schizophrenia often suffer from the so-called "metabolic syndrome" which is characterized by type 2 diabetes, visceral obesity, elevated lipid levels, and hypertension, and decreased sensitivity to insulin (for review see [32, 33]). This co-morbidity significantly contributes to an increased risk of cardiovascular diseases in mentally ill patients [33, 34]. In case of schizophrenia the increased incidence of the metabolic syndrome/type 2 diabetes is normally regarded as a result of the treatment of the patients with atypical neuroleptics (for recent reviews see [35–37]), and obesity represents a major cause for non-compliance of patients [38]. Recent work shows that treatment with typical neuroleptics may also increase the risk of developing the metabolic syndrome [37, 39]. The question, if drug-naïve patients with schizophrenia may already show symptoms of the metabolic syndrome/diabetes is controversially discussed (pro Ref. [40–45]; contra: Ref. [39, 46]). A family history of type 2 diabetes has been revealed in about 19% of schizophrenic patients [47] as compared to 1.2–6.3% in the general population [48]. Although it cannot be excluded that increased rates of glucose intolerance and/or diabetes in newly diagnosed and/or drug-naïve schizophrenia patients are partially due to shared lifestyle factors (i.e. reduced physical activity, impropriate nutrition and poorer health care [49]) a certain predisposition must be taken into account [45].

For whatever primary reason the peripheral glucose/insulin metabolism in schizophrenia is impaired, it should have an impact on brain metabolism, with considerable consequences for brain functioning. Indeed, brain imaging of patients with schizophrenia has revealed decreased brain glucose utilisation and reduced neuronal metabolism [50, 51]. Since insulin maintains energy balance and glucose homeostasis in the CNS, a disturbed glucose utilisation may well be the result of a compromised brain insulin signalling, as reviewed by Sharma et al. [52]. The interaction of insulin with its receptors leads to their stimulation, which triggers phosphorylation of tyrosine receptor kinase and activation of a downstream signal transduction pathway coupled to PI3K and protein kinase B/Akt. Akt is a multifunctional kinase regulating anti-apoptotic activities, cellular growth, and,

most important here, glucose metabolism [53–55]. Wondering if the known peripheral insulin resistance observed in many schizophrenics might have an impact on the insulin transduction cascade in the brain, Zhao et al. studied the functioning of cerebral IR in patients with schizophrenia and in a mouse model of the disease [36]. A key finding was that brain content and auto-phosphorylation of IR as well as insulin-dependent Akt signaling were severely depressed in a group of twelve patients with schizophrenia, most of which had received neuroleptics during lifetime. Similar results were obtained in the mouse model. Insulin resistance phenotype was induced in mice by treatment with the antipsychotic drug, clozapine. Treatment resulted in a functional inhibition of the cerebral IR, but the Akt activation status remained unaltered. Changes in GSK-3 alpha/beta were consistent with a net decrease in the enzyme activity, as opposed to that in schizophrenia. The results suggest that alterations in insulin-dependent Akt signaling in schizophrenia are similar to those observed in the cellular, but not animal models of insulin resistance. Interestingly, in the mouse model, clozapine ameliorates IR deficits at the GSK-3 alpha/beta level, which may, as the authors state, justify its role in treatment of schizophrenia. Overall, these results suggest that brain glucose metabolism is seriously compromised in schizophrenia. Of note, out of the twelve individuals with schizophrenia studied by Zhao and co-workers [36], only two had treatment with atypical neuroleptics, while nine had received typical neuroleptics and one individual was drug-free. Consequently, the observed changes in cerebral insulin metabolism in schizophrenia can hardly be attributed to atypical antipsychotics (see above). The involvement of Akt is of considerable interest, since recent evidence shows that Akt1 might be a potential susceptibility gene in schizophrenia [56]. The inhibition of IR signalling was accompanied by elevated contents of GSK-3 alpha and beta. Aberrant GSK-3 beta expression and/or activity in brains of patients with schizophrenia were repeatedly reported and might represent a new putative target of drug intervention [57]. Insulin may thus be of central importance for brain metabolism in schizophrenia. Several studies in schizophrenia described robust down-regulation of certain metabolic genes, in particular genes the products of which function in glycolysis and ATP generation. Insulin and IGF-1 were found to increase the expression of genes decreased in schizophrenia, including those involved in mitochondrial functions, glucose and energy metabolism, hydrogen ion transport, and synaptic function [58]. Moreover, similar to what has been reported for AD, the insulin-and Abeta-cleaving enzyme, IDE, was found to be reduced in its expression in neurons of the post-mortem dorsolateral prefrontal cortex of haloperidol treated, chronic patients with schizophrenia [49] (Fig. 3.1a). In experiments employing neuroblastoma cells and haloperidol-treated rats we could show that this reduced expression of insulin degrading enzyme in specific brain areas of schizophrenics is not caused by antipsychotic treatment [49]. Unfortunately (and at variance to the situation in AD), data about the brain insulin and glucose concentrations in schizophrenia are still lacking. Increased concentrations of glucose in the cerebrospinal fluid as well as alterations in glucose-metabolizing enzymes have been found in patients prodomal for psychosis [59].

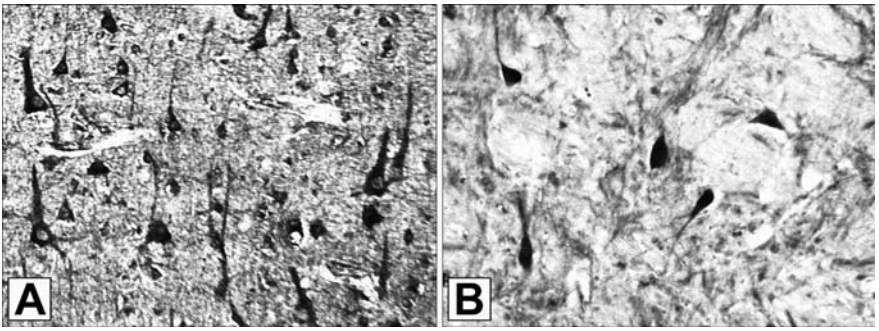


Fig. 3.1 Immunocytochemical localisation in human brain neurons of two enzyme proteins which are affected in schizophrenia subjects. (**a**) IDE in the prefrontal cortex. The cellular expression of IDE is reduced in schizophrenia. (**b**) Cathepsin K in striatal neurons and nerve fibres. The enzyme is up-regulated in haloperidol treated schizophrenics

3.4 Elderly Patients with Schizophrenia Should Have an Increased Frequency of AD Pathology – But They Don't

In the preceding two chapters we have tried to show that AD and schizophrenia share some clinical and biochemical features suggesting the existence of common pathogenic mechanisms. More similarities (but also obvious differences) between the two diseases are summarized in Table 3.1 and will not be discussed here in greater detail. The assumption that patients suffering from schizophrenia should be at higher risk to develop Alzheimer-like brain pathology is not new and came up before the striking brain metabolic abnormalities so common to both diseases were discovered. Indeed, there are some amazing parallels between AD and schizophrenia, including the occurrence of psychotic symptoms in both diseases, a predilection toward limbic areas, the presence of atrophic changes of the medial temporal region predominating in AD and in schizophrenia, as well as some common neurochemical peculiarities with regard to the dopaminergic, glutamatergic, and cholinergic neurotransmitter systems (reviewed in [60]). However, a major reason why investigators became convinced that the illness should lead to an increased Alzheimer pathology, is aging. Since age is a very important risk factor for the development of sporadic AD, diseases leading to premature aging of the brain and other organs are expected to show prevalence of AD-related alterations. The obvious argument that most patients with schizophrenia do not reach the age when typical hallmarks of AD become apparent in brains (65 years and older) has certainly to be considered. It becomes, however, alleviated because of (1) the aforementioned, accelerated aging process typical for the disease [30, 61] and the fact that (2) a sufficient number of elderly schizophrenics (aged 65 years and older) has been studied during the past two decades (see below). Another factor with potential relevance to AD pathology in schizophrenia is the antipsychotic medication of the patients. Neuroleptics are

Table 3.1 Similarities and differences between of schizophrenia and sporadic AD (after [60], modified and extended by work of others [74, 117–126])

Schizophrenia	AD
Hypothetical origin	
Probably neurodevelopmental	Neurodegenerative
Clinical features	
Delusions: persecutory, referential, bizarre	*Delusions*: persecutory, theft, infidelity
Hallucinations: auditory > visual	*Hallucinations:*visual > auditory
Flattened effect, avolition, apathy, poverty of speech and thought	Disengagement Apathy
Cognition largely stable (decline after age 65)	Permanent cognitive decline
Typical age of onset in early adulthood	Typical age of onset of familial AD: adulthood, of sporadic AD after 65
Remission possible	**Remission still impossible**
Neuroimaging	
Lateral/third ventricle enlargement	Lateral/third ventricle enlargement
Regional brain atrophy; temporal lobes, hippocampus, cerebellum and thalamus	*Diffuse cortical atrophy* prominently involving temporal lobes and hippocampus
Reduced cortical glucose metabolism	Reduced cortical glucose metabolism as the illness progresses
Decreased white matter integrity	Decreased white matter integrity
Neuropathology	
Regional atrophy of medial limbic structures	*Generalized atrophy* : medial limbic and pariental structures > frontal cortex
Hippocampus: atrophy, neuronal loss, pyramidal cell disarray; **no senile plaques, no NFTs**	Hippocampus: senile plaques, NFTs, granulovascular degeneration
Substantia innominata: sometimes cell degeneration	Substantia innominata: neurofibrillary Degeneration, sometimes cell loss
Reduced number of oligodendrocytes, decreased density of myelin	Reduced number of oligodendro-cytes, decreased density of myelin
Astrogliosis questionable	Focal astrogliosis (plaque-associated)
Immune/inflammatory markers	Immune/inflammatory markers
Re-entry in cell cycle (oligodendrocytes)	Re-entry in cell cycle (neurons)
Adult neurogenesis decreased	Adult neurogenesis decreased (mouse model)
Neurochemistry	
Overactivity of the dopaminergic system (disturbed acetylcholine/dopamine balance) (treatment: dopamine-blocking agents)	Underactivity of the cholinergic system disturbed dopamine/acetylcholine balance (treatment: cholinergic enhancement)
Disturbed glutamatergic system (hypofrontality)	Disturbed glutamatergic system
Underactivity of GABAergic system	GABAergic system spared
Dysbalance of neurotrophic factors (incl. neuregulin 1)	Dysbalance of neurotrophic factors (incl. neuregulin 1)
Disruption of the insulin signalling cascade	**Disruption of the insulin signalling cascade**

commonly associated with several adverse effects, including sedative, metabolic, and deleterious cognitive disturbances as well as structural brain changes [62, 63].

The recently discovered impairment of cerebral glucose/insulin metabolism typical for both diseases adds a strong point in favour of an increased risk for AD pathology in schizophrenia. While investigations on the linkage of schizophrenia and impaired brain insulin metabolism have just been beginning [36, 43, 49, 58], there is already a great body of evidence demonstrating strong impact of impaired cerebral insulin signalling on AD. Recent work gives reason to suppose that insulin resistance in the brain is most probably not a consequence (or side effect) of the developing dementia, but might rather be a primary event which clearly precedes the Abeta pathology and triggers their development [10]. Given the capability of cerebral insulin resistance to initiate and promote Abeta accumulation [26], it is reasonable to suggest a similar pathophysiological function in schizophrenia, the more as both in AD and schizophrenia a reduced expression and/or a functional deficit of the Abeta-clearing enzyme IDE has been reported [21–23, 49].

For all these reasons elder patients with schizophrenia are expected to have an increased frequency of AD. Amazingly enough, numerous neuropathological studies have convincingly shown that this is not the case. Baldessarini et al. [64] performed a meta-analysis of the ten reports on this topic available at that time, and came to the conclusion that there is no evidence in favour of AD-like neuropathology (SP and NFT) being more frequently found in the brains of patients with schizophrenia than in the brains of compared controls. Niizato and colleagues [65] evaluated the brains of 125 schizophrenic patients with no other major diseases with regard to the presence or absence of these neuropathological hallmarks. Of note, no quantitative or qualitative differences were found between the subgroup of individuals with schizophrenia (older than 75 years) and 12 age-matched normal controls. The frequency of subjects with schizophrenia meeting the neuropathological criteria of AD was equal to or even lower than that found in the general population in a third sample studied (post-mortem brains of 166 schizophrenics with a mean age of 72 years [66]). Although severe cognitive impairment is common among elderly patients with schizophrenia, this cognitive decline is not necessarily accompanied by AD pathology. Accordingly, Purovit et al. [67] could demonstrate that in their cohort 72% of the elderly patients with schizophrenia showed severe cognitive impairment during lifetime, but only in 9% of these individuals definite AD was diagnosed at autopsy using neuropathological criteria. A similar result was obtained in another study, where 68% of the patients with schizophrenia had severe cognitive impairment, but only 8% satisfied neuropathological criteria of AD [68]. Interestingly, among the schizophrenic subjects without AD, definite cognitive impairment was associated with higher levels of SP and NFT. The lack of an increased classical AD pathology in elderly schizophrenia patients was confirmed in more recent studies [69–72]. There is, however, evidence for oxidative DNA damage and coordinated cell-cycle activation in elderly subjects with “poor-outcome” schizophrenia [73, 74]. No abnormally elevated levels of AD-related biomarkers were detectable in the cerebrospinal fluid of schizophrenia subjects (reviewed in [75]), and, vice-versa, no biomarkers typical of schizophrenia were found in CSF

specimens of AD patients [43]. Overall, neuropathological and clinical practice provides convincing evidence against higher frequency and severity of AD pathology in post-mortem brains and bodily fluids of schizophrenics. The question is: Why?

3.5 Are Brains of Schizophrenia Subjects Protected from Developing AD Pathology?

Despite of the occurrence of strikingly analogous pathophysiological factors and mechanisms in patients with AD and schizophrenia, there is an obvious lack of increased AD pathology in brains of individuals with schizophrenia. One way to treat this result is to acknowledge that both diseases have different developmental origins as crucial predisposing factors for different neuropathological signs emerging in the aged brain (schizophrenia: aberrant brain development and connectivity; AD: neurodegeneration [76] and Table 3.1).

The other way is to ask for the existence and activity of mechanisms functioning to protect the brain from AD pathology in patients with schizophrenia.

Following this idea we will focus on a few aspects we find worth to be considered.

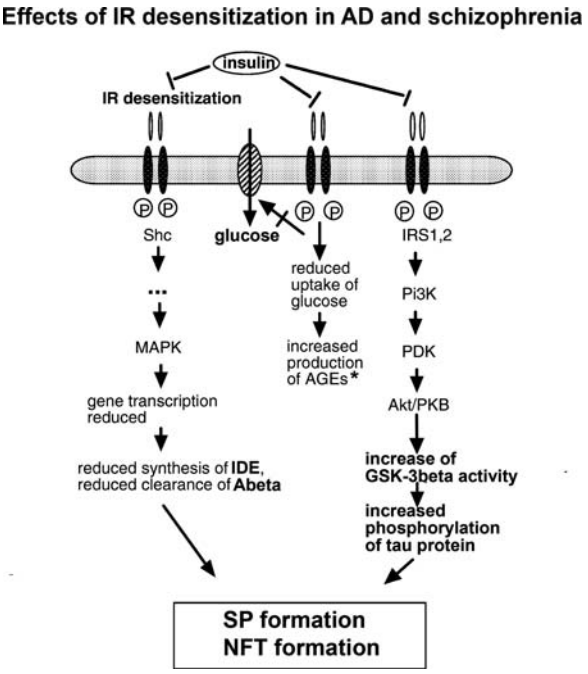
1. *Antipsychotic medication*. Nowadays, neuropathologists do rarely see brains at autopsy of non-medicated patients with schizophrenia. Therefore, the impact of neuroleptic treatment on brain structural changes must always be taken into account. The initial conjecture that long-term treatment with antipsychotics might support the development of AD pathology in chronic schizophrenia, has proven to be wrong (see above). Now we should ask the opposite: Might administration of neuroleptic drugs help to prevent AD pathology in schizophrenics? Before answering this question, one should ask: Do AD patients benefit from receiving antipsychotics (beyond improving the psychiatric symptoms and behavioural disturbances)? This question is justified, since nearly half of AD patients get antipsychotic treatment [77]. Unfortunately, the answer is no. Patients with AD who are receiving antipsychotic medications are at risk for further loss of memory because of the potential anticholinergic side effects of some antipsychotics [78]. Furthermore, several studies have shown that there is an increased long-term risk of mortality in patients with AD on antipsychotic medication, highlighting the urgent need to seek less harmful alternatives for the long-term treatment of neuropsychiatric symptoms in these patients (reviewed in [79]). But let us come back to the original question. When asking if schizophrenics might benefit from receiving neuroleptics in that they develop less AD pathology in late life, the answer would possibly be yes. A decade ago it was shown that in cultured cells, haloperidol can act as an inhibitor of certain proteinases involved in the enzymatic processing of APP to Abeta. Although these proteinases were poorly explored and not further specified at that time [80] the authors postulated their contribution to the reduced frequency of AD pathology in schizophrenia. In another study it was demonstrated that in cultured skin cells

of AD patients haloperidol efficiently countervails calcium ion imbalance and may thus help to reduce the neurotoxic effects of Abeta [81]. Moreover, an atypical neuroleptic, quetiapine has recently been shown to inhibit Abeta (25–35) aggregation in brains of mutant mice and in cell-free aqueous solutions and to block the fibrillar aggregation of Abeta [82]. Thus, neuroleptics might theoretically contribute to a reduced accumulation of brain amyloid in schizophrenia subjects, when administered early in life (actually, there is yet no evidence that neuroleptics might work in AD patients, when the pathologic process is already ongoing for decades). No information is yet available regarding a possible influence of antipsychotics on the second pathological hallmark of AD, the NFT (except that taking neuroleptics is significantly associated with more severe tangle pathology in dementia with Lewy bodies, but not with NFT in AD [83]). However, when taking stock of neuroleptics and AD pathology, one important aspect should be considered: it is the treatment with antipsychotics that brings about, or at least promotes, the serious metabolic problems of patients suffering from schizophrenia – with all the fatal consequences for brain metabolism (and possibly AD pathology). Therefore, the role of medication with neuroleptics is a Janus-faced one.

2. *Tobacco smoking.* A vast majority of individuals with schizophrenia are heavy smokers (recently reviewed by [84, 85]). The reasons for this nicotine abuse remain largely unknown. Ironically, ignoring the well-known adverse effects of smoking (which significantly contribute to patients' increased mortality), schizophrenics might really have some benefit of it. Individuals with schizophrenia may smoke to regulate mood, reduce stress and, via activation of the cholinergic system, improve cognition [85, 86]. As to AD pathology, smoking has been shown to attenuate Abeta deposition in some cortical areas of normal elderly individuals [87]. Hence, smoking may possibly contribute to reduced Abeta pathology in schizophrenia.
3. *Compensation by other Abeta clearing enzymes for reduced expression/activity of IDE.* The most important Abeta clearing enzyme, IDE, has been found to be less expressed and active in both AD and schizophrenia. Partial withdrawal of IDE activity might be of significance for amyloid formation. Furthermore, IDE is genetically linked to AD, diabetes type 2, and schizophrenia (reviewed in [49]). It is conceivable that other Abeta-removing enzymes like neprilysin, plasmin, thimet oligopeptidase, or angiotensin-converting enzyme[2] are secondarily up-regulated in schizophrenia, and thereby may compensate for partial IDE deficit. Due to the lack of information on this topic this assumption cannot be further substantiated. There is only one report on increased levels of plasmin in CSF of schizophrenis (in connection with inflammatory aspects of the disease [88]).
3. *Altered expression and/or activity of alpha-, beta- and gamma-secretases.* Abeta is produced from APP by proteolytic processing dependent on the beta-site APP-cleaving enzyme 1 (beta-secretase; BACE1) and gamma-secretase. Altered activities of these enzymes could therefore influence amyloid formation. However, compared to matched control cases, BACE1 expression was found to

be normal in brains of schizophrenics [89]. Gamma-secretase is a multiprotein complex comprising four subunits: presenilin(s), nicastrin, anterior pharynx-defective 1, and presenilin enhancer [90]. The activity of gamma-secretases is crucial for generation of Abeta. Missence mutations in either APP or the presenilins 1 or 2 are known to lead to early-onset familial AD (reviewed in [91]). Interestingly, presenilins 1 and 2 are possibly also associated with schizophrenia. A recent study showed that in peripheral leukocytes the expression of the gene coding for presenilin 2 was significantly lower in the group of patients with schizophrenia than in the control group (for detailed considerations see [92]). It remains to be elucidated, whether or not this altered expression of presenilin has functional consequences with regard to cerebral gamma-secretase activity. Finally, no work has yet been published on putative alpha-secretases that preclude by their activity the amyloidogenic pathway leading to Abeta, in schizophrenia.

4. *Glycogen synthase kinase-3beta.* GSK-3 is a constitutively active, serine/threonine kinase that plays important roles in a plethora of physiological processes ranging from glycogen metabolism to gene transcription [93]. It is abundantly expressed within the CNS. Once GSK-3 is activated it modifies different substrates by phosphorylation, including APP (mainly by isoform GSK-alpha) and tau protein (mainly by isoform GSK-3beta [94]). Dysregulation (over activity) of GSK-3 has repeatedly been implicated in all relevant aspects of AD pathogenesis including Abeta-induced neurotoxicity, tau hyperphosphorylation (leading to NFT), local SP-associated microglial activiation, neuronal loss and, last but not least, memory impairment [91, 93, 95, 96]. GSK-3 inhibitors are therefore under study for the treatment of AD [97]. As to schizophrenia there is consensus that GSK-3 beta is aberrantly expressed in the CNS of patients. However, while Zhao et al. [36] reported elevated levels of the protein in schizophrenia, a majority of investigators found the opposite (that is, reduced cerebral protein levels of the enzyme [98–101]). It has even been supposed that reduced GSK-3 expression might be the basis for key developmental brain abnormalities found in schizophrenia [98]. This would suggest that aberrant GSK-3 although being central to both diseases, yields quite opposite results: chronic hyperactivity in AD, finally leading to AD pathology, and reduced expression in schizophrenia, leading to neurodevelopmental disturbances early in life (but not to AD-like pathology later on) in schizophrenia. Hence, GSK-3 seems to be a promising candidate in search for possible protecting mechanisms against AD pathology. One question remains to be answered, namely, why GSK-3 is reduced in schizophrenia (and not increased – as found by Zhao et al. [36]). Atypical (but not typical) antipsychotics are known to either activate Akt (acting upstream to GSK-3) or to mimic Akt activity by increasing the phosphorylation of its substrates, GSK-3alpha and GSK-3beta [102, 103]. Moreover, GSK-3 is a downstream target of brain IR signalling and should become activated, when the IR is desensitized in schizophrenia (by analogy to what is proposed to happen in AD, see Scheme 3.1). To overcome this paradoxical situation it is necessary to look not only at expression levels of cerebral GSK-3 in schizophrenia, but also



Sch 3.1 summarises some metabolic effects of cerebral IR desensitization, which finally may lead to SP and NFT formation in AD, but not in schizophrenia (simplified after [127]). Differences in GSK-3 activity between AD and schizophrenia may be of crucial importance for the different outcome in both diseases. *Interestingly, impaired glucose utilisation in AD leads to the production and deposition of AGEs in AD, but not in schizophrenia (unpublished data). However, a treatment induced increase of the AGE-scavenger sRAGE may be found in sera of schizophrenics [128]

to take into account its enzyme activity status. Data on this topic suggests that phosphorylation levels of GSK3beta are reduced in schizophrenia, resulting in increased GSK-3beta activity (reviewed by Souza et al. [104]). Bringing both lines of evidence together, the consequence is far from being satisfactory: obviously, there are fewer GSK-3 molecules in schizophrenia brains, each of which may or may not have an increased enzymatic activity. From this consideration it is absolutely unclear, what the net outcome will be: More, less, or unchanged activity of GSK-3 in brains of schizophrenics compared to controls and, more importantly, to AD. The situation is further complicated by the circumstance that not only insulin, but also other signalling factors influence the Akt/GSK-3 system. For example, neuregulin-1 induced Akt phosphorylation has recently been shown to be decreased in schizophrenia, with possible consequences for GSK-3 activity [105]. Furthermore, GSK-3beta is a major constituent of both the canonical and non-canonical Wnt-signalling pathways. Aberrant Wnt-signalling has been demonstrated in AD and schizophrenia, and antipsychotic medication is thought to “normalise” Wnt signalling in schizophrenia subjects [91, 98, 106, 107]. Lastly, schizophrenia-associated GSK-3beta polymorphisms have recently been detected which might be of functional importance [104]. In sum, it must be stated that at present time it is not possible to decide, whether or not GSK-3 may be responsible for the absence of increased AD pathology in schizophrenia.

5. *Cathepsin K (Cat K) – just a speculation.* The gene coding for the cysteine proteinase Cat K is amongst the very few rat genes the expression of which is altered in the same direction by neuroleptics (haloperidol and clozapine), but in the opposite direction in the amphetamine-sensitized striatum [108]. We could show that this enzyme is up-regulated in brains of schizophrenics, which is most probably the result of neuroleptic medication [109] (Fig. 3.1b). The elevated expression of Cat K in schizophrenia might not only bring about consequences for general cerebral and extracerebral metabolism (ranging from altered proteolytic handling of neuropeptides and bone tissue turnover to blood glucose, insulin, and lipid levels [109–111]), but also for the development of AD pathology. Investigating the putative role of Cat K in extracerebral amyloidosis, Röcken and colleagues [112, 113] found that the enzyme possesses only endoproteolytic activity and does not generate any amyloid-like peptides. Thus, Cat K is an amyloid-retarding cysteine protease, which is able to modulate amyloid load in extracerebral amyloidosis. Unfortunately, no data is yet available as to the situation in the brain, but it is well conceivable (and we are currently about to test this conjecture) that Cat K may also play roles in cerebral amyloid degradation. In this context it would be very interesting to compare brain Cat K activities in patients with schizophrenia and AD. Finally, we will regard a particular aspect of Cat K activity, which might be of importance for the initial events leading to amyloid formation and accumulation. Recently, an exciting new model has been proposed which allows explaining the initial formation of amyloid seed. There is good evidence that N-terminally truncated and pyroglutamate-modified Abeta peptides are major constituents of amyloid deposits in sporadic and familial AD. Formation of pyroglutamate at the N-terminus confers resistance against cleavage by most aminopeptidases, increases toxicity of the peptides, and may seed Abeta aggregate formation [114]. The N-terminal pyroglutamate modification (occurring with glutamines at positions 3 and 11) is most probably carried out by the enzyme glutaminyl cyclase, the expression of which is up-regulated in the cortices of individuals with AD. This N-terminal modification of Abeta might be of crucial importance for the initiation of pathological cascades resulting in the development of AD [115]. To perform this fatal modification glutaminyl cyclase needs a free N-terminus glutamate. Since glutamines are at the positions 3 and 11 of the Abeta molecule (see Scheme 3.2), mechanisms must exist that cleave between Ala and Glu (positions 2 and 3), and between Try and Glu (positions 10 and 11). A cleavage between Glu and Val (positions 11 and 12), however, would definitely preclude any enzymatic modifications of N-terminus glutamine, and thus the generation of pyroglutamate-containing Abeta species. While several aminopeptidases exist which may cleave in a way that exposes N-terminal glutamine in Abeta, little is known about the latter possibility. Performing a Medline search we became aware of the fact that Cat K preferentially cleaves Glu-Val bondage(s) in proteoglycans [116]. While we are far from believe that Cat K is the only brain enzyme to be able to cleave at this position, we know at the same time that it is the only relevant one found elevated in medicated schizophrenia. Interestingly, patients with AD receiving neuroleptics (and thus

↓Cat K

Asp-Ala[2]-Glu[3]-Phe-Arg-His-Asp-Ser-Gly-Try[10]-Glu[11]-Val[12]-His-His-Glu-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val

Sch 3.2 Scheme showing the amino acid sequence of Abeta (1–40). Glutamines relevant for pyroglutamate modification are *underlined*. The putative Glu-Val cleavage site of Cat K is indicated by an *arrow*

possibly also having increased cerebral expression of Cat K) would not benefit, because pyroglutamate Abeta plays an immanent role as an initiator of amyloid seed only, and the further accumulation is supposed to go on with "normal" Abeta. Future studies will provide experimental results that confirm or dismiss our assumptions with regard to Cat K.

3.6 Conclusion and Further Direction of Research

As outlined, several mechanisms are currently under consideration that might contribute to brain protection from increased AD pathology in schizophrenia. Of those, for which experimental data already exist, the functional GSK-3 dichotomy in AD and schizophrenia seems to be most promising explanation. The potential role of Cat K as an amyloid-retarding enzyme remains to be elucidated in further research. An experimental confirmation of the assumed impact of the enzyme on cerebral abeta and amyloid processing, however, would have far-reaching consequences for our understanding of amyloid seed in general.

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Chapter 4
Assessing In Vivo Neurodegeneration in Schizophrenia Using Magnetic Resonance

Jean Théberge

Abstract Evidence that neuronal loss occurs over the course of neuropsychiatric disorders like schizophrenia, depression and bipolar disorder has stemmed from various invasive examinations in animal models or from post-mortem examinations of human tissues. The goal of neuroprotective interventions is to prevent the neuronal damage that is presumed to result from pathophysiological mechanisms in living human patients. Consequently, it is important that clinicians be able to assess if a given individual or group of individuals with similar diagnostic features presents signs of neuronal damage or neurodegeneration, thereby confirming the adequacy of neuroprotective interventions. Furthermore, an assessment of neuronal health is crucial to evaluating the success of neuroprotective treatments. This chapter reviews and discusses the application of magnetic resonance imaging and spectroscopy to the non-invasive assessment of indices of neurodegeneration, such as grey matter loss, N-acetylaspartate loss, and glutamatergic metabolite losses in vivo, in individuals with schizophrenia. The chapter also provides an outlook towards future applications of magnetic resonance in the monitoring of neuroprotective therapies.

Abbreviations, Symbols, Nomenclature

^{1}H	hydrogen, hydrogen nuclei, proton
^{31}P	phosphorus, phosphorus nuclei
B_0	static magnetic field, main field
CNS	central nervous system
Cr	creatine
CSF	cerebral spinal fluid
DLPF	dorsolateral prefrontal
γ	gyromagnetic ratio
GABA	γ-aminobutyric acid

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_4,

Gln	glutamine
Glu	glutamate
Glx	glutamate, glutamine and other overlapping metabolites
GroPCho	Glycerophosphocholine
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
Myo	myo-inositol
NAA	N-acetylaspartate
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
PCho	phosphocholine
PCr	phosphocreatine
RF	radiofrequency
STEAM	STimulated Echo Acquisition Mode
T	Tesla

4.1 Introduction

Over the last century, mental illnesses have been the subject of considerable research and debate. Historically attributed to demons, stress, anger, problems with living, and personal weakness [1], more recently, they have been attributed to neurological disease, with a great number of them now recognized to have a neuro-chemical basis. In fact, chemical intervention, in the form of antipsychotic medication, has become a first-line treatment approach for many psychiatric disorders.

Schizophrenia, the prototype psychotic disorder, has garnered considerable attention, both because it is one of the most common mental illnesses, with a lifetime prevalence of approximately one percent, and because of its often devastating effect on the patient, their loved one's, the health care system, and society. The mood disorders, major depression and bipolar disorder, also have sparked research interest, for many of the same reasons. In this chapter, the focus will be to review the role of magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) as tools to document and monitor neurodegenerative processes in schizophrenia.

4.2 About Schizophrenia

First described in 1809 by the French physician Philippe Pinel (1745–1829), *schizophrenia* is a diverse collection of symptoms that can be extremely debilitating to the patient and distressing to their families, friends and caregivers [2, 3]. It is associated with a vast array of psychologically distressing symptoms that include, among others, hallucinations, delusions, often total avolition, and sometimes violent behaviour. This being said, individuals with schizophrenia are more often a danger

to themselves than to others, which is reflected in suicide rates reaching as high as 4–10% [4].

In addition, the societal and economical burdens of schizophrenia are imposing. With a prevalence of roughly 1% [3] – which rises to 2% in first degree relatives, 9 and 13% in siblings and children, respectively, and 17 and 48% in fraternal and identical twins, respectively [5] – it is about as common as Alzheimer's disease and six times as common as insulin-dependent diabetes. And, because of frequent in-patient hospitalisations, profound losses in productivity, and the fact that disease onset generally is between the ages of 16 and 30 years old [3], the time of life when individuals are preparing for careers and families, the societal cost per patient is very high. For example, in the US, estimates that now are almost 2 decades old placed the annual direct and indirect costs as high as $17.3 and $15.2 billion, respectively [6]; likely, these figures represent only a fraction of today's costs. In Canada, more recent estimates of economical burden of schizophrenia have amounted to roughly $4 billion dollars or more than $130 per Canadian citizen, roughly half of this amount being direct costs related to health care expenditures and the remainder indirect costs largely related to lost productivity [7, 8]. Similarly, the total costs of schizophrenia in 2002 were estimated at approximately €2 billion (Euros) in Spain, with direct health care and informal care costs accounting for 53 and 47% of this, respectively; this total represented approximately 2.7% of all public health care expenditures for that year [9]. Overall, schizophrenia has been estimated to account for between 1.6 and 2.6% of all health care related costs in Western countries [10], and reported to cause significant health care expenditures in countries like India [11], Mexico [12], Brazil [13] and Nigeria [14].

The most widely recognizable symptoms of schizophrenia are those that have been categorized as *positive symptoms* (Fig. 4.1), which include hearing voices; bizarre delusions; the feeling of being controlled by outside forces (e.g., a local radio transmitter or alien satellite) or persecuted (paranoia); disorganized speech; formal thought disorders; and catatonia. The term 'positive symptoms' refers to the

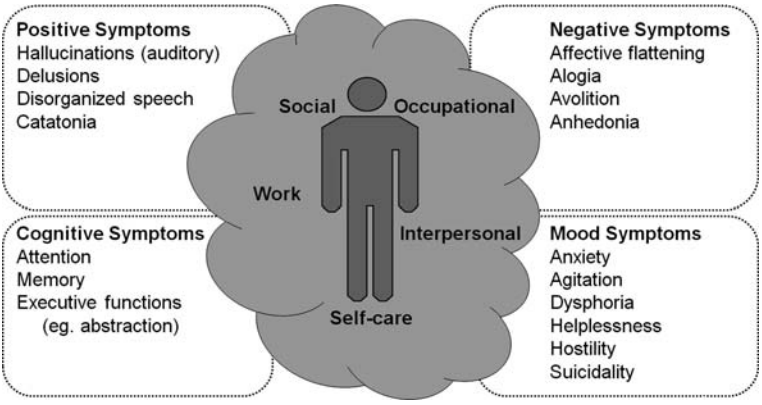


Fig. 4.1 Symptoms and impact on overall function

augmented experience of reality sufferers have relative to that of healthy individuals. However, schizophrenia sufferers also typically exhibit a host of *negative symptoms* that may be just or even more debilitating. Here, the term 'negative' symptoms refers to a diminished experience in individuals with schizophrenia relative to healthy individuals, and include a blunted affect (i.e., an apparent absence of emotions); problems with word and conceptual fluency; a lack of motivation (avolition); and an apparent inability to enjoy life (anhedonia). Outside both of these symptom categories are problems with attention and memory and numerous alterations in mood, such as increased agitation and anxiety, dysphoria, a sense of helplessness, hostility, and the afore-mentioned predilection for suicide. This distinction between positive and negative symptoms and the remaining cognitive and mood disruptions is especially important, in terms of treating schizophrenia, since the first class of symptoms appears to be the most responsive to currently available pharmacotherapy.

Moreover, it has become an increasingly common observation that the symptoms of schizophrenia actually worsen over time [3], in particular the negative symptoms, which often are the ones that prevent patients from living a productive life. A typical course of illness would include normal childhood and early teen years (Fig. 4.2). This generally is followed by a *prodromal phase* (labeled as *P* in Fig. 4.2), in later teenage years, during which mild symptoms start to appear, usually not always affecting function significantly, but often enough for parents or caregivers to notice personality and behavioral changes. For example, school performance may fall off; or the teen may become more socially isolated, or start using tobacco, alcohol or drugs. Those changes may not necessarily bring the individual to medical attention, as they may be easily mistaken for common rebellious adolescent behaviors. Then, suddenly, the individual suffers their first psychotic episode, classically associated with the exacerbation of a variety of positive symptoms, including mainly

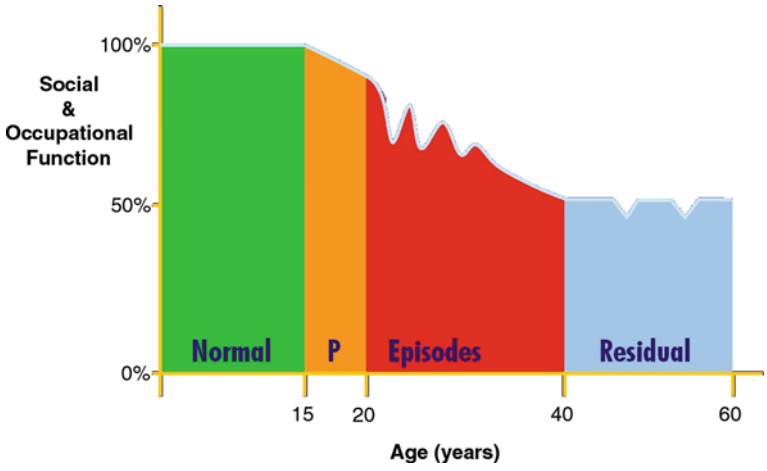


Fig. 4.2 Disease progression in schizophrenia (adapted from fig. 3.21 in: Stahl SM. Psychopharmacology of Antipsychotics. London, UK: Martin Dunitz Ltd; 1999)

auditory hallucinations and delusions. In this phase of the disease, episodes will often be resolved through treatment and possibly recur later during lapses in medication compliance. Over the next twenty or so years, patients generally experience large fluctuations in social and occupational function that can be more or less stabilized by medication. In general, antipsychotic medications tend not to alleviate negative symptoms as well as positive symptoms. Therefore, despite medication, negative and cognitive symptoms tend to progress until social and occupational function plateaus, usually by early middle age, at a level significantly lower than the premorbid level of functioning. The period of somewhat stable function that follows is called the *residual phase*, which is dominated by negative symptoms, but can be interrupted by periods of relapse; patients often remain in the residual phase for the remainder of their life.

Over the years, numerous theories have been espoused regarding the pathophysiology of schizophrenia. Initial efforts to explain the syndrome tended to look for abnormal anatomy [15]. Later, there was a shift towards neurochemistry and metabolism [15]. Since the widespread use of magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), there has been a concerted effort to look for both anatomical and physiological abnormalities, as well as for genetic explanations for them. There now is general acceptance that (1) there is a sizeable genetic and neurodevelopmental basis for schizophrenia [16]; (2) there is a sizeable neurochemical basis for schizophrenia, involving several neurotransmitter systems [2]; (3) clinically, a large proportion of patients experience deteriorating function over time despite treatment [3]; and (4) the schizophrenic brain demonstrates volumetric and neurochemical changes over time beyond the effects of normal aging.

4.3 Early Studies

Up until the last 50 or so years, those investigating brain changes in schizophrenia were limited to post-mortem examinations and, to a much lesser extent, pneumoencephalography [15]. Relatively little agreement stemmed from the results of these studies [15]. That brain atrophy beyond normal aging occurs in schizophrenia was well-recognized, with enlargement of the lateral ventricles first identified by Jakobi and Winkler [17], using pneumoencephalography; their report was published 1927. Numerous other structural abnormalities were noted, including localized and more general brain atrophy beyond that of normal aging; but not all patients had evidence of atrophy, and no universally-evident atrophic area or other structural abnormality was found [15]. Somewhat unexpected, due to early beliefs that the origin of schizophrenia lay within the outer cortex, was the relatively frequent identification of limbic system pathology. Perhaps the most major grievances expressed with these early studies were the inconsistent ways in which schizophrenia was defined, and the highly variable methodologies used. Nonetheless, many of the more consistent findings identified by these early techniques were noted again

by investigators using computed tomography (CT), including ventricular enlargement secondary to cerebral atrophy, as well as a variety of less consistent findings. An excellent review of these early studies was published by Weinberger et al. in 1983 [15].

None of these early studies addressed the issue of cerebral function, however. Moreover, a reliance on post-mortem examinations precluded the potential for monitoring changes over time; pneumoencephalography essentially has been abandoned; and the sensitivity of CT is generally not adequate to detect meaningful volumetric changes secondary to treatment, at least over the short-term. For these and other reasons, attention has been drawn away from merely documenting changes in cerebral volume, which could be due to losses in non-neuronal (for example, glial) cells, to assessing actual neurodegeneration, which explicitly refers to the loss of neurons or neuronal activity/function. The primary way by which this has been done has been by measuring the concentration of neurotransmitters believed to be involved in the disease. Dopamine sparked the most interest initially, for reasons provided in the next section. More recently, via the use of MRS, neuronal integrity and function has been assessed via measurements of N-acetylaspartate (NAA) [18–75] and glutamatergic neurotransmission has been assessed via measurements of glutamate (Glu) and glutamine (Gln) concentrations [54–56, 58, 76–86]. Other complementary indicators of cellular membrane integrity and turnover in schizophrenia such as phosphocholine and glycerophosphocholine have been measured with 1H-MRS and phosphorous (^{31}P)-MRS [86–92].

4.4 Neurotransmitters and Schizophrenia

Dopamine was the first neurotransmitter implicated in the pathophysiology of schizophrenia, primarily because the capacity of most antipsychotic drugs to block dopamine D2-receptors was found to influence their potency [93–95], but also because dopamine agonists, like amphetamines, could induce hallucinations. The earliest theoretical models for schizophrenia primarily implicated the sole action of dopamine on the ventral striatum (specifically, the nucleus accumbens) to explain schizophrenia [94]; and, up until very recently, antipsychotic medication generally has aimed to counteract this dopamine excess, via blockade of the D2-dopamine receptor [93, 95]. These anti-dopamine antipsychotic medications were effective at reducing hallucinations and other positive symptoms of schizophrenia, and became top-selling drugs in Canada [96] and the US [97], with annual sales approaching 10 billion dollars in the latter. However, even though there is substantial evidence supporting the role of dopamine in the pathophysiology of schizophrenia, the single action of dopamine does not account for all the symptoms of this disorder, especially those classified as negative or cognitive symptoms; nor do dopamine theories alone explain the disease's often progressive clinical course despite treatment targeting dopamine receptor blockade.

More recently, proponents of glutamatergic models of schizophrenia have stepped forward, since these chemicals offer a somewhat better explanation (1) for the changes in dopaminergic activity that are observed, and (2) for the negative symptoms patients experience [98]. For example, drugs that block N-methyl-D-aspartate (NMDA) glutamate receptors can induce both positive and negative symptoms of schizophrenia, in healthy volunteers and in schizophrenics [99–101]. Increased glutamatergic activity could be responsible for excitotoxic damage that spreads through the basal ganglia thalamo-cortical pathway [102], ultimately reaching regions of the ventral tegmentum that control the release of dopamine in the ventral striatum (see Fig. 4.3). The anterior cingulate and the thalamus are part of this pathway and both receive glutamatergic input. The anterior cingulate plays a role in the integration of affective components into behaviour [103], while the thalamus is a sensory gateway and also plays a role in the learning of new behaviours [104], something which most schizophrenics find inherently difficult. Abnormal glutamatergic inputs or neuronal damage within these regions could cause dysfunction that is consistent with the symptoms of schizophrenia. Glutamatergic models of schizophrenia have successfully explained results gleaned from experiments in rat models of schizophrenia [105] but have yet to be fully substantiated in humans. In addition, pharmacological agents that act upon glutamate receptors, such as ketamine, have been administered to humans and shown to exacerbate schizophrenic symptoms [100, 103]; and, although no glutamate-based antipsychotic currently is used widely in humans [99], such drugs are under investigation. Clinical trials assessing the co-administration of standard antipsychotic medication plus either glycine [106], D-serine or D-cycloserine [107–111], all substances that stimulate NMDA glutamate receptors, have been performed with generally favorable outcomes; and, as will be detailed later in this chapter, the encouraging results of a phase II clinical trial using a single novel anti-psychotic drug that activates glutamate receptors also have been published within the past couple of years [112].

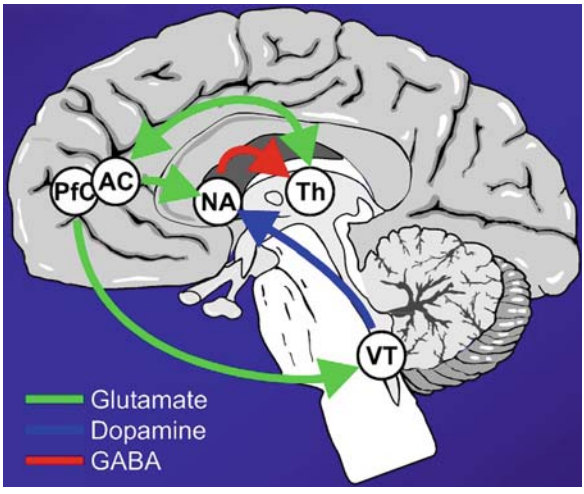


Fig. 4.3 The limbic basal ganglia thalamocortical pathway. A brain circuit potentially implicated in schizophrenia

4.5 Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS)

4.5.1 Brief History

The interaction of radiofrequency (RF) electromagnetic radiation with the nuclei of certain atoms when placed in strong magnetic fields was discovered independently, in 1945, by Purcell [113] from the Massachusetts Institute of Technology (MIT) and Bloch [114] from Stanford. This phenomenon is now referred to as *nuclear magnetic resonance* or NMR. This discovery led to the measurement of the magnetic moments of several atomic nuclei. Nuclei possessing a nuclear magnetic moment were quickly investigated and the interest in NMR would have certainly died amongst the physics community if it were not for the discovery of a phenomenon called *chemical shift* a few years later. Proctor and Yu [115] and Dickinson [116] first observed chemical shift in 1949, their experiments revealing the dependence of NMR signal frequencies on the nuclei's chemical environment (via electronic shielding). Interest in a non-destructive chemical analysis tool sparked tremendous excitement in chemistry research, such that NMR became the tool of chemists. However, it was not until the 1970s that NMR was used to study living tissues [117]. Lauterbur [118] and Mansfield [119] described the first principles of magnetic resonance imaging during the same period. Nowadays, several techniques exist by which to localize the origin of NMR signals in vivo. Signal contributions from very abundant compounds (water, lipids) can be suppressed [120], and quantum mechanical formalism used to filter out signals from other unwanted compounds [121]. Spectroscopy and imaging techniques can be combined to yield in vivo chemical maps of disease conditions [122]. Those technological advances have brought ^{1}H NMR out of the physics lab and into the hospital for clinical and research applications. Somewhere in the course of this transformation, NMR, a field of research laden with acronyms, lost its leading 'N', for 'nuclear', because of its association, in popular culture, with ionizing radiation.

4.6 Basic Magnetic Resonance Principles

When exposed to a high-intensity magnetic field, which we can label B_0, atomic nuclei that possess a magnetic moment naturally rotate around the axis of the field at a characteristic frequency (Larmor precession frequency). The orientation of individual nuclei is a mixture of two eigenstates, in which the longitudinal component of the magnetic moment comes to lie either parallel or *anti-parallel* to the direction of B_0. Statistically, within a large population of nuclei such as that found in a small volume of human brain tissue, a slightly larger probability exists for the magnetic moment of the individual nucleus to be in a mixture of states dominated by the parallel eigenstate rather than dominated by the anti-parallel state. Therefore, a net amount of magnetisation, M_0, is established in the tissue at the macroscopic

level along the direction of B_0. This phenomenon is beautifully illustrated in a 2008 teaching article by Lars Hanson [123]. An immediate consequence of this phenomenon is that tissues exposed to larger magnetic fields will magnetize to a greater degree, resulting in more macroscopic magnetisation to work with, per unit volume of tissue. We will see in the paragraphs below how macroscopic magnetization of human tissues can be used to produce maps of the spatial distribution of water molecules (anatomical MRI) or quantify the chemical content of a localized area (MRS).

The usual unit used to indicate the strength of static magnetic fields utilized in MR is the Tesla (T). A magnetic field intensity of 1.5 Tesla, as currently routinely used in clinical MRI scanners, is approximately 30,000 times greater than the background magnetic field of the Earth, which hovers at around 0.5 gauss (1 Tesla = 10,000 gauss).

4.7 Basic MR Scanner Hardware

An MR scanner is composed of several concentric electromagnetic wire windings that fit into one another to produce the various fields required for MRI or MRS. More specifically, the MR machine has three basic components: (1) a primary winding that produces the large static magnetic field; (2) three gradient coils that produce fields that vary linearly with position; and (3) a radiofrequency coil used to manipulate the tissue's macroscopic magnetization and receive the MR signals.

The *primary winding* must support the high electrical currents necessary to produce high intensity magnetic fields within the scanner bore in the range of 1.0 Tesla and above. Consequently, this winding is typically composed of a superconductive material kept below its superconductive threshold in a bath of liquid helium thermally insulated from the rest of the equipment by a cryostatic chamber. Most MRI machines commonly used in clinical MRI use magnetic fields of 1.5 to 3 Tesla, but MRI scanners used for human research purposes use a main field varying from 1.5 Tesla up to 10 Tesla [124].

In addition to this main magnet, MRI devices have three *gradient coils*, which normally fit within the bore of the cryostatic chamber and are designed to expose either the whole body or only the head to a weak magnetic field linearly changing with position along one of three orthogonal axes. Their role is mainly to allow spatial localization of the MR signal; i.e., to produce a change in the resonant frequency (or relative change in phase) of the precessing tissue magnetization that depends linearly on tissue position. *Radio frequency (RF) coils* are used to transmit and receive electromagnetic signals at the resonant frequency of a given nuclei to manipulate the orientation of the local macroscopic magnetisation vector. When an appropriate RF pulse is transmitted, the local macroscopic magnetization, normally aligned with the main magnetic field, will tip out of alignment and begin a precession motion around the axis of the main field. With the RF coil now in receive mode, the precessing magnetization will induce a voltage within the RF coil that varies in time at its resonant frequency. This oscillating voltage is referred to as the MR signal.

4.8 Magnetic Resonance Imaging (MRI)

By carefully applying combinations of RF pulses and magnetic gradients, one can introduce a known relationship between precession frequency (or precession phase) and position along a given axis. This careful combination of RF pulses and magnetic field gradients is known as a pulse sequence and the art of developing pulse sequences is known as pulse programming. The MR signal received by the RF coil is a combination of signals from all atomic nuclei within the sphere of influence of the RF coil. For a given pulse sequence, one can use mathematical tools to identify the spatial origin of signals received based on the known relationship between precession frequency and position that was purposely introduced. In this manner, signal localization can be achieved along 1–3 spatial dimensions. When this procedure is conducted using the MR signal produced by hydrogen nuclei within water molecules, the resulting maps of the spatial dependence of the signal intensity are called MRI images. The intensity of the signal is represented on a gray scale and is determined by the concentration of water within each tissue (proton density) as well as the characteristic time of return to equilibrium (alignment with B_0) of the macroscopic magnetization in each tissue (T_1 and T_2 relaxation).

4.9 Magnetic Resonance Spectroscopy

Although MRI is the most broadly spread application of the magnetic resonance phenomena in human medicine, one is not limited to obtaining MR signals from the water molecules or from the hydrogen nuclei altogether. Molecules other than water also contain hydrogen nuclei and can produce MR signals with specific signatures, which are based upon their specific molecular configuration and the chemical shift of their resonance, caused by varying degrees of electronic shielding provided to any given hydrogen nuclei within the molecule. Molecules that contain more than one hydrogen nuclei close in space may also exhibit interactions between nuclei that will produce characteristic splitting of their associated resonances. It is, therefore, clear how MRS can be of use in the non-invasive analysis of brain chemistry.

The list of nuclei with magnetic moments does not stop at hydrogen. In vivo MRS of phosphorus (^{31}P), carbon (^{13}C), lithium (^{7}Li) and fluorine (^{19}F) nuclei have been performed successfully in humans [125].

From a technical standpoint, the hardware required for ^{1}H-MRS is identical to that used for routine MRI, and the basic NMR theory is also completely analogous to what has been described previously for MRI. For MRS of nuclei other than hydrogen, specially designed RF coils must be used to allow for reception and transmission at a vastly different resonant frequency. Otherwise, the major differences between MRI and MRS occur within the pulse sequences used to obtain an MRS signal from a localized tissue structure.

4.9.1 In Vivo Proton Spectroscopy of the Human Brain

Hydrogen is one of the numerous atoms whose nucleus possesses a magnetic moment. Because of its small mass (a single proton), the hydrogen nucleus exhibits the largest *gyromagnetic ratio* (γ; i.e., the ratio of its magnetic moment to its angular momentum). Combined with its high natural abundance (99.985%), its high NMR sensitivity (proportional to γ^3) makes it the easiest nuclei to observe. Most molecules found in the brain contain at least a few hydrogen atoms and have the potential of producing an NMR spectral line. Unfortunately, if the molecule's concentration is below 1 mM, or if the molecule is tightly bound to macromolecules, it may be difficult or impossible to obtain a signal from it. In the human brain, many metabolites have concentrations between about 1 and 10 mM and are sufficiently free to produce measurable NMR signals. The signal water generates is considerably larger than any other signal, due to the large concentration of water in brain tissue (~55.5 M), the signal being approximately 10,000 times greater than for any other metabolite. This explains why the spatial resolution of MRI can easily reach $1 \times 1 \times 1$ mm,while ^{1}H-MRS examinations are limited to volumes of interest approximately $1 \times 1 \times 1$ cm or larger. In spectroscopy, the disproportionately large NMR signal from water must be actively suppressed, lest it overwhelm the rest of the spectra. Once the water peak issue has been addressed, the in vivo metabolite spectrum is revealed. An example short echo time STEAM spectrum with usual peak assignments is presented in Fig. 4.4.

Approximately thirteen metabolites can be quantified in vivo in brain tissue: N-acetylaspartate, glutamate, glutamine, GABA, aspartate, N-acetylaspartylglutamate, glucose, choline, creatine, phospho-creatine, myo-inositol, scyllo-inositol, and taurine; and each has its own specific in vivo line-widths (Table 4.1).

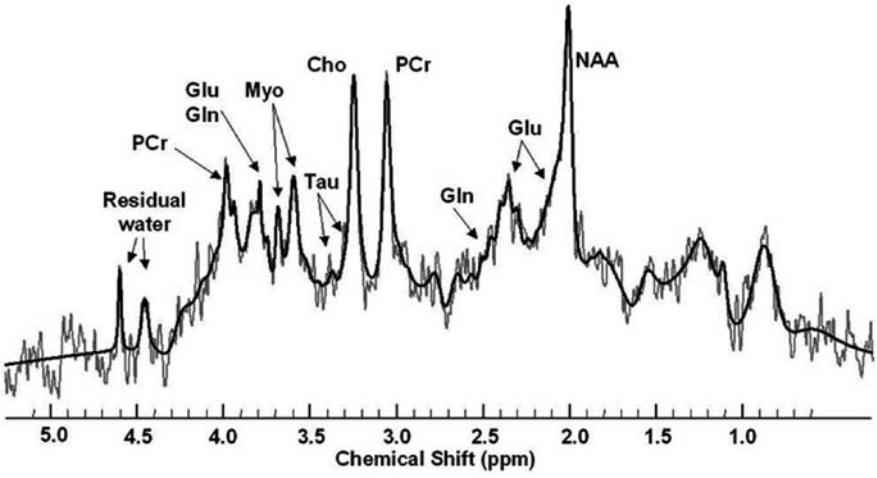


Fig. 4.4 Typical in vivo STEAM spectrum from the left anterior cingulate. (1.5 cc voxel, 4.0 Tesla, TE = 20 ms, 256 averages, 2 Hz exp. filter)

Table 4.1 Thirteen metabolites routinely measured in human brain 1H-MRS

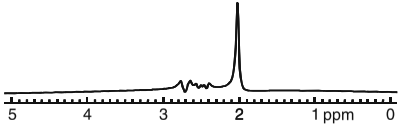
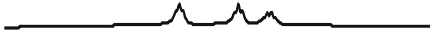
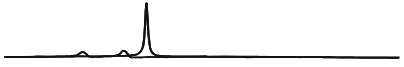
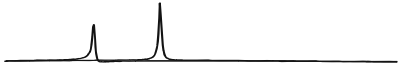
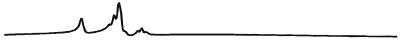
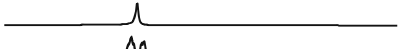
Metabolite	In vivo concentration ($mmol/kg_{ww}$)	Number of protons	Spin system*	STEAM20 spectrum (in vivo linewidths)**
N-acetylaspartate	7.9–16.6	7	S_3, ABX, D	
Glutamate	6.0–12.5	5	AMNPQ	
Glutamine	3.0–5.8	5	AMNPQ	
GABA	0.2–1.4	6	$A_2M_2X_2$	
Aspartate	1.0–1.4	3	ABX	
N-acetylaspartyl-glutamate	0.6–2.7	11	S_3, ABX, $AMNP_2$	
Glucose	1.0	7	ABC-MNO-X, AB-MNOP-X	
Choline	0.9–2.5	13	S_9, M_2,M_2	
Creatine	5.1–10.6	6	S_3, S_2, S	
Phosphocreatine	3.2–5.5	7	S_3, S_2, S, S	

Table 4.1 (continued)

Metabolite	In vivo concentration ($mmol/kg_{ww}$)	Number of protons	Spin system*	STEAM20 spectrum (in vivo linewidths)**
Myo-Inositol	3.8–8.1	6	2DD, 4S	
Scyllo-Inositol	0.3–0.6	6	S_6	
Taurine	0.9–1.5	4	A_2X_2	

*Singlets produced by n magnetically equivalent protons are denoted by S_n. Doublets and Doublets of Doublets are denoted D and DD respectively. Multiplets produced by n spins are noted M_n

**Only resonances upfield of water are shown. The Glutamate and Glutamine spectra only show resonances associated with the MNPQ spins of their respective spin systems. The glucose spectrum represents the resonances from the beta anomer only.

The metabolites that generally have received the most attention in the schizophrenia neurodegeneration literature have been N-acetylaspartate, glutamate, glutamine and, to a lesser extent, choline, creatine, and metabolites measurable by ^{31}P-MRS. In brief, *N-acetylaspartate* (NAA) is an amino acid, typically considered to be a marker of neuronal integrity and function. Glutamate (Glu) is another amino acid and the main excitatory neurotransmitter of the brain; in fact, about 85% of all energy expenditure in the brain relates to supporting glutamatergic neurotransmission [126]. *Glutamine*(Gln), an amino acid synthesized from glutamate within astrocytes, is a precursor and storage form of glutamate. *Cholines* generally are markers of cell membrane integrity, with the choline peak (also called the trimethylamine peak or TMA) containing contributions from free choline (Cho), phosphocholine (PCho) and glycerophosphocholine (GroPCho), free choline only contributing about 5% of the choline signal; the choline peak typically is associated with cellular membrane metabolism. Other chemicals of interest are *phosphocreatine* (PCr), which is involved in energy metabolism; *myo-inositol* (Myo), which can serve as a marker of astrocyte density; and *γ-aminobutyric acid* (GABA), the main inhibitory neurotransmitter of the brain.

4.10 Volumetric MRI Studies of Schizophrenia

As described earlier, ventricular enlargement in schizophrenia, signifying brain atrophy, was first documented in 1927 through pneumoencephalography, and later was confirmed by numerous other research groups, by a variety of means that have included both direct post-mortem examinations, CT, and most recently, MRI. By virtually all these means, volumetric reductions have been identified in several cortical regions, especially frontal and temporal, and several sub-cortical regions, in particular the hippocampus [15, 17, 127–130]. Most MRI studies have used manual or semi-automated methods to delineate the contour of brain structures of interest, and have estimated volumes by counting the number of *voxels* (a 3-dimensional volume element) within this contour and multiplying this count by the known volume of the imaging voxel. In the past decade, two meta-analyses have been published assessing voxel-based volumetric studies in schizophrenia patients. In the first of these, published in 2000, Wright et al. analyzed 58 studies, spanning from 1988 to 1998, in which a total of 1588 independent schizophrenia patients were compared against a similar number of controls gleaned from the same studies. The vast majority of these controls were healthy and unrelated, though there was a single study with 15 monozygotic twins who were discordant for schizophrenia, three small studies (total $n = 68$) whose controls were unrelated but had minor illnesses, and a single study with 20 controls for whom such details were not provided. Forty-five of the 58 studies used a 1.5 Tesla MR machine, though the range was from 0.25 to 2 T. Analysis revealed a mean 2% decrease in overall cerebral volume and a mean 26% increase in total ventricular volume among patients

[129]. Areas that were particularly reduced in size were the left parahippocampus (by 7%), the right and left amygdyla, and left amygdyla/hippocampus (all by roughly 6%), and the right parahippocampus and amygdyla/hippocampus (both by 5%). Interestingly, in the twin study, in 12 of 15 discordant pairs, the schizophrenic twin was identified by blinded visual inspection of cerebrospinal fluid spaces [131]. Quantitative analysis again revealed a smaller left and right hippocampus in 14 and 13 affected twins, respectively. Similarly, in schizophrenic versus unaffected twins, the lateral ventricles were larger on the left in 14 and on the right in 13, and the third ventricle larger in 13. None of these differences were identified in seven sets of monozygotic twin controls without schizophrenia. The authors concluded that these anatomical differences were both consistent and not entirely explained by genetics.

In a more recent meta-analysis in which only voxel-based volumetric studies were included, Honea et al. identified 15 studies, incorporating 390 schizophrenic patients and 364 healthy volunteers [128]. Studies spanned from 2001 to May 2004; eleven used 1.5 T scanners, three 1.0 T, and one a 2.0 T scanner. In total, 50 regions of the brain were identified as having been decreased in volume in at least one of the 15 studies, but only two sites exhibited a decreased volume in the majority of studies, both of these sites on the left side: the left medial temporal lobe and the left superior temporal gyrus (in 9 and 8 of 15 studies, respectively). Areas that were reduced in volume in half the studies were the left medial and the left inferior frontal gyrus, the left parahippocampal gyrus, and the right superior temporal gyrus. The authors further compared the twelve studies performed in chronic patients against the three in whom patients were amid their first schizophrenic episode; and, albeit with two few first-episode studies for a statistical comparison of proportions, the authors nonetheless noted certain discrepancies: the left medial frontal gyrus was reduced in 64 and 0%, and the right anterior cingulate reduced in 27 and 100% of studies with chronic versus first-episode patients, respectively. As a final comparison, they noted that the range of smoothing kernels used across the studies was from 4 to 12 mm, and re-assessed the results according to whether a small (4–8 mm) or large (10–12 mm) smoothing kernel had been used. They concluded that, in general, a greater percentage of studies in which a smaller smoothing kernel was used demonstrated volume reductions in small structures, like the medial temporal lobes, and suggested that this reduced sensitivity of larger kernel devices could account for some of the inter-study discrepancies.

Recently, given technical advances and the development of complex algorithms, the question of whether or not schizophrenic patients have reduced cortical thickness has been addressed in a few studies, including treated patients [127, 132], first-episode (FE) patients [133, 134], and those with childhood- or adolescent-onset schizophrenia [135]. In each study, significant broad-based cortical thinning was noted. In one study of 33 treated patients versus 32 healthy volunteers, patients demonstrated cortical thinning involving the orbitofrontal cortices bilaterally; the left inferior frontal, inferior temporal, and occipitotemporal cortices; and the right medial temporal and medial frontal cortices [132]. In another study of treated patients published just this year, subvoxels were counted from 3-dimensional MRI

scans collected using a 1.5 T scanner to assess 155 affected patients, 192 affected siblings, and 196 normal controls [127]. It was determined that patients, but not their unaffected siblings, had significant cortical thinning relative to healthy controls, especially involving the frontal lobes, but also in temporal, parietal, occipital and limbic regions, with temporal lobe reductions most pronounced on the right. Narr et al. have published the results of two recent studies on first-episode patients versus controls, and documented regional gray matter thinning in frontal, temporal and parietal heteromodal association cortices bilaterally [133], and within cingulate, occipital and frontopolar cortices [134], suggesting that regional reductions in cortical thickness and gray matter concentration are present at disease onset. Similarly, White et al. identified significant decreases in cortical thickness in children and adolescents with schizophrenia, relative to age-matched controls, which were most pronounced in the cortical tissue underlying the sulci [135]. Patients also exhibited relatively flattened curvature in the sulci and more steeped or peaked curvature in the gyri.

Up until the past few years, volumetric studies in schizophrenia patients generally have been cross sectional, assessing patients at a single stage of disease, most commonly after treatment has been initiated. Recently, a small number of longitudinal studies have been done to assess volumetric brain changes over time in these patients. In 2003, Sporn et al. published the results of a longitudinal study in which one or more follow-up scans were obtained at approximately 2-year intervals for 39 patients with childhood-onset schizophrenia (mean age 14.5) and 43 matched controls [136]. Rates of brain volume reduction were significantly higher among patients than among controls, with the rate of gray matter reduction related to pre-morbid impairment and baseline symptom severity. Unexpectedly, those with a higher rate of gray matter reduction responded better to treatment. Similarly, Thompson et al. studied adolescents with schizophrenia, and detected striking anatomical profiles of accelerated grey matter loss, when subjects were rescanned prospectively with MRI three times at 2-year intervals [137]. The earliest deficits they uncovered were in parietal brain; but, over the course of 5 years, these deficits progressed anteriorly into the temporal lobes to include the sensorimotor and dorsolateral prefrontal cortices and frontal eye fields. Moreover, these changes mirrored psychotic symptom severity and the neuromotor, auditory, visual search, and frontal executive impairments they observed in patients, even controlling for medications and IQ. Of particular note was that, early in the disease, temporal grey matter loss was absent, but it became quite prominent later on; and only later during the course of observation were changes identified in dorsolateral prefrontal cortex and the superior temporal gyri, two regions consistently affected in adult studies.

Cahn et al. [138] and Ho et al. [139] studied patients deemed to be early in the course of their illness, and both teams of investigators also revealed progressive gray matter loss. In the first study, 34 first-episode schizophrenia patients who had taken antipsychotic medication for less than 16 weeks and 36 matched healthy controls underwent MRI at baseline and after one year, with clinical outcomes measured at two years [138]. Both total brain volume and gray matter volume decreased

(by 1.2 and 2.9%, respectively), and lateral ventricle volume increased (by 7.7%) in patients versus controls. The decrease in global gray matter volume correlated significantly with outcome and, independently, with a higher cumulative dose of antipsychotic medication. Moreover, at 5-year follow-up, a greater decrease in total brain volume predicted a higher negative symptom score and a lesser likelihood of living independently; and a greater decrease in gray matter volume predicted higher positive and negative symptom scores, lower-level functioning, and a decreased likelihood of living independently [140]. Five-year follow-up MRI in the same patient population identified an association between a longer duration of psychosis, a greater decrease in gray matter volume, and a greater increase in ventricular volume [141].

Ho et al. studied the longitudinal progression of structural brain abnormalities in 73 recent-onset schizophrenic patients and 23 controls and identified, in patients, accelerated enlargement of cortical sulcal cerebrospinal fluid spaces, progressive reduction in frontal lobe white matter volume, and a reciprocal increase in frontal lobe cerebrospinal fluid volume [139]. Patients with poor outcomes exhibited more lateral ventricular enlargement over time than patients who did well; progressive loss of frontal lobe white matter volume and increased frontal lobe CSF fluid volume were associated with greater negative symptom severity; and reduced frontal lobe gray and white matter volumes correlated with poorer executive functioning.

In a 5-year longitudinal study, Van Haren et al. obtained MRI whole brain scans from 96 patients who had had schizophrenia for an average of over 10 years, and from 113 healthy matched controls, and identified excessive decreases in gray matter density in patients in the left superior frontal area, left superior temporal gyrus, right caudate nucleus, and right thalamus, relative to controls [142]. Excessive gray matter density decline involving superior frontal gray matter was associated with an increased number of hospitalizations, whereas lesser decreases in this area were associated with a higher cumulative dose of either clozapine or olanzapine over the scan interval.

Meanwhile, Mathalon et al. studied 24 men with chronic schizophrenia versus 25 controls, subjects in both groups undergoing two brain MRI imaging scans, an average of four years apart [143]. Again, schizophrenic patients exhibited a faster rate of volume decline than controls, specifically of right frontal gray matter and bilateral posterior superior temporal gray matter; and CSF volume expanded in the right frontal sulci, left lateral ventricle, and bilateral prefrontal and posterior superior temporal sulci. Again, there were associations between brain volume loss and both positive and negative symptoms. And Wood et al. longitudinally evaluated hippocampal and temporal lobe, as well as whole-brain and intracranial volumes at two time points, approximately two years apart, in 30 patients with first episode psychosis and 12 with chronic schizophrenia, relative to 26 healthy controls [144]. Whole-brain volume diminished significantly over the follow-up interval in both patient groups, but the rate of this volume loss was no different in first episode versus chronic patients, and no other differences versus controls were noted.

Other longitudinal studies that have assessed focal brain areas have identified specific volume losses over time, such as enticing evidence demonstrating an association between progressive gray matter volume reductions in the right posterior fusiform gyrus and low extroversion scores [145]; and progressive volume decreases in the left Heschl gyrus and planum temporale [146], and left superior temporal gyrus [147]. One limitation in terms of interpreting these studies is that the 'focal' decreases may have been part of a global decline in brain volume, rather than truly being selective.

Several other problems arise when using volumetric studies as a measure of disease progression in schizophrenia. One potential problem is that volume loss has not always correlated with predicted losses in function [148]. Another is that volumetric changes are highly non-specific, being a normal part of aging, and being associated with behaviours like cigarette smoking and heavy alcohol use [149, 150], both of which may be particularly common among those with psychiatric illness. Volumetric losses do appear to be independent of increases in CSF, however [151]. Perhaps even more limiting a problem is that volume loss does not necessarily represent the loss of neurons, since about 90% of all brain cells are glial, and quantitative MRI cannot discriminate between glia and neurons. It was Deicken et al., in 1999, who published work comparing the use of MRI and MRS in schizophrenia, performing quantitative MRI and ^{1}H MRS on the right and left hippocampal regions in 23 chronic schizophrenic patients and 18 controls [33]. Relative to controls, the schizophrenia patients demonstrated no change in hippocampal volumes on either side, but significantly decreased NAA in the hippocampal regions bilaterally. There also was no correlation between hippocampal volumes and NAA in either subject group. The authors concluded that hippocampal NAA, measured via MRS, may be a more sensitive measure of neuronal loss than volumetric measurements using MRI; and that, in schizophrenics, reduced hippocampal NAA may be evidence of neuronal dysfunction or damage, instead of neuronal loss.

4.11 Magnetic Resonance Spectroscopy and Schizophrenia

The ability of proton magnetic resonance spectroscopy (^{1}H MRS) to measure levels of brain metabolites and neurotransmitters non-invasively makes it an ideal tool by which to further our understanding of schizophrenia.

Abnormal levels of brain metabolites and neurotransmitters have been identified in numerous post-mortem studies [56, 152]; but these results add little to the discussion of what happens over time in the living patient. Consequently, the study of the pathophysiology of schizophrenia has been forced to turn toward non-invasive techniques by which to measure neurotransmitter and metabolite activity in vivo. Among the various approaches has been the use of 1H-MRS, with NAA, glutamate/glutamine, choline and creatine being the metabolites most commonly evaluated. Prior research has shown that ^{1}H-MRS measurements of these metabolites are reasonably reproducible [78, 153].

4.12 N-Acetylaspartate in Schizophrenia

NAA is an amino acid that is present in high concentrations throughout the CNS. From the standpoint of MRS, advantages of NAA are (1) that it is the most common metabolite detectable by MRS, so that the precision of its measurement exceeds that of all other metabolites; and (2) that it is found almost exclusively in neurons, and not in glial cells [154, 155]; consequently, levels of NAA in brain tissue generally have been interpreted as indicating the quantity (or concentration) of viable neurons in the tested area [156].

Dating back to the last decade of the 20th century, numerous MRS studies have been published examining levels of NAA in the brain of living schizophrenic patients [18–67]. It is beyond the scope of this chapter to review every single one, but Table 4.2 presents essential details from those studies published this decade. As can be seen, about half of these studies have been small, with fewer than 20 subjects having schizophrenia, though studies have tended to get larger, and hence to have greater statistical power to detect inter-group differences, as the decade has progressed. Most studies have involved patients who have had schizophrenia for some time and have been on treatment, though some studies involved first episode (FE) patients [19, 45, 51, 54, 55, 69, 71, 74], and two studies evaluated first-episode drug-naïve (FEDN) patients [61, 72]. Two studies were restricted to children and adolescents [46, 53]. The vast majority of studies were controlled, though matching, even for just age and gender, often was not noted. Most controls were healthy volunteers, though one study used unaffected siblings as the control group [27].

In general, levels of NAA, whether measured in absolute terms or as a ratio to the level of creatine (NAA/Cr), have been reduced in patients versus controls, but there is some degree of inconsistency between results. A relatively recent review and meta-analysis of the literature on schizophrenia and MRS-detectable brain metabolites, has been written by Steen, Hamer and Lieberman [63]. In this paper, Steen et al. identified 64 published English-language papers that used ^{1}H-MRS to measure NAA concurrently in 1256 schizophrenia patients and 1209 healthy controls. Field strength was 1.5 T in 88% of the studies, and 77% of the studies focused on patients with chronic disease. Consistent with a review of Table 4.2 in this chapter, NAA was reported to be reduced in a broad range of tissues in the brains of schizophrenic patients, relative to controls; but most notably in the hippocampus and in both gray and white matter of the frontal lobe cortex. Although some have hypothesized and tested for differences in NAA levels in those with early versus late disease, and in white matter (WM) versus gray matter (GM), the literature fails to support this with any conviction. As mentioned earlier in this section, most studies have been underpowered, with only three of the 64 studies included in Steen's meta-analysis sporting the 80% power necessary to detect a 10% NAA reduction in patients, and none adequately powered to detect a 5% NAA reduction.

Table 4.2 Human MRS studies examining CNS levels of N-acetylaspartate (NAA) in schizophrenia (SCZ)

Authors	Year published	Area(s) examined	Patients/Controls	Findings
Rusch et al. [58]	2008	hippocampus, amygdyla, DLPF cortex	29 SCZ/32 CL	Glutamate, not NAA, low and related to executive functioning.
Sigmundsson et al. [60]	2008	DLPF cortex	25 SCZ with 'deficit syndrome'/26 matched CL	NAA low in SCZ, and negatively correlated with symptom severity (esp. +ve Sx); positively correlated with social function.
Molina et al. [52]	2007	prefrontal cortex	11 SCZ, 13 Bipolar/10 CL all male	low NAA in both SCZ and bipolar d/o.
Aydin et al. [19]	2007	corpus callosum	12 FE, 16 chronic/28 matched CL	NAA levels low in both FE and chronic SCZ.
Zabala et al. [69]	2007	DLPF cortex	8 FE-SCZ, 15 FE psychosis/32 CL; all adolescents	NAA/water low on left in FE-SCZ.
Tang et al. [68]	2007	DLPF WM, medial temporal WM, occiptal WM	40 SCZ/42 CL	NAA low in medial temporal white matter bilaterally.
Galinska et al. [45]	2007	L. frontal and temporal lobes, thalamus	30 FE-SCZ/19 CL	No reductions in NAA/Cr or NAA/H2O ratios vs. controls.
Szulc et al. [66]	2007	multiple selected regions	58 treated SCZ/21 CL	NAA low in thalamus in SCZ taking typical anti-psychotics; not low in SCZ taking atypical anti-psychotic drugs
Stanley et al. [61]	2007	L. DLPF cortex	15 FEDN-SCZ, 3 schizoaffective/61 matched CL	NAA low in SCL patients, but there was an age effect, with only early-onset patients having low NAA.
Ohrmann et al. [55]	2007	DLPF cortex	15 FE-SCZ, 20 chronic/20 matched CL	low NAA, glutamate/glutamine and choline NAA Levels correlate with verbal learning and memory.
Shimizu et al. [59]	2007	posterior cingulate gyrus (PCG), medial temporal lobes	19 chronic SCL/18 matched CL	NAA/Cr low in PCG in SCZ vs. CL.
Molina et al. [51]	2006	DLPF cortex	34 SCZ (17 FE)/50 CL	No NAA correlation with volume losses.

Table 4.2 (continued)

Authors	Year published	Area(s) examined	Patients/Controls	Findings
Wood et al. [71]	2006	L. prefrontal and temporal cortex	46 FE-SCZ	NAA/Cr ratio in prefrontal cortex predicts 18-month outcome.
Jessen et al. [48]	2006	L. frontal lobe, L. ant. cigulate gyrus, L. superior temporal lobe	19 'at-risk', 21 SCZ/31 CL	NAA/Cr and NAA/Cho low in L. frontal lobe; NA/Cr low in ant. cingulate gyrus – in SCZ and at-risk groups vs. CL
Tanaka et al. [70]	2006	frontal lobe	14 chronic SCZ/13 CL	NAA low; choline, creatine and NAA/Cre not reduced NAA correlated with negative symptoms and card sorting
Szulc et al. [65]	2005	frontal and temporal lobes	14 SCZ treated with risperidone	NAA and myo-inositol increase with Rx; positive symptoms correlate positively with pre-Rx NAA in frontal lobe, but negatively in temporal lobe. Glx corrleates with negative symptoms in temporal lobes.
Jakary et al. [47]	2005	mediodorsal and anterior thalamus	22 male SCZ/22 male CL	NAA lower in SCZ at both locations; no reduction in Cho or Cr.
Ende et al. [41]	2005	cerebellum and pons	14 SCZ/14 CL	NAA low in cerebellar cortex and vermix in SCZ.
Molina et al. [50]	2005	DLPF cortex	16 recent-onset, 19 chronic SCZ/	Chronic SCZ had low NAA/Cr ratios on left vs. both other groups.
Ohrmann et al. [54]	2005	DLPF cortex	18 FE-SCL, 21 chronic SCZ/21 matched CL	Chronic SCZ had low NAA and Glx vs. both other groups.
Theberge et al. [72]	2004	L. anterior cingulate, L. thalamus	19 FEDN-SCZ	Thalamic NAA negatively correlatd with duration of prodrome; Cho positively correlated with duration of untreated psychosis.
Blasi et al. [26]	2004	multiple brain regions	17 SCZ/17 matched CL	NAA/Cr low in hippocampus vs. CL.
O'Neill et al. [53]	2004	multiple brain regions	11 children and adolescent SCZ/20 matched CL	NAA low in thlamus; Cho high in superior ant. Cingulate, frontal cortex and caudate head.

Table 4.2 (continued)

Authors	Year published	Area(s) examined	Patients/Controls	Findings
Wood et al. [74]	2003	L. medial temporal and L. DLPF cortex	56 FE, 30 at high risk/21 CL.	No significant inter-group differences noted.
Ende et al. [40]	2003	hippocampus, thalamus, basal ganglia	13 treated SCZ/13 match CL	Low NAA in hippocampus and thalamus; no difference in creatine, phosphocreatine or choline
Bertolino et al. [25]	2003	hippocampus and DLPF cortex	24 schizophreniform disorder/24 CL	reduced NAA/Cre ratios at both locations
Delamillieure et al. [36]	2002	hippocampus, thalamus, prefrontal cortex	17 SCZ/14 CL	No reduction in NAA, choline or myo-inositol
Yamasue et al. [75]	2002	anterior cingulate gyrus	16 SCL/15 CL	Low NAA/Cho and high Cho/Cre ratio in SCZ vs. CL.
Bustillo et al. [28]	2002	DLPF cortex	10 minimally-treated SCZ/10 CL 1-year longitudinal study	No inter-group differences at baseline; frontal lobe NAA declined with treatment, but was not correlated with outcome.
Hagino et al. [46]	2002	frontal lobe	13 young SCZ/13 CL	Low NAA/Cre in L. inferior frontal cortex; NAA/ phosphocreatine positively correlated with learning performance
Auer et al. [18]	2001	thalamus and white matter	32 acutely-ill, treated SCZ/17 matched CL	Thalamic NAA low in SCL; Cho, Cr and mI elevated in WM
Ende et al. [39]	2001	thalamus	15 stable treated SCZ/15 matched CL	Low NAA in mediodorsal thalamus bilaterally; R. and L. correlated.
Steel et al. [62]	2001	prefrontal and temporal lobes	10 SCL/10 CL	non-statistically significant 10-15% reduction in NAA
Bertolino et al. [24]	2001	multiple brain areas	23 SCL; longitudinal within-subject clinical trial	NAA rose significantly in DLPF cortex during drug treatment
Tibbo et al. [73]	2000	cerebellum	12 R.-handed male SCL/12 CL	No inter-group differences in NAA/Cr or Cho/Cre.

Table 4.2 (continued)

Authors	Year published	Area(s) examined	Patients/Controls	Findings
Callicott et al. [29]	2000	prefrontal	36 SCL	Regionally selective correlation between NAA/Cr and negative symptoms
Block et al. [27]	2000	L. frontal lobe and basal ganglia	SCL patients/unaffected family members	Low frontal NAA/Cr in patients.
Omori et al. [56]	2000	L> thalamus and frontal lobe	20 SCL/16 matched CL	low NAA/Cr and Cho/Cr in thalamus vs. CL.
Deicken et al. [34]	2000	thalamus	17 treated male SCL/10 male CL	Low NAA bilaterally.
Delamillieure et al. [35]	2000	medial prefrontal	22 SCZ (5 deficit syndrome [DS])/ 21 CL	The 5 SCL patients with Deficit syndrome had lower NAA/Cr and NAA/ phosphocreatine than SCL without DS or CL.
Ende et al. [38]	2000	anterior circulate gyrus	19 stable treated SCL/16 CL	NAA low vs. controls; those on typical anti-psychotic drugs had lower mean NA than those on atypical drugs.
Fukuzako et al. [43]	2000	L. medial temporal lobe	40 treated SCL 40 CL	low NAA/Cr in SCL; lower NAA/Cr in those with undifferentiated vs. paranoid schizophrenia.
Bertolino et al. [23]	2000	DLPF cortex	13 SCL/13 CL	NAA low and strongly correlated with working memory.

NAA N-acetylaspartate, *Glx* glutamate/glutamine/GABA, *Cho* choline, *Cr* creatine, *mI* myo-inositol, *SCZ* schizophrenia, *FE* 1st episode, *FEDN* 1st episode, drug naïve, *L.* left, *R.* right, *DLPF* dorsolateral prefrontal, *WM* white matter

Most patients included in published studies already were on treatment prior to assessment, even many of the first episode patients. This may be important in terms of understanding the role of NAA in schizophrenia, given that some studies have demonstrated rapid changes, sometimes within weeks, on drug therapy [24, 38, 65–67]. Consequently, it is unclear what role, if any, NAA has in the course of disease. To date, for example, the role of NAA in health remains unknown. It has been shown to be involved in myelin synthesis [157] and in osmoregulation [158, 159], and it may have an anti-inflammatory role as well [160]. In one study in monkeys, an association was shown between NAA concentrations in the dorsolateral prefrontal (DLPF) cortex and the striatal availability of dopamine D2 receptors, the former identified by MRS and the latter via in vivo microdialysis [22], but this has not been confirmed in humans. In general, the assumption has been that, because NAA is present almost exclusively in neurons and in relatively high concentrations, declining levels mean either neuronal death/loss, or decreased neuronal function. But some even are questioning this [161]. This is one among many reasons that recent interest has turned somewhat towards the glutamatergic system, assessed via measurements of glutamate and glutamine as well as other neurotransmitters (namely GABA) and other metabolites.

In fact, in another more recent, but less comprehensive review that was published by Reynolds and Harte [162], the authors make the case that NAA might best be considered a marker of glutamatergic function, and that dysfunction of the glutamate-glutamine cycle might have the more central role in the underlying pathophysiology and neurodegenerative processes of this disease.

4.13 The Glutamatergic System in Schizophrenia

As already stated, glutamate is by far the most important excitatory amino acid in the brain. Glutamatergic neurons comprise the major excitatory pathways that link the cortex, limbic system, and thalamus, all regions that have been implicated in the pathophysiology of schizophrenia, based upon previously-mentioned volumetric, histologic and spectroscopic studies [163]. However, relative to NAA, interest in glutamate and its metabolites in the pathophysiology of schizophrenia only has been kindled within about the past decade. One potential reason for this is that, to detect and discriminate abnormalities in glutamate and glutamine, ^{1}H-MRS studies generally require shorter echo times and stronger fields than the 1.0 to 1.5 T fields used in most studies performed in the last decade of the 20th century. More recently, investigators have utilized up to 3.0 T [80, 84] and 4.0 T [81, 82, 164] devices with shorter echo times, which have allowed for the discrimination of glutamate and glutamine peaks. This level of discrimination is critical to the ability to quantify glutamate and glutamine levels separately with reasonable precision. Because glutamate is omnipresent within neurons and glial cells, measurements of glutamate levels by 1H-MRS can be difficult to interpret in terms of glutamatergic neurotransmission.

To properly understand whether glutamatergic neurons are more or less activated than normal, one must be able to assess the rate of the glutamate-glutamine cycle occurring within the synapses of glutamatergic cells. The glutamate levels detected by 1H-MRS include a large fraction of glutamate not directly part of the glutamate-glutamine cycle. However, of the fraction of glutamate used for neurotransmission and released in the synaptic cleft, some is actively up-taken by the astrocytes which isolate glutamatergic synapses from the extracellular space. This must occur to regulate synaptic glutamate levels and prevent excitotoxic damage. The astrocytes are the only cells that possess the enzymes required to convert glutamate into its storage form, glutamine, which does not have the excitotoxic properties of glutamate. As needed, glutamine can be shuttled back to the neuron and reconverted to glutamate for the next cycle of glutamate release. It therefore stands to reason that levels of glutamine measured by 1H-MRS may be a better proxy of the rate of the glutamate-glutamine cycle than levels of glutamate themselves [80, 81]. Unfortunately, the weak MRS signals of glutamine make its concentration measurements difficult, so that the use of high field scanners and short echo time pulse sequences becomes advantageous.

The case that Reynolds and Harte make in their review for the glutamatergic system being worthy of investigation harkens back to a 2002 study on mesial temporal lobe epilepsy, in which a highly-significant association between hippocampal N-acetylaspartate and glutamate content was observed [165]. However, it was five years earlier that Bartha et al. published a study demonstrating increased glutamine levels in the medial prefrontal cortex of never-treated schizophrenia patients, relative to healthy controls, speculating that this finding most likely reflected decreased glutamatergic activity [76]; and a couple of years before that when Olney and Farber first proposed the combined dysfunction of dopamine and N-methyl-D-aspartate (NMDA) glutamate receptors as playing a pathophysiological role in schizophrenia [166].

As the conviction that dopamine excess is the crucial neurochemical abnormality in schizophrenia has waned, and recognition that NAA decreases may merely be an indicator that some other process has increased, a ‘glutamate hypothesis’ for schizophrenia has emerged [163, 167–170]. Tsai has described this hypothesis succinctly:

> The ‘glutamate hypothesis’ of schizophrenia has emerged from the finding that phencyclidine (PCP) induces psychotic-like behaviors in rodents, possibly by blocking the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, thereby causing increased glutamate release. N-acetyl aspartylglutamate (NAAG), an endogenous peptide abundant in mammalian nervous systems, is localized in certain brain cells, including cortical and hippocampal pyramidal neurons. NAAG is synthesized from N-acetylaspartate (NAA) and glutamate, and NAA availability may limit the rate of NAAG synthesis. Although NAAG is known to have some neurotransmitter-like functions, NAA does not. NAAG is a highly selective agonist of the type 3 metabotropic glutamate receptor (mGluR3, a presynaptic autoreceptor) and can inhibit glutamate release. In addition, at low levels, NAAG is an NMDA receptor antagonist, and blocking of NMDA receptors may increase glutamate release. Taken together, low central NAAG levels may antagonize the effect of glutamate at

NMDA receptors and decrease its agonistic effect on presynaptic mGluR3; both activities could increase glutamate release, similar to the increase demonstrated in the PCP model of schizophrenia. [170]

Both ketamine and phencyclidine (PCP) have been shown to cause hallucinations and other positive and negative symptoms of schizophrenia, when given to animals or humans [80]. In addition, these two drugs have been demonstrated to both stimulate the release of glutamate in prefrontal cortex [171–173] and to be cytotoxic [166, 174]. There is evidence now that this cytotoxicity may be directly related to the enhanced release of glutamate [169, 175].

To this author's knowledge, the first reported attempt to measure glutamate levels in live brain using MRS was by Choe et al. in 1994 [86]. In this study, a 1.5 T magnetic field was used to examine right-side prefrontal white matter in 23 drug-naïve schizophrenics and 10 healthy controls. The results were an elevated glutamate/glutamine to creatine ratio (Glx/Cr), as well as depressed ratios of NAA to creatine, and choline compounds to creatine. Since then, there have been several further attempts [54–56, 58, 76–85], almost all initially using 1.5 T magnetic fields, but more recently using 3.0 and 4.0 T (Table 4.3). In these studies, the levels of glutamine and glutamate appear to differ depending upon the stage of disease and the tissue studied. For example, in one of the few longitudinal studies that have been done, to determine whether glutamatergic changes in patients with schizophrenia correlate with grey-matter losses over the first years of illness, Théberge et al. evaluated left anterior cingulate and thalamic glutamatergic metabolite levels using 4.0 T MRS, and measured grey-matter volumes via MRI in 16 patients with first-episode schizophrenia before and after 10 and 30 months of drug treatment, and in 16 healthy controls on two occasions 30 months apart [83]. They found elevated glutamine levels in the anterior cingulate and thalamus of never-treated patients, but thalamic levels of glutamine declined to the point of being significantly reduced by 30 months. Limited grey-matter reductions were seen in patients at 10 months, but grey-matter loss was widespread by 30 months. Moreover, parietal and temporal lobe grey-matter loss correlated with thalamic glutamine loss. The authors suggested that these findings could indicate either neurodegeneration or a plastic response to reduced subcortical activity. Prior to this, the investigators had used both 1H-MRS and 31P-MRS to study the left anterior cingulate and left thalamus in 9 and 8 schizophrenia patients and controls, respectively, since phosphate is a marker of cell membrane integrity, to address the issue of neuronal damage. They identified a significant positive correlation between glutamine and phosphoethanolamine (PEtn) in the left anterior cingulate, and a significant negative correlation between NAA and glycerophosphocholine (GroPCho) in the left thalamus of patients, but no significant correlations in controls. They argued that the former correlation could reflect a link between neurotransmission alterations and membrane phospholipid metabolism alterations, and the latter the presence of neurodegeneration. To date, there is early evidence that the latter is taking place, again through the use of 31P-MRS studies [88–91], but considerable research, via MRS and likely other means, will be necessary before this issue is adequately clarified.

Table 4.3 Human MRS studies examining CNS levels of glutamate/glutamine in schizophrenia (SCZ)

Authors	Year published Field strength	Area(s) examined	Patients/Controls	Findings
Stone et al. [80]	2009 3.0 T	anterior cingulate, L. hippocampus, L. thalamus	27 at risk/27 CL	At risk group had lower glutamate in thalamus, higher in anterior congulate.
Rusch et al. [58]	2008 2.0 T	hippocampus, amygdyla, DLPF cortex	29 SCZ/32 CL	Glutamate, not NAA, low and related to executive functioning.
Théberge et al. [83]	2007 4.0 T	L. anterior cingulate and thalamus	16 FEDN_SCZ/ 16 match CL 30-month prospective study	High Glx in never-treated patients; Glu levels low after 30 months drug treatment; progressive GM atrophy noted
Ohrmann et al. [55]	2007 1.5 T	DLPF cortex	15 FE-SCZ, 20 chronic/20 matched CL	Low NAA, glutamate/glutamine and choline NAA Levels correlate with verbal learning and memory.
Wood et al. [85]	2007 3.0 T	R. and L. dorsal and rostral cingulate	15 male SCZ/15 male CL	NAA levels low; Glx no different than CL.
Ohrmann et al. [54]	2005 1.5 T	DLPF cortex	18 FE and 21 chronic SCZ/21 matched CL	Chronic patients had lower NAA and Glx
Tibbo et al. [84]	2004 3.0 T	R. medial frontal lobe	20 adolescents at high risk/matched low-risk CL	Glx elevated
Théberge et al. [82]	2003 4.0 T	L. anterior cingulate and thalamus	21 chronic SCZ/ 21 CL	glutamate and glutamine low in ACG; glutamine high in thalamus.
Théberge et al. [81]	2002 4.0 T	L.anterior cingulate gyrus thalamus	21 never-treated SCZ/ 21 CL	Glutamine elevated at both locations; other meabolites not different vs. CL
Kegeles et al. [78]	2000 1.5 T	hippocampus	10 male SCZ/ 10 male CL	Glx/Cho elevated on R.
Bartha et al. [77]	1999 1.5 T	L. mesialtemporal lobe	11 SCZ/ 11 CL	No differences in NAA, Glx or other metabolites
Omori et al. [56]	1997 7.0 T	thalamus, frontal pole, cerebellar vermis	post-mortem study 8 SCZ/ 8 CL	NAA, glutamate and valine ‘tended to be low’

Table 4.3 (continued)

Authors	Year published Field strength	Area(s) examined	Patients/Controls	Findings
Bartha et al. [76]	1997 1.5 T	L. medial prefrontal cortex	10 never-treated SCZ/ 10 CL	normal NAA; glutamine elevated
Stanley et al. [79]	1996 1.5 T	DLPR cortex	13 FEDN and 12FE and 12 chronic SCZ/ 24 CL	normal NAA; low Glx
Choe et al. [86]	1994 1.5 T	R. prefrontal WM	23 drug naïve chronic SCZ/10 CL	Low NAA/Cr and Cho/Cr; elevated Glx/Cr

NAA N-acetylaspartate, *Glx* glutamate/glutamine/GABA, *Cho* choline, *Cr* creatine, *mI* myo-inositol, *SCZ* schizophrenia, *FE* 1st episode, *FEDN* 1st episode, drug naïve, *L.* left, *R.* right, *DLPF* dorsolateral prefrontal, *WM* white matter, *GM* gray matter

As a final argument for the pathophysiologic role of glutamate/glutamine in schizophrenia, and hence for the need to monitor levels in vivo, the results of several clinical trials assessing the co-administration of standard antipsychotic medication and a secondary drug that stimulates NMDA glutamate receptors have been published. In one double-blind, placebo-controlled, 6-week, crossover treatment trial in which 22 treatment-resistant schizophrenic patients were given 0.8 g/kg per day of glycine to supplement their usual anti-psychotic drug, glycine treatment was well tolerated and caused a significant reduction in negative symptoms, unrelated to alterations in extrapyramidal effects or depression, as well as a significant improvement in Brief Psychiatric Rating Scale (BPRS) scores [106]. Pre-treatment glycine serum level was strongly negatively correlated ($r = 0.80$) with clinical response; in other words, the lower the baseline glycine level, the greater the clinical response. The results have been somewhat variable in the five trials in which D-cycloserine was given to supplement another drug, largely dependent on the nature of the co-administered drug [107–111].

Most recently, encouraging results have emerged from a randomized, three-armed, double-blind, placebo-controlled phase II clinical trial comparing placebo, olanzapine, and LY2140023, a pro-drug for LY404039, a selective agonist for metabotropic glutamate 2/3 (mGlu2/3) receptors that has demonstrated antipsychotic potential in animal studies. LY2140023-treated patients exhibited statistically-significant improvements in both positive and negative symptoms relative to those on placebo, and no differences relative to those on olanzapine [112, 176]. Patients on LY2140023 did not differ from placebo-treated patients with respect to prolactin elevation, extrapyramidal symptoms or weight gain, side effects typically associated with D2-receptor blockers.

4.14 Conclusions and Future Directions

The mandate of this chapter was to discuss the current status of magnetic resonance imaging and spectroscopy in psychiatric illness, for which schizophrenia was selected for discussion, in terms of the detection and monitoring of neurodegenerative changes. If the goal of neuroprotective interventions is to prevent neuronal damage, it is crucial for clinicians to be able to identify and monitor such neuronal damage in live patients. Both MRI and MRS point to progressive brain changes in individuals with schizophrenia, which include regional losses of brain volume, independent enlargement of ventricles, and alterations in brain metabolites, among which glutamate, glutamine may have the greatest pathophysiological relevance. The combined use of ^{1}H-MRS and ^{31}P-MRS in the same subjects may allow for more definitive conclusions on the occurrence of neurodegeneration and the ability of neuroprotective agents to stop or reverse damage.

Note: The literature review for this chapter was done using the Medline search engine at http://www.ncbi.nlm.nih.gov/PubMed/ and the following search terms: MRI and volumetric loss and grey matter loss (12 abstracts listed); MRI and

volumetric loss (232); MRI and 'white matter changes' (604); 'neurodegeneration' and 'schizophrenia' (121); 'MRI' and 'schizophrenia' (904); 'N-acetylaspartate' and 'schizophrenia' (148); 'MRS' and 'schizophrenia' (175); 'spectroscopy' and 'schizophrenia' (419); 'glutamate' and 'schizophrenia' (1318); 'glutamine' and 'schizophrenia' (115); and '31P' and 'schizophrenia' (55). Additional references were identified in the reference lists of papers and books reviewed. Only English and French-language articles are referenced in the text and tables.

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Chapter 5
Vulnerability of the Brain to Neuropsychiatric Disorders Resulting from Abnormal Thyroid Hormone or Vitamin D Homeostasis

Sarah J. Bailey and Peter J. McCaffery

Abstract Nutritional modification is an approach to augment protection of the brain from psychiatric disease that is both inexpensive and with fewer side-effects than most psychoactive drugs. Two factors derived from nutritional sources that regulate gene transcription via nuclear receptors are thyroid hormone and vitamin D. Both of these factors thus lie at a nexus of environmental and genetic regulation of gene expression. Changes in both pathways have been associated with schizophrenia and depression but unlike dietary supplements such as omega-3 fatty acids, their mode of signalling is well understood. This chapter details the association of thyroid hormone and vitamin D with these psychiatric disorders. Likely targets for these two nuclear receptor regulators include the dopamine receptors, serotonergic pathways, hippocampal neurogenesis as well as components of the developing brain. The association of the nuclear receptor signalling pathways with several diseases suggests that they are less likely to be responsible for the unique features of each disease but are involved in aspects common to the disorders, as has been proposed for genes such as DISC1. An imbalance in the thyroid hormone and vitamin D pathways may contribute to depression and schizophrenia and restoration of homeostasis may provide a route by which the brain may be protected.

Abbreviations

ATP	Adenosine triphosphate
BDNF	Brain derived neurotrophic factor
CNS	Central nervous system
Cnr	Cannabinoid receptor
CSF	Cerebrospinal fluid
GABA	Gamma-aminobutyric acid
GDNF	Glial cell line derived neurotrophic factor
5-HT	5-hydroxytryptamine

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_5,

5HTT	5-hydroxytryptamine transporter
5-HT1A	5-hydroxytryptamine receptor 1A
N-CoR	Nuclear receptor co-repressor
NGF	Nerve growth factor
PI3K–PKB	Phosphatidylinositide 3-kinase-protein kinase B
RA	All-*trans* retinoic acid
SAD	Seasonal affective disorder
Scn4b	Sodium channel subunit
SMRT	Silencing mediator for retinoid and thyroid hormone receptors
T_4	Thyroxine
T_3	Triiodothyronine
TH	Thyroid hormone
TR	Thyroid hormone receptor
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
TTR	Transthyretin
VD	Vitamin D
VDR	Vitamin D receptor

5.1 Introduction

5.1.1 Dietary Factors as Neuroprotective Agents

Neuroprotective mechanisms can be enhanced by dietary factors. Usually such interest focuses on dietary restriction or on antioxidant nutrients, such as vitamins A, C and E, since oxidative stress has been implicated in the mechanisms leading to neuronal cell injury in various disease states such as the neurodegenerative disorders [1] (see Chapter 17 in this book). In this chapter we describe two dietary factors that are not antioxidant, but rather act via nuclear receptors to influence neuronal behaviour; vitamin D (VD) and thyroid hormone (TH). Interaction with nuclear receptors leads to gene regulation and it has been suggested that both VD and TH may have neuroprotective actions through this route. As ligand activated regulators of transcription the VD and TH receptors are a focus point of interaction between the environment (i.e. the external factors that alter levels of their ligands) and genetics (via the genes regulated by these transcription factors).

5.1.2 Thyroid Hormone Signalling

Thyroid hormone (TH) regulates basal metabolic rate and cellular metabolism of protein, fat and carbohydrates and is necessary for the growth and development of multiple tissues including muscle, bone and the central nervous system [2]. TH is essential for the development of the nervous system [3] and deficiency during embryonic or postnatal development leads to cretinism and severe stunting of brain

maturation. TH insufficiency can result from impaired thyroid gland function or from a lack of dietary iodine necessary for the synthesis of TH. Indeed iodine deficiency remains a widespread cause of mental retardation in a number of developing countries. Thus adequate TH levels are a prerequisite for the normal development and function of the nervous system.

The production of TH is under the control of the hypothalamic-pituitary-thyroid axis. The hypothalamus induces the pituitary via thyrotropin-releasing hormone (TRH) to secrete thyroid stimulating hormone (TSH), which in turn acts on the thyroid gland to produce TH (Fig. 5.1). Homeostasis of TH in the brain is under

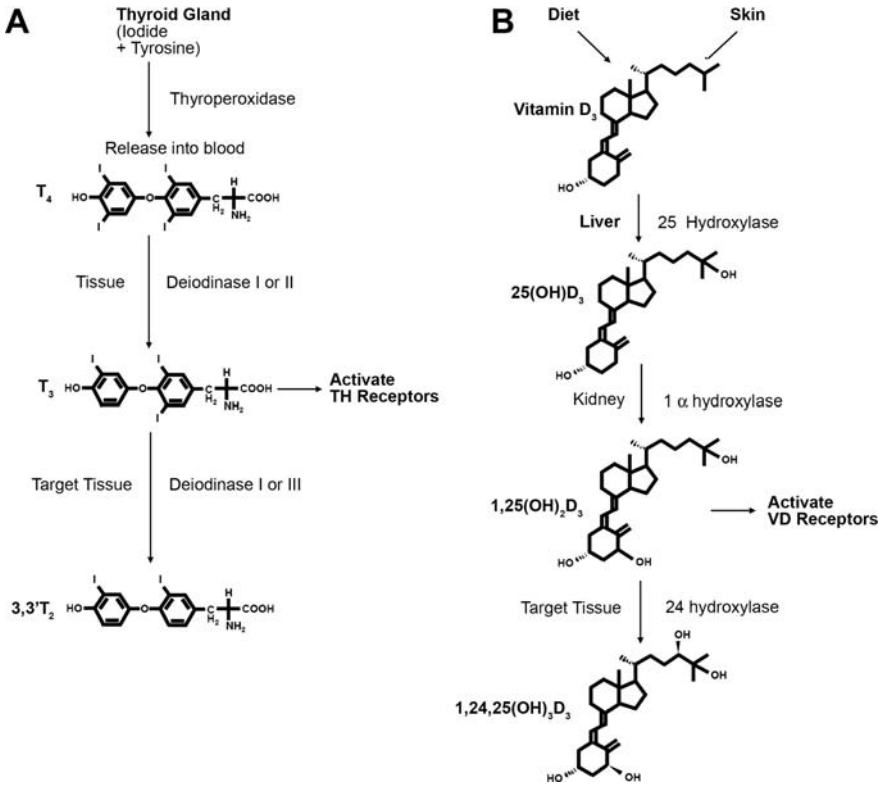

Fig. 5.1 Synthesis of active thyroid hormone and vitamin D from their precursors. (**A**) In the thyroid gland thyroperoxidase catalyzes the iodination of tyrosine in thyrogobulin which are coupled together to generate T_4 (as well as T_3 and other variants) and which is released into the circulation. Tissues which express deiodinase I or II can deiodinate T_4 to the more active T_3, the high affinity ligand for the TH receptor (although T_4 can also activate the receptor). Deiodinase I or III in target tissue will turn off the signal by further deiodination of T_3 to the lower activity $3,3'T_2$. (**B**) Vitamin D_3 is either derived from the diet of generated in the skin by the action of uv light on 7-dehydrocholesterol. Carried in the circulation to the liver vitamin D_3 is hydroxylated to $25(OH)D_3$. In the kidney it is further hydroxylated to $1,25(OH)_2D_3$, the form with high affinity for the VD receptor, which is then released into the circulation to act on target tissues, which also express the 24 hydroxylase to turn off the signal by hydroxylation of $1,25(OH)_2D_3$ to $1,24,25(OH)_3D_3$

tight control and brain TH levels can be maintained over an extended time even when an animal becomes hypothyroid [4]. The thyroid gland produces two major forms of TH, triiodothyronine (T_3) and thyroxine (tetraiodothyronine, T_4). TH is taken up into cells by a variety of transporters including those of the organic anion, monocarboxylate and L-type amino acid transporters [5] (Fig. 5.2). Such transport mechanisms are not only important in the uptake of TH but also in the transport of T_3 between cells. T_4 is the main circulating form of the hormone in plasma and is converted to the more active T_3 in tissues via the removal of an iodine atom by deiodinase I or II, with deiodinase II being a prominent form in the human brain [6] (Fig. 5.1). The majority of T_3 present in the brain is generated by deiodination of T_4 [7]. Surprisingly though both T_4 and T_3 are present in the brain at similar concentrations, differing from peripheral tissues where T_4 levels are much greater than T_3 [8]. Once inside the cell both T_3 and T_4 can bind to the nuclear TH receptor (TR), although T_3 is the more active form and T_4 has lower affinity for the receptor. The two subtypes of the TR, TRα and TR β act directly on gene transcription, mainly as retinoid X receptor heterodimers [9], as ligand-regulated repressors or activators of target genes.

In the brain the predominant isoforms of TR, TRα1, TRβ1 and TRβ2, are all expressed. TRα1 is the major isoform expressed during embryonic development, although TRβ1 expression increases before birth [10]. TRα1 and TRβ1 also exhibit a widespread distribution in the adult brain in distinct spatiotemporal patterns [11]

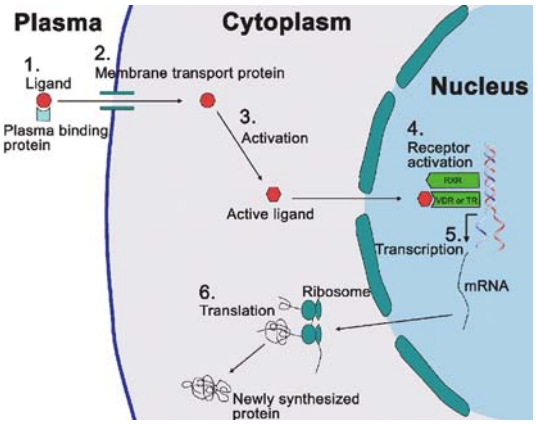

Fig. 5.2 Action of vitamin D and thyroid hormone to activate gene transcription. A binding protein, VD binding protein or transthyretin for TH, carries the ligand in the circulation (1). The ligand is then transported across the membrane, MCT8, MCT10 and OATP1C1 performing this for TH and VD transported by receptor-mediated endocytosis dependent on the membrane receptors megalin and cubilin (2). In some cases enzymes present in the cytoplasm convert the ligand to a form with greater affinity for the receptor, as is the case for conversion of T_4 to T_3 by deiodinase (3). The ligand enters the nucleus and binds to its specific receptor (VDR or TR) dimerized to RXR (4). This activates the transcription of genes that have the appropriate hormone response element in their promoter (5). The resultant mRNA is transported to ribosomes in the cytoplasm to be translated to functional protein (6)

suggesting that these receptor subtypes mediate different physiological actions of TH. Expression of the TRβ2 isoform is more restricted being localized to the hypothalamus and anterior pituitary where it plays a major role in the feedback regulation of the hypothalamic-pituitary-thyroid axis [12, 13].

TRs bind T_3 with high affinity and in turn bind to thyroid hormone response elements in target genes thus activating gene transcription (Fig. 5.2). TRs also function as repressors with unliganded receptors recruiting transcriptional co-repressors (e.g. N-CoR , SMRT) to suppress basal gene expression. Most T_3-mediated gene expression is positively regulated and negative gene regulation is less well documented [9]. TH actions via TRs, for instance, are widely implicated in the development of the nervous system. Although TRs also function in the adult brain, surprisingly few neuronal genes have been shown to be directly regulated by TH. In models of TH insufficiency during development [14] or in the adult [15], as well as hyperthyroidism in the adult brain [16], a number of differentially regulated neuronal genes have been identified. Some of these genes contain thyroid hormone response elements suggesting they are direct targets of TH while others are downstream genes indirectly regulated by TH. Often though, genes regulated by TH in the developing brain are insensitive to TH in the adult [3]. Alternatives exist to TH's gene regulatory role and non-genomic effects of TH have been reported to be important, certainly during brain development [17].

Abnormal thyroid function can lead to hyperthyroidism and hypothyroidism, related to altered levels of T_4 or T_3, but also to subclinical conditions that reflect changes in plasma TSH levels with no change in T_4/ T_3 [2, 18]. Opinions vary on the "normal" acceptable range for TH levels but as screening for thyroid disease becomes more common, subclinical thyroid disorders are being diagnosed more frequently in young, middle-aged and elderly populations although the relevance to TH disease or the benefits of treatment have not been proven [18]. TH dysfunction has been implicated in a number of neurological conditions, including cognitive impairment, Alzheimers disease, cerebrovascular disease and neuropsychiatric diseases [19–22]. Thus, although TH has a more dominant role in the developing CNS than in the adult, it must still be involved in key tasks in the adult brain, including control of "embryonic" like phenomena such as neuroplasticity. Here we consider the involvement of TH in schizophrenia and in depression in terms of its potential neuroprotective role for the adult brain.

5.1.3 Vitamin D Signalling

Vitamin D (VD) is a steroid hormone best known for its role in the endocrine control of calcium homeostasis. However, evidence is now accumulating to suggest that VD also plays a physiological role in the regulation of the immune system, cardiovascular system, the pancreas and the brain.

VD has two major forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The latter is derived from plant sources and is not produced in the body. Vitamin D_3 in contrast is produced in the skin on exposure to ultraviolet radiation

from the precursor 7-dehydrocholesterol (Fig. 5.1). Given this endogenous production, vitamin D_3 is not formally a vitamin although dietary sources (primarily oily fish) can provide some of the body's vitamin D_3 needs. Vitamin D_3 is itself inactive and must be metabolized in the liver to 25-hydroxyvitamin D_3 (25(OH) D_3) which in turn is hydroxylated to 1α, 25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, calcitriol) in the kidney (Fig. 5.1). Both of these metabolites are transported in the blood bound to VD binding protein. However, only the active metabolite,1,25-$(OH)_2D_3$, enters target cells where it binds to the VD receptor (VDR) (Fig. 5.1). The VDR is a member of the steroid hormone nuclear receptor family which heterodimerizes with the retinoid X receptor and binds to a VD response element in the promoter region of regulated genes. In addition to these genomic actions, more rapid non-genomic responses, via activation of a plasma membrane associated VDR, have also been reported to occur in certain cell types [23].

A physiological role for vitamin D_3 in the brain has been suggested based in part on the presence of the VDR and also the enzyme 1α-hydroxylase, which generates the active 1,25-$(OH)_2D_3$ metabolite and is not, as once thought, restricted to the kidney but is present in both neurones and glia [24]. This indicates that there is local production of the active metabolite 1,25-$(OH)_2D_3$ and that autocrine or paracrine signalling to neighbouring cells is likely. Most brain regions that were positive for 1α-hydroxylase immunolabelling were also positive for the VDR, although discrete distribution patterns for both proteins were evident between cell layers and subregions [24]. The VDR has been identified in rat [25, 26], hamster [27] and human [24] brain. In the adult human brain, the VDR is present in neurones and glia of the prefrontal cortex, hippocampus, hypothalamus, amygdala, substantia nigra and other regions [24]. The VDR is also widely distributed in the embryonic brain, from studies in the rat, most prominently in the neuroepithelium and proliferating zones of the CNS from embryonic day 12 [28].

The actions of VD in the brain include immune modulation and neuroprotection [29, 30]. The neuroprotective effects of VD are not fully understood but appear to be exerted via regulation of calcium homeostasis, for example, via downregulation of calcium channel expression in neurons [31, 32] or by induction of calcium binding proteins [33]. Additional mechanisms include the regulation of neurotrophic factors, for example, the expression of nerve growth factor and neurotrophin 3 is stimulated by 1,25-$(OH)_2D_3$ [34, 35]. Antioxidant pathways are also capable of regulation by 1,25-$(OH)_2D_3$, for example by upregulating glutathione production in glia [36], which may contribute to the neuroprotective actions of the hormone.

What is the correct amount of VD that is required for "normal" function in the brain? This is largely unknown. Estimates of VD sufficiency are all based on its role in calcium homeostasis and maintenance of healthy bones. It has been suggested that about half of the elderly in North America and two thirds of the rest of the world are not getting adequate amounts of VD for bone maintenance [37]. VD status is assessed by measuring plasma 25(OH) D_3 levels since its availability depends on adequate access to vitamin D_3 (both dietary sources and that produced by the skin) and, unlike 1,25-$(OH)_2D_3$, 25(OH) D_3 is not regulated by the endocrine system. In the brain 1,25-$(OH)_2D_3$ is likely to be synthesized and to act locally which makes it

difficult to predict the relationship between plasma 25(OH) D_3 levels and physiological function. Nonetheless, low levels of VD have been suggested to be contributory to a number of neurological conditions including epilepsy [38, 39] impaired cognitive function [40, 41] and multiple sclerosis [42]. Here we focus on the evidence that VD is involved in the pathology of schizophrenia, in which case a vitamin deficiency in the developing brain is proposed, and depression, where it is the adult brain that is potentially influenced.

5.2 Schizophrenia and Thyroid Hormone

5.2.1 Loss of Thyroid Hormone Homeostasis in Schizophrenia

TH's regulation of both developmental and mature function of the CNS means that, as with VD, there are two alternative points at which TH may impact on schizophrenia. However, the dominant evidence points to abnormal TH signalling leading to psychosis in the mature brain rather than during development and a family history of thyroid disorders occurs more frequently in schizophrenics [43]. Although no link has yet been found with mutations in the TH receptor [44] or the plasma TH binding protein, transthyretin [45], an imbalance of TH, in either deficiency or excess, results in a range of psychological disturbances, including depression, and behaviours that overlap with the positive symptoms of schizophrenia. "Myxedema madness" [46, 47] is a well-characterized condition in which hypothyroidism leads to delirium, hallucinations and psychosis. Although outwardly contradictory, the opposite condition of hyperthyroidism also results in delirium and psychosis [48]. It seems that, as with other nuclear ligands that regulate the brain, such as retinoic acid (RA) [49], their endogenous function can be disrupted by either excess or deficiency of the ligand, but in either direction the behavioural results may overlap.

5.2.1.1 Altered Thyroid Hormone Function Test in Schizophrenia

The effects of hyper- or hypothyroidism to induce symptoms of schizophrenia led to the testing of thyroid function in schizophrenics. Several studies found a relationship between thyroid disorders and schizophrenia e.g. MacSweeney et al. [50] and Baumgartner et al. [51], however, as detailed below, the types of changes seen in schizophrenia are not uniform but vary from study to study. For instance one report described elevation of free T_3 and T_4 in 19% of schizophrenic patients, with greater frequency in paranoid schizophrenics [52]. It was found that patients who showed improvement in their abnormal TH homeostasis with treatment tended to be those who improved in behaviour [53, 54] and the severity of the altered TH homeostasis correlated with the seriousness of the psychiatric symptoms [55]. Other studies have suggested that high levels of TSH correlate with a poorer response to treatment whereas a reduced response of TSH to TRH correlates with a better treatment

response [56]. In contrast Baumgartner *et al* reported no change in TSH or T_3 but higher levels of T_4 correlating with schizophrenia severity and the extent to which the patient responded to treatment [57].

The frequency of correlation of schizophrenia with abnormal thyroid function tests varies significantly between studies some showing as low as 9% [58] or very little change over the course of treatment [59], while some studies find a correlation frequency of up to 36% although with no clinical thyroid illness [60]. Othman et al. found a high frequency of correlation between abnormal thyroid function test and schizophrenia, but these abnormalities were variable in form, leading to the author's suggestion that schizophrenia was linked to some disruption in central regulation of the hypothalamic pituitary thyroid axis [61]. This inter-patient variability in thyroid status though is perhaps not surprising given that, as described earlier, psychosis results from either extreme of TH state. Although it is evident that a number of antipsychotic drugs alter TH homeostasis [62] a decrease in TH is evident even in drug-free schizophrenics [63] and thus drug treatment is not the single cause for abnormal thyroid homeostasis in schizophrenia.

5.2.1.2 Abnormal Thyrotropin-Releasing Hormone Levels in Schizophrenia

TRH is the factor released by the hypothalamus to induce the pituitary to secrete TSH to regulate the release of TH by the thyroid gland. Several studies have suggested a link between the receptor for TRH and schizophrenia. The drug phencyclidine induces schizophrenia like symptoms in humans, and a search for genes in the prefrontal cortex altered in the phencyclidine-induced rat identified a large rise in the transcript for the TRH receptor [64]. A quantitative study of the receptor using radiolabelled TRH also revealed higher levels of the TRH receptor in the dentate gyrus of schizophrenics compared to controls [65], although lower levels were detected in various regions of the amygdala. As evident though from the expression of TRH receptor in brain regions outside of the hypothalamus, its function is not limited to control of TSH release. Indeed, TRH is expressed in many brain loci and is even present outside the CNS and, beyond its role in the hypothalamic pituitary thyroid axis, TRH is likely to function as a neuromodulator or perhaps neurotransmitter [66]. Thus it is possible that changes in TRH receptor reported in schizophrenia are unrelated to alterations in TH homeostasis.

5.2.2 The Role of Thyroid Hormone in Schizophrenia and Potential Mechanisms

The lack of consistent change in TH signalling means it is unlikely that the disruption of this signal is the primary cause of schizophrenia, and the changes are likely to be complementary to additional genetic and environmental causes. Analysis of hypothyroidism due to iodine deficiency in newly admitted psychiatric patients did not indicate a link between mood disorder, or any specific psychiatric illness [67]. It was proposed that the link found in other studies was due to the effects of stress

on thyroid homeostasis or drugs used to treat psychiatric disease. Further, TRH was not found to be an effective treatment for schizophrenia [68] or at least no effect was evident related to a change in endocrine function [69].

Despite these negative findings, the frequency of change in TH homeostasis in schizophrenia, and the effects of hyper or hypothyroidism to induce pyschosis, implies that one or more aspects of thyroid signalling are likely to be a component of the underlying molecular mechanism of schizophrenia. It would also suggest that thyroid treatment may be beneficial, but would need to target the varied changes in signalling that seem to occur in individual patients. The mechanism by which TH may promote schizophrenia is unknown but several candidate genes have been suggested in the recent review by Palha and Goodman [70]. For instance, the dopaminergic hypothesis of schizophrenia proposes an overstimulation of the dopamine signalling system [71]. Alterations in thyroid levels in the rat can modify dopamine receptor expression [72], specifically hypothyroidism results in an increase in the D2 dopamine receptor [73] and increases sensitivity to D2 receptor agonists [74], potentially enhancing dopaminergic signalling.

With regard to the neurodevelopmental hypothesis of schizophrenia, proposing that abnormal brain development is a key contributor to schizophrenic pathology, myelination is one process potentially impacted by hypothyroidism. Although not conclusive there are strong hints of abnormalities in white matter in schizophrenia [75, 76]. TH deficiency profoundly influences oligodendrocyte differentiation [77] and thus relatively small fluctuations in TH during postnatal development may alter white matter and connectivity in the brain. However, as already discussed, changes in TH signalling are not usually considered part of the developmental errors that may give rise to schizophrenia.

5.3 Schizophrenia and Vitamin D

5.3.1 Neurodevelopmental Effects of Vitamin D Deficiency and Schizophrenia

VD deficiency during embryonic development has been proposed as a risk factor for schizophrenia as part of the neurodevelopmental hypothesis for this disorder [78, 79]. Deficiency of this vitamin during pregnancy is relatively prevalent even in western countries [80]. The requirement for sunlight to generate this vitamin has been suggested as an explanation for the preferential birth of individuals diagnosed with schizophrenia during the winter and spring months [81–83] as well as the higher frequency of schizophrenia in dark skinned migrants into countries at high latitudes [84, 85] and the higher incidence in cities compared to rural areas [86, 87] leading to low VD levels [88]. It should be noted though that alternative explanations exist for the winter/spring bias of birth of schizophrenic individuals [89] and not all studies even agree with this association [90] while the greater rates of schizophrenia

in dark-skinned migrants or individuals in urban versus rural areas could be due to social stress [91]. As described below though, there is good reason to believe that a deficiency of VD will result in brain abnormalities with the potential to engender schizophrenia.

5.3.2 Animal Model Studies on Vitamin D Signalling Deficiency and Disrupted Brain Development

5.3.2.1 Vitamin D Deficiency: Changes in Genes and Protein Expression

Deficiency of VD during rat embryonic and postnatal development leads to significant morphological changes in the adult brain such as enlargement of the ventricles together with a decline in neuroprotective nerve growth factor (NGF), although there is no change in glial cell line derived neurotrophic factor (GDNF) or brain derived neurotrophic factor (BDNF) [92]. VD depletion applied only during embryonic development similarly results in a decline in NGF [92]. A screen of protein changes in the adult rat following VD deficiency during embryogenesis found changes in the frontal cortex and hippocampus of proteins involved in diverse pathways including mitochondrial function, cytoskeletal control, synaptic plasticity, chaperoning and neurotransmission [93]. Particularly intriguing is that of the 36 proteins found to change, 13 have been linked with schizophrenia [93]. Some of the most interesting of these are required for mitochondrial function, specifically mitochondrial ATP synthase beta-chain and subunit VIa of cytochrome *c* oxidase and a second subset involved in pre-synaptic function and neurotransmission, including synapsin-2 and gap-43. A screen for alterations in gene expression in the brain following embryonic VD deficiency found changes in 74 genes (two-fold or more) involved in the same pathways [94]. Sixteen of these were genes linked with schizophrenia. Surprisingly only 4 of these overlapped with those found in the proteomic study [93, 94], possibly implying limitations in this approach in that the changes detected in gene expression may have been insufficient to lead to alterations in protein levels detectable in the protein screen. It should be noted though that the analysis of transcripts was performed on whole brain whereas the analysis of protein was limited to hippocampus and frontal cortex.

A further candidate for VD regulated pathways in schizophrenia include the phosphatidylinositide 3-kinase-protein kinase B (PI3K–PKB) pathway which has been proposed to be linked with schizophrenia [95] and is activated by VD [96]. Surprisingly, one set of proteins not on the list are the neuronal calcium binding proteins, such as parvalbumin and calbindin which are markers of cortical GABAergic interneurons repeatedly described as reduced in schizophrenia [97]. Such proteins may have been considered a target of VD regulation given its importance as a regulator of calcium homeostasis, however none of these genes appear to be regulated by VD, not even calbindin [98].

5.3.2.2 Vitamin D Deficiency: Changes in Behaviour

Studies in the rat indicated that prenatal VD deficiency did not alter learning or memory in standard tasks such as spatial learning in a radial maze however there were more subtle effects such as impairment of latent inhibition (considered a marker of schizophrenia) as well as a decline in holeboard habituation [99]. It was found that antipsychotic drugs such as haloperidol could completely restore habituation in rats prenatally depleted of VD [100]. Exposure of developing Sprague-Dawley rats to VD deficiency immediately after conception (with postnatal VD repletion), which presumably does not result in complete VD depletion until later development, still results in MK-801 (an NMDA receptor antagonist) induced hyperlocomotion as an animal model of schizophrenia [101]. 129/SvJ but not C57BL/6 J mice when depleted of VD during development show spontaneous hyperlocomotion and both strains exhibit increased exploration but showed no change in a battery of other tests covering, for instance, social behaviour, depressive-like behaviour, anxiety or sensorimotor gating [102]. It should be noted that neither the behavioural nor biochemical effects of prenatal VD deficiency are due to an indirect effect of such deficiency on the hypothalamic pituitary axis [103].

5.3.2.3 Loss of Vitamin D Receptor has Negligible Effect on Brain Development

It has been previously shown that deficiency in growth regulatory factors, such as TH, is much more damaging for the developing and mature animal than loss of receptors. It is presumed that this is because, in the case of receptor deficiency, TH responsive genes are left in a neutral state. In contrast, they are chronically repressed in the presence of receptor but absence of ligand because unliganded receptors can act as potent transcriptional repressors [3]. This is likely also the case with VD since studies with the VDR null mutant mouse have shown little effect on behaviour with no change in spatial memory, olfaction, taste or hedonic response (as a measure of depressive-like behaviour) [104]. It has been suggested that VDR mutation results in greater anxiety [105] but these results may reflect muscular and motor impairments [106].

VDR mutation is likely to only have minor effects on the developing human brain as demonstrated in a study by Ozer et al. that examined an inbred family overloaded with both psychosis and a rickets–alopecia syndrome unresponsive to VD [107]. This latter disorder maps to chromosomal region 12q12–q14 where mutations in the VD receptor occur [108]. However the psychosis and rickets – alopecia syndrome are inherited independently suggesting that the loss of VD signalling does not increase the susceptibility to psychosis. Analysis of VD receptor missense variants found no correlation between schizophrenia and the most frequent M1T variant [109]. Similarly an analysis of single nucleotide polymorphisms (SNPs) in the VDR found no association with schizophrenia [110]. Thus, whereas deficiency of the ligand for VDR is damaging to the developing brain, leading to behavioural

traits in mice reminiscent of schizophrenia, loss of the receptor itself presents few problem and this type of genetic defect is unlikely to be part of schizophrenic etiology.

5.4 Depression and Thyroid Hormone

5.4.1 Thyroid Hormone Homeostasis in Depression

Changes in the hypothalamic-pituitary-thyroid axis function have long been recognized in depressed patients [111–114]. Further, TH has been used in the treatment of depression, most commonly as an augmentation strategy with antidepressant treatments [115, 116]. Hypothyroid and hyperthyroid conditions have also been associated with changes in mood. Virtually 100% of patients with severe hypothyroidism also have depression [117]. The precise mechanisms underlying the interaction of TH with depression has not yet been elucidated; consequently it is difficult to establish a causal relationship between TH and depression. However the potential neuroprotective effects of TH could be important in the pathogenesis of depression particularly in relation to the neurotrophic effects of TH.

As with schizophrenia, both a deficiency and an excess of TH may produce a similar outcome in depression. Whereas depression is a common feature of hypothyroidism, hyperthyroidism gives rise to more heterogeneous psychiatric symptoms including both depression and elevated anxiety. Conversely, the majority of patients with primary depression have normal TH status. However it has been shown that approximately 25% of depressed patients have alterations in their TH function evident in the form of elevated T_4 levels and blunted TSH response to TRH stimulation while successful treatment of depression reverses these changes [111–113]. T_3 levels have been reported to be normal or in more severely depressed patients to be reduced [112]. There is some debate as to whether brain TH levels mirror those seen in the plasma, so that normal systemic TH levels may not reflect abnormal amounts in the brain. What is not understood, however, is how altered TH homeostasis may be linked to depression. One hypothesis relates to the role of 5-hydroxytryptamine (5-HT, serotonin) in inhibiting the release of TRH from hypothalamic neurons [112, 118]; the lowered 5-HT levels found to be associated with depression would lead to raised TRH secretion resulting in increased TSH release and stimulation of T_4/T_3 production by the thyroid. On the other hand, TH itself feedbacks on 5-HT homeostasis [115, 119]. In adult hypothyroid rats there is increased turnover of 5-HT, as evidenced by increased 5-HIAA levels, whereas in thyroid intact rats T_3 suppresses 5-HT turnover. TH has also been shown to regulate the expression of 5-HT2 receptors although both increases [120] and decreases [121] in the receptors have been reported in hyperthyroidism. In addition to changes in 5-HT function, TH is also capable of regulating the expression of noradrenaline receptors. Hypothyroidism is associated with a reduction of, and hyperthyroidism an increase in, the expression of α and β adrenergic receptors in cerebral cortex [120, 122].

5.4.2 Thyroid Hormone Receptor Function

As already mentioned, few neuronal genes have been shown to be directly regulated by TH. Microarray studies have been applied to adult rat brains exposed to T_3 (hyperthyroid model) and only eight genes were demonstrated to be upregulated including Ca^{2+}/Calmodulin-dependent protein kinase B and Na-dependent neurotransmitter transporter among others [16]. A wider more recent screen used only striatal tissue from hypothyroid rats treated with either single or multiple doses of T_3 [15]. Repeated dosing produced a smaller change in gene expression than acute doses, with 18 genes identified as upregulated including the cannabinoid receptor (Cnr1), the extraneuronal monoamine transporter (Slc22a3) and a voltage-gated sodium channel subunit (Scn4b) among others [15]. The ability to regulate genes such as cannabinoid receptors and monoamine transporters which have been implicated in depression may provide a mechanism for the effects of TH on mood. Alongside this the ability of TH to regulate 5-HT and noradrenergic receptor expression would also be anticipated to contribute to depression [118, 119].

In adult rat brain TRα1 has high expression in the olfactory bulb, hippocampus and cerebellar cortex. In hypothyroid rats TRα1 expression is increased in hippocampus and cortex and is normalized by T_4 administration [123]. TRα1 dominant negative mutant mice show an increase in anxiety-related behaviours, in both the open field and the elevated plus maze test, which is reversible by T_3 treatment [124]. However, the behavioural phenotype of these mice is complicated by a locomotor dysfunction as a result of delayed post-natal cerebellar development. In humans there is a high degree of comorbidity of depression with anxiety and they share similar neuronal circuitry and molecular involvement [125] so it is possible that depression behaviours are also altered in these TRα1 mutant mice but this has not been reported.

5.4.3 Thyroid Hormone in Animal Models of Depression

Hypothyroid rats show an increase in immobility in the forced swim test that is consistent with increased depression-related behaviour that can be reversed with TH treatment [126]. These changes in behaviour may reflect a change in serotoninergic function. Hypothyroid rats also show a decrease in cortical 5-HT2A receptors but not 5-HT1A receptors or 5-HTT sites [127]. On the other hand, Sandrini et al. [121] showed that acute and chronic treatment with T_3 in adult rats could decrease the expression of 5-HT2 receptors but not 5-HT1A receptor subtypes. Others have shown that hypothyroidism in rats is associated with a decrease in 5-HT synthesis [128, 129] and that hyperthyroidism is associated with increased 5-HT synthesis [130, 131].

TH status has also been shown to affect adult neurogenesis in rats and mice [132–136] and a decline in hippocampal neurogenesis has been implicated in some of the hippocampal aspects of depression [137]. A short period of adult onset

hypothyroidism caused a decrease in the number of proliferating cells and immature neurons in the subgranular zone of the rat hippocampus [136]. These authors also showed that these changes were accompanied by an increase in immobility in the forced swim test, consistent with increased depression-related behaviour. Both the behavioural and neurogenic effects were reversed with chronic TH treatment [136]. The effects of hypothyroidism on neurogenesis have been shown to be mediated by TR α expressed in neural progenitor cells, but not by TRβ, in mice [135]. A set of factors potentially downstream of TH in the control of neurogenesis are the neurotrophins; BDNF and NT3, and the receptors TrkA and p75ntr, are all regulated by TH [14]. This highlights a potential neuroprotective role for TH in hippocampal adult neurogenesis, a process implicated in the therapeutic response to antidepressants [138]. Antidepressant treatments of all classes can increase BDNF expression whereas stress, which can promote depression, reduces BDNF expression [139].

Transthyretin (TTR) is the transport molecule that binds TH in the plasma and is therefore a critical determinant of free T_4 and T_3 levels. TTR levels have been shown to be reduced in CSF of depressed patients [140] and this correlates with suicidal ideation and low 5-HT function [141]. In TTR null mice however depression-related behaviour is decreased, while exploratory behaviour is increased and this is thought to be associated with increased noradrenergic function in limbic forebrain [142]. The TTR null mice also have reduced TH levels in the CSF [142] although the distribution of TH in the brain is entirely normal [143]. The effects of knocking out TTR are likely to be complex since its function is to transport both TH and retinol which have important effects on the development of the nervous system as well as regulating mood [144].

5.4.4 Thyroid Hormone Treatments in Depression

Thyroid hormone, particularly T_3, has been used to accelerate or augment antidepressant response [111, 115, 116, 145]. The role of T_3 as an augmentation strategy in the treatment of treatment-resistant depression, has been demonstrated even in depressed patients with normal TH status [116]. Augmentation therapy refers to the use of an additional compound that is not by itself antidepressant, in addition to an antidepressant to which the patient has little or only partial response. In the late 1960s and early 1970s many studies suggested that the addition of T_3 at the start of a trial with a tricyclic antidepressant reduced the usual lag in therapeutic onset (reviewed by [116]). Altshuler et al. [146] conducted a meta-analysis of double-blind placebo-controlled trials showing that T_3 had a significant effect on the time to response compared with placebo. Similarly, Aronson et al. [147] conducted a meta-analysis of eight controlled trials and showed that patients treated with T_3 augmentation of tricyclic antidepressants were twice as likely to respond as controls. However, Gitlin et al. [148] reported no difference in augmentation response between T_3 and placebo. Most of the literature focuses on T_3 augmentation of tricyclic antidepressants and the evidence base for other antidepressants, for example

the selective serotonin reuptake inhibitors, is more limited but has been shown for sertraline [149]. While approximately half of patients appear to respond to T_3, the optimal dose, treatment period and long-term efficacy of T_3 as an augmentation strategy remains to be determined [145].

As T_3 is largely derived from the deiodination of T_4 in the CSF it has generally been assumed that both hormones would have equivalent therapeutic effects. However T_3 is preferable to T_4 as an augmenter in unipolar depression [150]. Joffe and Singer [151] found T_3 to be significantly more effective than T_4 in augmenting the response to tricyclic antidepressants (desipramine, imipramine). Bunevicus et al. [152] suggested that combining T_4 and T_3 in patients on thyroid replacement therapy improved measures of mood and cognition better than T_4 alone. Alternative approaches to elevating TH levels are inconsistent but have included administration of TRH which produces rapid antidepressant effects and TSH can accelerate the effects of tricyclic antidepressants [111].

What are the mechanisms by which T_3 may be beneficial as a treatment for depression? These are largely unknown, although it has been proposed that T_3 may act through correction of suboptimal thyroid function and possibly by direct effects of TH on the brain [115, 145]. The tricyclic antidepressants imipramine (relatively 5-HT selective) and desipramine (relatively NA selective) are both augmented by thyroid hormones indicating that effects on both serotoninergic and noradrenergic systems are involved. As suggested above TH can positively regulate adult neurogenesis and increase 5-HT levels. Both of these effects would be predicted to accelerate and augment the therapeutic effects of antidepressants.

5.5 Depression and Vitamin D

The most convincing evidence of a role for VD in depression comes from studies of patients with seasonal affective disorder (SAD). SAD patients are thought to be exposed to less sunlight and therefore the formation of vitamin D_3 might be abnormally low in these patients in winter. VD deficiency or low serum levels of the precursor $25(OH)D_3$ is associated with low mood and depression in humans [153–155]. The reverse has also been shown. Supplementing with VD for 5 days during late winter had a significant positive effect on mood in healthy subjects [156] or on well-being in endocrine outpatients with low $25(OH)D_3$ levels [157]. It has even been suggested that in SAD patients VD supplementation may reduce depression more than phototherapy [158]. There is a seasonal variation in the levels of $25(OH)D_3$ (the inactive precursor for active VD) in plasma, with peak values occurring during autumn, although there is no seasonal change in the serum concentrations of $1,25\text{-}(OH)_2D_3$ [159]. Low 5-HT levels in the brain have been linked to the symptoms of SAD and it has been suggested that VD may play a role in seasonal cycles of mood due to regulation of 5-HT [160, 161]. Although this has yet to be substantiated, the 5-HT system is thought to be involved in the positive response to light therapy in SAD patients [162].

Further evidence of a role for VD in depression comes from genetic studies of VDR polymorphisms. Five VDR polymorphisms (*Cdx-2, FokI, BsmI, ApaI* and *TaqI*) have been associated with a number of phenotypes including bone mineral density and risk for fractures [163, 164]. Kuningas et al. studied the influence of these 5 polymorphisms on cognitive function and depressive symptoms in an elderly population ($\geq$ 85 yrs). Carriers of *ApaI* variant-allele had better cognitive functioning together with less depressive symptoms assessed by the 15-item geriatric depression scale [164]. These data suggest that the *ApaI* VDR polymorphism may confer a protective effect against age-related changes in neuronal function.

Animal studies of VD and mood disorders have produced apparently conflicting results. As described earlier, mice lacking the VDR display exhibit only minor changes in behaviour. These include though reduced exploration in a range of tests including the holeboard open field and elevated plus maze indicating an increased anxiety-related behavioural phenotype [105] although this may be attributable at least in part to impaired motor performance [165]. These authors also demonstrated a reduction in the time spent immobile in the forced swim test which would be considered an *anti-depressant* effect in this commonly used model of depression-related behaviours. This is somewhat surprising given the discussion above of patient studies indicating that low levels of VD are associated with pro-depressant effects. However, Burne et al. [106] further showed that the motor deficit in VDR null mice did not significantly alter swimming activity per se. Rather, VDR null mice have difficulty floating perhaps because of muscle weakness or an indirect effect on calcium availability. Subsequent experiments showed that in different paradigms of depression-related behaviours, VDR mutant mice show no change in depression-related indices in either the tail suspension test [166] or in the sucrose preference test [104].

The mechanisms underlying a potential role for VD in depression may include regulation of adult neurogenesis. One study looking at long-term treatment with 1,25-$(OH)_2D_3$ in aging rats revealed a higher density of hippocampal neurons in supplemented animals suggesting that VD may stimulate adult neurogenesis [167]. It is clear then that VD can regulate adult brain gene expression but the outcomes for depression-related behaviours in animal models are difficult to interpret because of a pronounced locomotor effect of manipulating VD levels.

5.6 Conclusions and Future Directions

In this chapter we described two nutritionally derived regulatory factors, TH and VD, which act through members of the same nuclear receptor family. Evidence was provided for their neuroprotective action in both schizophrenia and depression, and deficiency in these factors may lead to susceptibility to these psychiatric diseases. However, the evidence for these relationships is not equal in all cases and the conclusion will focus on the strongest of these associations; the protective

role for VD in schizophrenia, acting in the developing brain and TH's link with depression in the adult brain.

5.6.1 Vitamin D, Brain Development and Protection from Schizophrenia

The most explicit effect of nutrition in schizophrenia is evident in the increased incidence of the disorder resulting from famine during the mother's first trimester [168, 169]. Certainly, some of the neuropathological changes in schizophrenia could occur early during neural development, such as the reduced brain volume and ventricular enlargement [170]. However these changes can also reflect defects later during cortical development since the reduction in size likely reflects reduced neuropil and neuronal size rather than decline in neuronal numbers and the effects can be region specific including cortical temporal lobe regions [171]. In particular, the decline in white matter in fibre tracts such as the corpus callosum [172], potentially contributing to dissociative thinking, and cognitive deficits in schizophrenia, imply abnormalities during later stages of brain development.

A couple of studies have alluded to the link between VD levels during later development and schizophrenia. In a small study, third trimester maternal levels of calcidiol were measured as an indicator of VD status (26 cases, 51 controls). These were not associated with the risk of schizophrenia in the offspring, however the subset of dark-skinned individuals (7 cases, 14 controls) showed a trend of correlation [173]. If VD deficiency during development promotes schizophrenia then supplementation of the general population should reduce this risk and such was found in males following supplementation during the first year of life [174]. However, although the effect size was quite large the confidence levels were imprecise and a difference was not evident in females. It is of note though that such an effect would imply a requirement of VD on CNS development not just during the third trimester but also postnatally, and that disruption of these postnatal events promotes schizophrenia.

One mechanism by which VD may contribute to neuroprotection is through regulation of the neurotrophin NGF which, unlike GDNF or BDNF, declines in expression in the adult as a result of VD developmental deficiency [92]. Either through the direct requirement of VD as a ligand for the VD nuclear receptor, or through secondary routes such as promotion of NGF expression, VD developmental deficiency leads to loss of a wide range of proteins in frontal cortex involved in tasks including, as previously described, mitochondrial function, cytoskeletal control, synaptic plasticity, chaperoning and neurotransmission [93]. Maintenance of sufficient VD levels during development will be essential to maintain normal levels of these proteins.

That VD has less of a neuroprotective action in the adult is implied, for instance, in a study by Schneider et al. [175]. This small study investigated the relationship of VD with schizophrenia, alcoholism and major depression and found

levels in all three disorders to be lower than in controls. The absence of any specificity to one particular disease led the authors to speculate that this signified malnutrition in these groups [175].

5.6.2 Thyroid Hormone and Depression

As already described, both hypothyroidism and hyperthyroidism have been associated with depression and TH is only neuroprotective when its balance is maintained correctly and that it is damaging when levels are either to high or too low. Although explicit thyroid disease is not evident in the majority of depressed patients a significant number may show subclinical signs [176] and, for instance, TH feedback to the hypothalamus can be disrupted in depression with decreased TRH transcript in neurons of the hypothalamic paraventricular nucleus [177]. Some cases of depression where thyroid disease is not detected may be due to the difficulty in defining exactly when TH homeostasis is abnormal. A major difficulty is that the complexity of TH homeostatic regulation means that no single measure will accurately determine what the TH levels are in any particular tissue, such as the brain. The standard treatment for hypothyroidism is T_4 therapy, titrated until serum TSH levels are between 0.5 and 2.0 mIU/L [178] but this does not restore correct T_3/ T_4 levels in plasma and tissues. In animal models such a balance required treatment with T_3 and T_4 together but treatment of hypothyroid patients with such a combined therapy has generally not been successful to alleviate cognitive difficulties (reviewed by Escobar-Morreale et al. [179]).

In cases where hypothyroidism is clearly diagnosed then major depressive disorder commonly results. Use of TH as an adjunctive to antidepressants was first proposed in 1969 [180] and T_3 improves the response to tricyclic antidepressants, possibly to a greater extent in women [146]. It would be predicted that such an augmentative effect would be strongest in those with subnormal thyroid function and depression in patients with skin dryness, high cholesterol and complaints of cold may suggest hypothyroidism; however, an effect can be evident in patients with a normal TH response [181]. Supraphysiological doses of TH have been found to be effective in cases of anti-depressant resistant unipolar and bipolar depression [182] and improves function in prefrontal and limbic brain areas [183]. Possible insight into the mechanism of action of such treatment is provided by the observation that high doses of TH do not result in the same increase in circulating TH that would occur in normal individuals suggesting that this may be correcting a hidden defect in TH function [184].

One potentially revealing influence of hypothyroidism on the brain is the reduction in cerebral blood flow, frequently reported in a region specific manner [185–187]. Such localized reductions in blood flow also occur in depression, which can at least be partially regionally correlated with the changes in hypothyroidism [186]; although it may be noted that another study found hypothyroidism and depression to show cerebral blood flow changes in different regions, with the exception of pre- and post-central gyri [187]. Regional cerebral blood flow is even

reduced in cases of mild hypothyroidism and, surprisingly has been found not to be restored to normal levels with treatment and restoration of the euthyroid state [185], although other studies on transient hypothyroidism have shown variability in restoration of blood flow with treatment [188]. Other imaging studies have found hypothyroidism to result in more global effects on the brain, reducing cerebral blood flow and glucose metabolism, at least when hypothyroidism is of short duration due to thyroidectomy; hypothyroidism in these cases however can still be correlated with depression. [189]. Positron emission tomography (PET) has also been used to identify changes in cerebral metabolism in prefrontal and limbic brain regions in patients with bipolar depression and treatment with T_4, altered metabolism correlating with improvement in depression.[183]. Thus brain imaging studies may point the way to the regions affected by hypothyroidism and determine the points of overlap with thyroid dependent and independent depression.

5.6.3 Thyroid Hormone and Effects on Cognition

TH likely plays an essential function in adult working memory [190] and hypothyroidism results in subtle cognitive impairments [191]. Cognitive deficits are evident in depression and a decline in TH signalling may be a factor in this. Cognitive difficulties are also evident in schizophrenia and this may be a point of overlap between the two disorders with regard to the contribution of altered thyroid hormone signalling. Zhu et al. [192] showed that hypothyroidism and subclinical hypothyroidism results in a decline in human executive memory performance correlated with a decrease in activation, as determined by fMRI of frontoparietal regions and such deficits could be improved with TH treatment. One likely mechanism by which TH supports working memory is through the requirement of TH for some aspects of neuroplasticity. Hypothyroidism in the adult rat model leads to a reduction in paired-pulse facilitation and LTP of postsynaptic potentials when investigated in the dorsal hippocampo-medial prefrontal cortex and this decline is reversed by replacement of TH [193]. Thus TH supports synaptic plasticity at least in some brain regions required for learning and memory. A potential mediator of this control is neurogranin (RC3), a postsynaptic substrate for protein kinase C which is phosphorylated during long term potentiation and regulates calcium mediated signalling [194]. Neurogranin is directly regulated by thyroid hormone [195] and the human gene has a thyroid hormone response element in its promoter [196]. Neurogranin knockout mice perform poorly in the morris water maze and exhibit decreased LTP. Studies with TH receptor knockout mice suggest that neurogranin may contribute to the weakened learning and memory in this mutant [197] and similarly may play a role in the decline in striatal synaptic plasticity in the hypothyroid mouse [198]. Neurogranin may also provide a molecular relationship between TH and schizophrenia given the large reduction reported of neurogranin in the prefrontal cortex of schizophrenics [199]. A recent genetic association study also pinpointed neurogranin as a gene linked with schizophrenia [200].

5.6.4 Summary

Modification of nutrition provides a route of neuroprotection from psychiatric disease that is both inexpensive and with few side-effects. The diet in western countries is suboptimal; although not deficient in quantity it can be insufficient in certain micronutrients required, for instance, for neurotransmitter synthesis [201]. Other micronutrients may be beneficial for psychiatric disorders when given at hyperphysiological doses, as suggested for omega-3 fatty acids [202]. A range of vitamins have been proposed to protect against psychiatric diseases (see Chapter 17 in this book). We have previously detailed the micronutrient factor, vitamin A, that regulates transcription through a member of the nuclear receptor family of transcription factors, and which may influence depression, schizophrenia and autism [144]. We described in this chapter the essential requirement for VD during development and how this protects against abnormal development that may promote schizophrenia. Certainly, disruption of TH signalling during embryonic development will also result in embryonic abnormalities, including those that show some parallels to the abnormalities evident in schizophrenia, e.g. myelination and synapse formation. However, it is difficult to build up a case of linkage based simply on these abnormalities, given that such changes are evident following alterations in many other embryonic regulatory factors. The better rationale is for TH's influence on the adult where either excess or deficiency can result in symptoms of depression. Similarly, although the potential for VD deficiency to influence the adult brain exists, particularly in the case of SAD, it is unlikely to be contributory to most cases of depression and stronger evidence exists for its requirement in the developing brain. Although the evidence is not conclusive [203] the VD deficient mouse may provide a useful model of schizophrenia [204] and has provided candidate genes regulated by VD and whose abnormal expression may contribute to depression [93, 94].

Acknowledgements We would like to thank Dr. Michelle Lane for her reading of the text and excellent suggestions as well as thanking Jemma Ransom for her reading of the section on schizophrenia.

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Chapter 6
Phosphatidylinositol 3-Kinase/Glycogen Synthase Kinase and Mitogen-Activated Protein Kinase Signalling Cascades in Neuronal Cell Survival: What the Neurotrophins Have Taught Us and Implications For Neuropsychiatric Disorders

Stephen D. Skaper

Abstract Cellular diversity in the nervous system evolves from the concerted processes of cell proliferation, differentiation, migration, survival, and activity-dependent synaptic remodelling. Identification of the molecular components responsible for these processes is tied strongly to our knowledge of neurotrophic factors, their cognate receptors, and associated signal transduction pathways. Neurotrophic factors (e.g. nerve growth factor, brain-derived growth factor, glial cell line-derived neurotrophic factor) initiate signalling by activation directly or indirectly of transmembrane receptors which, in turn, regulate the activity of critical cellular proteins by posttranslational modification. The mitogen-activated protein kinase/extracellular signal-regulated kinase and phosphatidylinositol 3-kinase (PI 3-kinase)/Akt signalling pathways are the two principal pathways mediating neurotrophic pro-survival action. In the case of PI 3-kinase/Akt activation, survival appears to involve phosphorylation and inactivation of the downstream pro-apoptotic effector glycogen synthase kinase-3 (GSK-3). Intriguingly, Akt/GSK-3 signalling may be impaired in schizophrenia, and that the NMDA antagonist phencyclidine reportedly can induce neuronal cell death and behavioural deficits that mimic some symptoms of schizophrenia, while decreasing Akt activity and increasing GSK-3 activity. In addition, several clinical studies suggest that certain forms of schizophrenia may be associated with changes in the genetic code of neurotrophic factors. Although controversial, an association of the *Akt1* gene with schizophrenia has been claimed in some populations. This chapter will provide an overview of these signalling cascades in neuronal cell survival, an appraisal of findings in support of altered PI 3-kinase/Akt/GSK-3 signalling in neuropsychiatric disorders, and the potential for their manipulation as a neuroprotective strategy.

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_6,

Abbreviations

BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CREB	cAMP-response element binding protein
GSK-3β	glycogen synthase kinase-3β
MAP	mitogen-activated protein
MEK	MAP-ERK kinase kinase
MEKK2	MAP kinase-activated protein kinase-2
NGF	nerve growth factor
NMDA	N-methyl-D-aspartic acid
NT-3	neurotrophin-3
NT-4	neurotrophin-4
PCP	phencyclidine
PI 3-kinase	phosphatidylinositol 3-kinase
PLC-γ1	phospholipase C-γ1
Rsks	ribosomal S6 kinases
SH2	Src homology 2
SOS	son of sevenless

6.1 Introduction

In mammals and other vertebrates, soluble peptide growth factors play essential roles in intercellular communication. They exert their effects by signalling through surface membrane receptors that interact with diverse types of intracellular second-messenger systems. In a sometimes surprising manner, many growth factors have been found to subserve a wide variety of functions by acting on many cell types at different stages of development or in adult life. One of the major advances of cellular neuroscience has been to recognise that much of the cellular damage resulting from such central nervous system (CNS) insults as stroke, trauma, and neurodegenerative disease may be caused by a limited number of endogenously generated molecules with neurotoxic activities. Less well-developed is the idea that endogenous mechanisms exist to provide neuroprotection, and that endogenous molecules may be produced specifically to service neuroprotective signalling functions.

Neurotrophic factors are secreted proteins that promote neurite outgrowth, neuronal cell differentiation and survival both in vivo and in vitro. Since their discovery in the 1950s, neurotrophic factors have raised expectations that their clinical application to neurodegenerative diseases might provide an effective therapy for what are now untreatable conditions. The importance of this class of molecules is illustrated by the severe neurological deficits found in animals in which a neurotrophic protein or its corresponding receptor has been deleted by homologous recombination [1]. Independent lines of investigation have shown the neurotrophins to protect against neuronal dysfunction and death in animal models of injury and neurologic disease [2–11]. Moreover, clinical studies indicate that certain forms of schizophrenia may

be associated with changes in the genetic code of neurotrophic factors [12, 13]. These data raise the exciting possibility that neurotrophins, or drugs which activate their pro-survival signalling pathways may act to protect neurons in disease as well as normal neurons. This article will discuss the biology of neurotrophins, their signalling pathways involved in promoting neuronal cell survival, and potential role in neuropsychiatric disorders.

6.2 Neurotrophins and Their Receptors

Neurotrophins were identified as promoters of neuronal survival, but it is appreciated that they regulate many aspects of neuronal development and function, including synapse formation and synaptic plasticity [14–17]. The first neurotrophin, nerve growth factor (NGF), was discovered during a search for survival factors that could explain the deleterious effects of deletion of target tissues on the subsequent survival of motor and sensory neurons [18]. NGF is part of the neurotrophin family of approximately 25 kDa polypeptides which function as homodimers, and which share a high degree of structural homology and includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) [19]. Neurotrophins are found in both mammals and lower vertebrates, while the neurotrophin homologues NT-6 and NT-7 were cloned in fish [20, 21]. These do not have orthologues in mammals or birds and appear to interact with the same receptors as the mammalian proteins. The neurotrophins exhibit actions on distinct, as well as partially overlapping, subsets of peripheral and central neurons [22, 23]. Individual neurons may also be responsive to more than one neurotrophin at a given time or at subsequent times during development. Moreover, the actions of neurotrophic factors are not restricted to developing post-mitotic neurons. Indeed, these factors can act on dividing neuroblasts [24].

The neurotrophins and their genes share homologies in sequence and structure, including the existence of multiple promoters [25]. The protein product of each gene includes a signal sequence and a prodomain, followed by the mature neurotrophin sequence. Regulation of neurotrophin maturation may be an important post-transcriptional control point that limits and adds specificity to their actions. Neurotrophin activity is regulated by the proteases responsible for conversion of the proneurotrophins to mature neurotrophins. Interest in these proteases has been stimulated by studies showing that proneurotrophins are secreted from cells and are biologically active [26, 27]. Proneurotrophins bind with high affinity to $p75^{NTR}$, and activate signalling pathways controlled by this receptor, which in many cells results in promotion of apoptosis [26, 28, 29] attributable to concomitant interaction with the sorting receptor sortilin [30], a member of the Vps10p family of receptors.

The mature neurotrophin proteins are non-covalently associated homodimers. The neurotrophins share a highly homologous structure [31–35] and are members of a large superfamily of growth factors that contain a tertiary fold and cysteine “knot”. These features are present in transforming growth factor-β, platelet-derived growth

factor, human chorionic gonadotropin, vascular endothelial growth factor, and others. The cysteine knot consists of three disulfide bonds that form a true knot of the polypeptide chain. Two cysteines that make up the knot are missing from human NT-6. Neurotrophin residues are generally divided into two categories, conserved or variable, based on sequence alignments [23]. Amino acid residues implicated in neurotrophin binding that are conserved are likely to represent a common interface to the Trk receptors, while the unique ones may represent elements of specificity [36]. Detailed discussions of neurotrophin structure and molecular evolution have appeared elsewhere [37–40].

The neurotrophins interact with two entirely distinct classes of receptors, Trks (tropomyosin receptor kinases) and $p75^{NTR}$, the first discovered member of the tumour necrosis factor receptor superfamily. The latter was initially identified as a low-affinity receptor for NGF [41, 42], but was subsequently shown to bind each of the neurotrophins with approximately equal nanomolar affinity [43–45] but lacks a catalytic motif. $p75^{NTR}$ binds NGF along the interface between two NGF monomers and binding results in a conformational change in NGF that alters the monomeric interface on the opposite side of the NGF dimer, eliminating the potential for binding of one NGF dimer to two $p75^{NTR}$ monomers. In contrast to interactions with $p75^{NTR}$, the neurotrophins dimerise the Trk receptors, resulting in activation through transphosphorylation of the kinase motif present in their cytoplasmic domains. The four neurotrophins exhibit specificity in their interactions with the three members of this receptor family with NGF activating TrkA [46–48], BDNF and NT-4 activating TrkB [49–51], and NT-3 activating TrkC [52]. In addition, NT-3 can activate the other Trk receptors with less efficiency [50, 51, 53, 54] (Fig. 6.1).

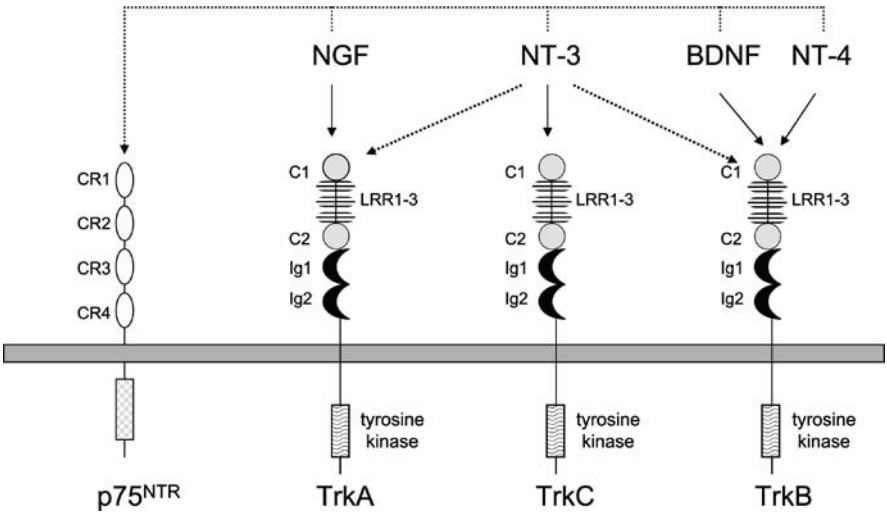

Fig. 6.1 Neurotrophins and their receptors. The neurotrophins display specific interactions with the three Trk receptors: NGF binds TrkA, BDNF and NT-4 bind TrkB, and NT-3 binds TrkC. In some cellular contexts, NT-3 can also activate TrkA and TrkB albeit with less efficiency. All neurotrophins bind to and activate $p75^{NTR}$. CR1-CR4, cysteine-rich motifs; C1/C2, cysteine-rich clusters; LRR1-3, leucine-rich repeats; Ig1/Ig2, immunoglobulin-like domains

In vitro studies indicate that the presence of $p75^{NTR}$ potentiates activation of TrkA by suboptimal concentrations of NGF, although it does not appear to potentiate the activation of other Trk receptors similarly by their ligands [55, 56], and that $p75^{NTR}$ collaborates with TrkA to form high-affinity (10^{-11} M) binding sites for NGF [57]. The major site at which neurotrophins interact with the Trk receptors is in the membrane-proximal immunoglobulin(Ig)-like domain. The three-dimensional structures of the domain in each of the Trk receptors has been solved [58]. In addition, the structure of NGF bound to the TrkA Ig domain has also been determined [36]. These results have made it possible to identify residues in the neurotrophins and the Trk receptors that account for the specificity observed in their interactions [59, 60].

Trk receptor function is modulated by $p75^{NTR}$ on several levels—by promoting ligand binding, by promoting accessibility to neurotrophins through promotion of axonal growth and target innervation, and by promoting endocytosis and retrograde transport to membrane compartments where internal engagement of neurotrophins with Trk receptors may promote signalling. For example, $p75^{NTR}$ inhibits activation of Trk receptors by non-preferred neurotrophins both in vivo and in vitro [61, 62]. The presence of $p75^{NTR}$ potentiates activation of TrkA by suboptimal concentrations of NGF, although it does not appear to potentiate the activation of other Trk receptors similarly by their ligands [63, 64], and collaborates with TrkA to form high-affinity binding sites for NGF [65]. In addition to the promotion of binding of NGF to TrkA, $p75^{NTR}$ can promote retrograde transport of several neurotrophins [66]. $p75^{NTR}$ may reduce ligand-induced Trk receptor ubiquitination, thereby delaying Trk internalisation and degradation [67], or promote Trk receptor endocytosis through polyubiquination and subsequent internalisation to endosomal compartments, resulting in enhanced signalling [68]. These findings each suggest a mechanism by which $p75^{NTR}$ may promote axon growth and target innervation in vivo and in vitro [69, 70]. Significant, albeit incomplete, sensory and sympathetic deficits have been observed in mice lacking $p75^{NTR}$, although the precise $p75^{NTR}$ function(s) responsible for preventing these deficits remains to be clarified [71–73].

6.3 Neurotrophin Signalling Pathways

Many extracellular signals transduce their cellular responses by regulating tyrosine phosphorylation of their target proteins. Ligand-induced oligomerisation of receptor protein tyrosine kinases and autophosphorylation have been established as a general mechanism for the activation of growth factor receptors, as well as many other families of cell surface receptors [74]. The Trk receptors are typical receptor tyrosine kinases whose activation is stimulated by neurotrophin-mediated dimerisation and transphosphorylation of activation loop kinases [75]. The cytoplasmic domains of the Trk receptors contain several additional tyrosines that are also substrates for phosphorylation by each receptor's tyrosine kinase. When phosphorylated, these residues form the cores of binding sites that serve as a scaffolding for the recruitment of a variety of adaptor proteins and enzymes that ultimately propagate the neurotrophin signal [76]. Within the activated Trk molecule the phosphotyrosines and

their surrounding amino acid residues create binding sites for proteins containing phosphotyrosine-binding or Src homology 2 (SH2) domains. The major pathways activated by the Trk receptors are Ras, Rac, phosphatidylinositol 3-kinase (PI 3-kinase), phospholipase C-γ1 (PLC-γ1) and their downstream effectors [75, 76]. In addition, endocytosis and transfer of Trk receptors to different membrane compartments control the efficiency and duration of Trk-mediated signalling, in part because many adaptor proteins are localised to specific membrane compartments [77].

6.3.1 The Ras-MAP Kinase Pathway

Activation of Ras is required for normal neuronal cell differentiation and also promotes survival of many neuron subpopulations. Interestingly, $p21^{ras}$ may mediate NGF signals in some neurons (for example, avian sensory or rat sympathetic neurons) but not in others (avian sympathetic neurons), despite the ability of NGF to activate Ras in all Trk-expressing neurons [78–80]. Transient activation of Ras is mediated by the adaptor protein Shc, which is recruited to its recognition site on activated Trk via interactions with the Shc PTB domain [81]. This sets in motion the Ras-mitogen-activated protein (MAP) kinase cascade. Trk-mediated phosphorylation of Shc creates a phosphotyrosine site on Shc that recruits another SH2 domain-containing protein, Grb2, which is bound to the nucleotide exchange factor SOS (son of sevenless). Activation of Ras may also be facilitated by neurotrophin-dependent phosphorylation and activation of the Ras G protein-releasing factor, RasGRF1 [82]. A Grb2-SOS complex translocates to the plasma membrane, where SOS activates the small G protein $p21^{ras}$ by replacing GDP with GTP [76]. Active Ras stimulates signalling through the protein kinases Raf-1, B-Raf, MAP-ERK kinase kinase (MEK), and p38 MAP kinase [83–86]. Activation of one or more of these kinases leads to the phosphorylation and activation of MEK and the MAP kinase isoforms, ERK-1 and ERK-2 [83, 85, 87] (Fig. 6.2).

Introducing activated forms of upstream regulators of MAP kinase, including Ras [88], Raf [89], or MEK [90], mimics NGF by inducing neurite outgrowth in PC12 pheochromocytoma cells. The dominant negative forms of Ras [91], Raf [92], and MEK [90] block the neurite outgrowth in PC12 cells stimulated by NGF. A specific inhibitor of MEK, PD098059, which has no activity at PI 3-kinase or other serine-threonine or tyrosine kinases [93] arrested neurite outgrowth but did not block survival in PC12 cells terminally differentiated with NGF [94]. In neurons, p38 is probably activated by a pathway initiated by Ras-mediated binding and activation of the exchange factor Ra1GDS, which results in activation of Ra1 and recruitment of Src [95]. p38 MAP kinase in turn activates MAP kinase-activated protein kinase-2 (MEKK2). Ras also triggers a signalling cascade through sequential activation of Wnk1, MEKK2 and MEK5 that results in activation of ERK5 (also known as big MAP kinase 1) [96, 97]. Selective p38 inhibitors promote the in vitro survival of chicken embryo sensory [94, 98], sympathetic, ciliary, and motor neurons [99].

Each of the above MAP kinase cascades has overlapping, as well as distinct targets within the cell [99]. ERK1/ERK2 and ERK5 phosphorylate and activate the

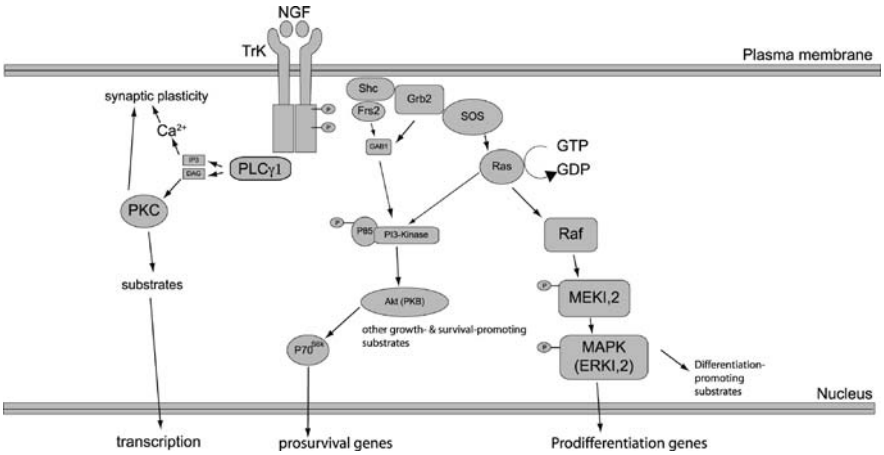

Fig. 6.2 Neurotrophin signalling. Depicted are interactions of NGF (exemplar neurotrophin) with Trk and major intracellular signalling pathways activated. Neurotrophin binding to Trk receptor leads to dimerisation and autophosphorylation. The linker Shc binds to phospho-Y490 on Trk and to a Grb2-SOS complex. SOS is a nucleotide exchange factor that activates Ras by replacing GDP with GTP. Activated Ras interacts directly with the serine-threonine kinase Raf. The activated Raf leads to the sequential activation of MEK, the mitogen-activated protein kinase-ERK kinase (MAPK). MAPK translocates to the nucleus, where it phosphorylates transcription factors and promoting neuronal cell differentiation. Activation of PI 3-kinase through Ras or Gab1 promotes survival and growth of neurons. Activation of PLC-γ1 results in activation of Ca^{2+}- and protein kinase C-regulated pathways that promote synaptic plasticity. See text for further details (pp. 8–13)

ribosomal S6 kinases (Rsks) ($pp90^{rsks}$) [100]. Rsks and MEKK2 each phosphorylate transcription factors such as cAMP-response element-binding protein (CREB), which activate transcription of genes essential for differentiation and survival of neurons [100]. In addition to shared targets, the different MAP kinase cascades also have distinct transcription factors as targets [99]. For example, ERK5, but not ERK1/ERK2, activates myocyte enhancer factor 2 [101], while ERK1/ERK2, but not ERK5, activates ELK-1 [102]. The MAP kinase cascades feedback to attenuate and terminate responses through phosphorylation of intermediates and activation of phosphatases. As an example, ERK and Rsk mediate phosphorylation of SOS, which results in dissociation of the SOS-Grb2 complex [103].

6.3.2 The PI 3-Kinase and PLC-γ1 Pathways

Even in rat sympathetic neurons where Ras is mediating survival, it is clear that other signalling pathways are activated, because ERK1 and ERK2 do not mediate the Ras-dependent survival effects [104–106]. Generation of P3-phosphorylated phosphoinositides by PI 3-kinase promotes survival of many neurons, and there is evidence to suggest that Ras can signal through PI 3-kinase [107]. In many neurons, Ras-mediated activation of PI 3-kinase initiates the major pathways through which survival signals are conveyed [108]. For example, in PC12 cells a common

tyrosine residue(s) may be responsible for coupling Trk to the Ras signalling pathway and to PI 3-kinase [109]. Both couplings appear to occur via two common components, Shc and Grb-2, with Grb-2 docking further to pathway-specific proteins (Fig. 6.2). Recruitment of Gab1 (Grb2-associated binder-1) by phosphorylated Grb2 permits subsequent binding and activation of PI 3-kinase [110]. In addition, in some neurons, Trk receptor activation results in phosphorylation of insulin receptor substrate-1, which also permits recruitment and activation of PI 3-kinase [111]. PI 3-kinase may mediate neurotrophin survival effects, for example, that of NGF on PC12 cell survival [112]. Pharmacological agents that suppress PI 3-kinase activity block the capacity of insulin-like growth factor-1 [113] and BDNF [114] to sustain the survival of cerebellar granule neurons upon growth signal withdrawal.

The 3-phosphoinositides generated by PI 3-kinase trigger the activation of two protein kinases, the serine-threonine kinase Akt (also known as protein kinase B) [115], and the p70 ribosomal protein S6 kinase [116]. Akt controls the activities of several proteins important in promoting cell survival, including substrates that directly regulate the caspase cascade, such as BAD, a pro-apoptotic Bcl-2 family member [117]. Phosphorylated BAD is sequestered by 14-3-3 proteins, preventing its pro-apoptotic actions. Akt also regulates the activity of several transcription factors. Through phosphorylation of the forkhead transcription factor, FKHRL1, it promotes association of this transcription factor with 14-3-3 proteins, thereby sequestering it in the cytoplasm and preventing it from activating transcription of several genes whose products promote apoptosis [118]. Experiments with pharmacological inhibitors, as well as expression of wild-type and dominant-inhibitory forms of Akt, demonstrate that Akt but not p70 ribosomal protein S6 kinase mediates PI 3-kinase-dependent survival [114, 119, 120].

Trk kinase activation results in PLC-γ1 recruitment to a docking site similar on all Trks. The docked PLC-γ1 is activated through Trk-mediated phosphorylation and then hydrolyses phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate and diacylglycerol. Inositol trisphosphate triggers mobilisation of Ca^{2+} cytoplasmic stores while diacylglycerol stimulates diacylglycerol-regulated isoforms of protein kinase C. These two signalling molecules activate numerous intracellular enzymes, including all protein kinase C isoforms, Ca^{2+}-calmodulin-dependent protein kinases and other Ca^{2+}-calmodulin-regulated targets. PLC-γ1 activation results in activation of, e.g. protein kinase C-δ, which mediates NGF-promoted activation of MEK and ERK1/ERK2 [121]. The activity of PLC-γ1 has been implicated in the ability of TrkB receptors to modulate synaptic transmission and long-term potentiation [122, 123].

6.4 Neurotrophins and Glycogen Synthase Kinase-3

Differentiated cells, including neurons in the CNS, require the presence of survival factors to suppress the intrinsic cell death machinery and thereby avoid apoptosis [124]. The regulation of apoptosis by survival factors is therefore critical for normal development and proper functioning of multicellular organisms. In addition, abnormal apoptosis in CNS neurons may play a significant role in

neurodegenerative diseases [125]. Survival growth factors protect neurons from a variety of pro-apoptotic stimuli [18, 126], and one of the protective mechanisms has been attributed to the activation of the PI 3-kinase signal transduction pathway [112, 127–129]. Although effectors downstream from PI 3-kinase that mediate neuronal cell survival have not been completely identified, one likely candidate is Akt [130]. Akt phosphorylates and inhibits glycogen synthase kinase-3β (GSK-3β; also known as tau protein kinase I) [119, 131, 132], a proposed mechanism by which neurons become resistant to apoptotic stimuli [133–135]. GSK-3β may also be regulated by other PI 3-kinase-dependent, but Akt-independent, pathways [136, 137]. Recombinant over-expression studies using active or dominant negative proteins have led to the proposal that GSK-3 may be a relevant effector of PI 3-kinase-mediated neuronal cell survival [133, 134, 138].

GSK-3 is a multifunctional serine/threonine kinase that was originally identified as a regulator of glycogen metabolism [139]. Ubiquitously expressed in eukaryotes [140], GSK-3 occupies a central stage in many cellular and physiological events, including Wnt and Hedgehog signalling, transcription, insulin action, cell-division cycle, circadian rhythm, patterning and axial orientation during development, and others [141]. The GSK-3 kinase family is highly conserved throughout evolution. In humans, two genes encode two distinct but closely related GSK-3 forms, GSK-3α (51 kDa) and GSK-3β (47 kDa). They display 84% overall identity (98% within their catalytic domains) with the main difference being an extra Gly-rich stretch in the N-terminal domain of GSK-3α. However, they are not interchangeable functionally, as demonstrated by the embryonic-lethal phenotype observed when the gene that encodes GSK-3β is knocked out. Recently, GSK-3β2, an alternative splicing variant of GSK-3β that contains a 13-amino acid insertion in the catalytic domain, has been identified [142]. Only GSK-3β, however, is highly enriched in the brain [143], where it has been implicated in several CNS disorders, such as Alzheimer's disease [144], schizophrenia [145, 146], and bipolar disorders [144, 145].

In the insulin signalling pathway, insulin, through its tyrosine kinase receptor activates PI 3-kinase-mediated signalling, which results in Akt-mediated inhibition of GSK-3. In addition to mediating insulin's effects, PI 3-kinase and Akt have distinct properties as mediators of the actions of neurotrophic molecules and this represents the most established pathway by which GSK-3 exerts its neurotrophic/neuroprotective effects. For example, BDNF binding to TrkB results in receptor dimerisation, autophosphorylation of multiple tyrosine residues in the TrkB cytoplasmic domain, and subsequently modulation of intracellular signalling pathways including activation of PI 3-kinase, of which a primary target is Akt (Fig. 6.3). Akt phosphorylates GSK-3, among many other targets. The precise downstream mechanisms that mediate GSK-3's actions in neurotrophic pathways are not fully understood, but are believed to include effectors such as p53, CREB, heat-shock factor-1, c-Jun, and Bax [144, 147, 148].

Post-mortem studies reported a reduction in cortical volume, a decrease in the density of neurons and glial cells, as well as neuronal cell atrophy in the prefrontal, orbital, and cingulated cortices, amygdale, and other brain areas in patients with bipolar disorder [149, 150]. Structural imaging studies showed a reduction in gray matter volume in orbital and medial prefrontal cortices, ventral striatum,

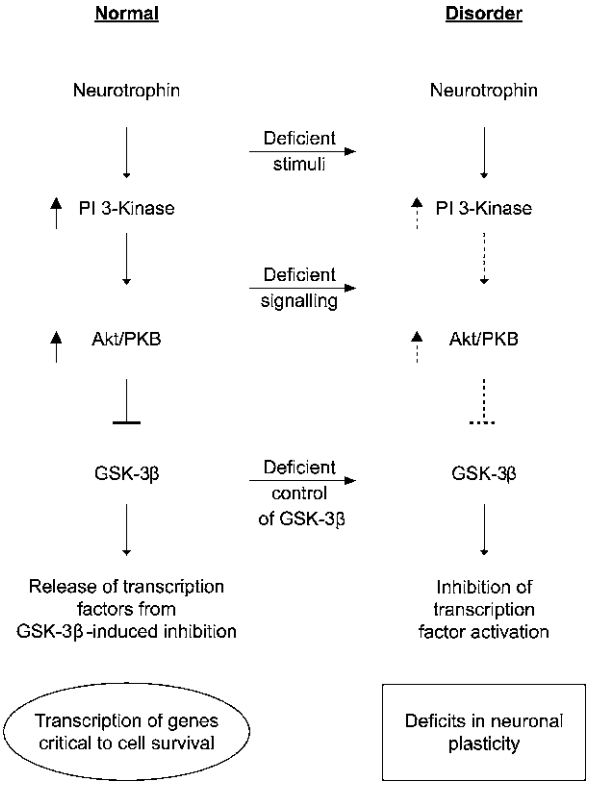

Fig. 6.3 GSK-3 is a component of neurotrophin signalling pathways. Neurotrophins such as BDNF act through their cognate Trk receptors to activate PI 3-kinase, Akt, and inhibit GSK-3. Many effectors have been implicated in GSK-3's neurotrophic actions, including transcription factors (e.g. heat-shock factor-1, c-Jun, p53, and cyclic AMP response element binding protein) and recently the pro-apoptotic bcl-2 family member BAX

and hippocampus as well as enlargement of the third ventricle in bipolar patients [149–151]. Functional imaging has also revealed multiple abnormalities of regional blood flow and glucose metabolism in these same limbic and prefrontal cortical structures [151, 152]. Thus, both neuroimaging and post-mortem investigations suggest that the pathology of mood disorders may involve cell- and structural-based impairments in function and plasticity, consequences that perhaps could be modified by the neurotrophic effects of pharmacological agents. Intriguingly, the recent discovery of reduced Akt concentrations and decreased phosphorylation of Ser9 of GSK-3β (and, presumably increased GSK-3β activity) in the brains of patients with schizophrenia, and the compensatory effect induced by the antipsychotic drug haloperidol indicate that alteration in Akt and GSK-3β contributes to this neuropsychiatric disorder [153].

6.5 The PI 3-Kinase/Akt/GSK-3 Pathway as a Target for Neuroprotection

As mentioned above, GSK-3 is inhibited by Akt-mediated phosphorylation, which may play a critical role in regulating neuronal cell survival. Recombinant genetic studies indicate that GSK-3 can play a key role in the survival of some neuronal

cell populations in response to apoptotic triggers [133, 134, 138, 154]. However, interpretations based on the latter alone require caution, given the possible artefactual perturbation of normal patterns of the temporal and spatial regulation of protein kinase-mediated signalling complexes. It is becoming apparent that cellular responses to GSK-3 modulation can vary according to the nature of the inhibitory signal [155, 156]. Thus, the cellular consequences of GSK-3 inhibition may vary according to cell type and range of environmental stimuli. Moreover, given the potential degree of cross-talk and dynamics of GSK-3 regulation, the impact of overexpressing recombinant mutant GSK-3 and/or protein inhibitors of GSK-3 activity towards some substrates may be quite distinct from pharmacological inhibition of endogenous pools of active GSK-3 in some cell types.

Lithium has been in widespread clinical use for over 3 decades, primarily for the treatment of bipolar disorder and unipolar depression, although its therapeutic target remains uncertain [157]. Long-term lithium treatment increases total gray matter content [158] and enhances levels of N-acetyl-aspartate, a marker of neuronal cell viability, in the brain of bipolar patients [159]. In addition, bipolar subjects with past lithium or valproic acid (VPA; another mood-stabilising drug) exposure tend to have greater amygdalar gray volume than control patients without such exposure [160]. Interestingly, the loss of the subgenual prefrontal cortex volume found in bipolar patients was essentially suppressed in patients receiving protracted lithium or VPA [161].

Lithium directly inhibits GSK-3 activity in an ATP non-competitive manner, with an IC50 of $\sim$2 mM [162, 163], and has been used extensively as a tool to support a role for GSK-3 in neuropathological settings. Several studies have shown that lithium inhibits tau hyperphosphorylation, degeneration, and cognitive deficits in animal models [164–167], and can protect cultured neurons against amyloid β-peptide-induced neurotoxicity [168]. Therapeutic levels of lithium have been claimed to reduce production of amyloid β-peptides in a mouse model of Alzheimer's disease, by interfering with amyloid precursor protein cleavage [169]. Lithium may ameliorate also the in vivo neurotoxicity of the HIV-gp120 envelope protein [170], and prion peptide toxicity to neurons in vitro [171]. Neuroprotection against glutamate excitotoxicity in vitro, and in animal models of excitotoxin injury (e.g. cerebral ischemia, quinolinic acid infusion) have been described for lithium [172]. Lastly, treatments with GSK-3 inhibitors including a clinical dose of lithium to rats with thoracic spinal cord transection or contusion injuries induced significant decending corticospinal and serotonergic axon sprouting in caudal spinal cord and promoted locomotor functional recovery [173].

Interpretation of lithium's action is complicated by the many additional activities of this agent, including inhibition of polyphosphate 1-phosphatase, inositol monophosphatase, casein kinase-II, mitogen-activated protein kinase-2 and p38-regulated/activated kinase [174, 175], as well as the activation of PI 3-kinase/protein kinase B and c-Jun N-terminal kinase in cellular assays [176, 177].

More than 30 inhibitors of GSK-3 have been identified to-date [142, 178]. A number of these have been co-crystallised with GSK-3β and all localise within the ATP-binding pocket of the enzyme. Several of these molecules have been applied as neuroprotective agents, and some examples are discussed below.

Two novel potent and selective small-molecule ATP-competitive inhibitors of GSK-3 activity are the structurally distinct maleimides SB-216763 and SB-415286, that inhibit purified GSK-3α in vitro with IC_{50}s of 78 nM and 74 nM, respectively, and show similar potency towards purified GSK-3β [179]. Neither compound exhibits significant activity towards any member of a panel of two dozen other related protein kinases, including Akt and 3-phosphoinositide-dependent protein kinase-1. SB-216763 and SB-415286 promote the survival of cerebellar granule neurons and sensory neurons following neurotrophic factor withdrawal, or inhibition of PI 3-kinase activity with LY294002 [180], the latter inactivating the PI 3-kinase/Akt survival pathway, thereby activating GSK-3β (Fig. 6.4). Inhibition of neuronal cell death correlated with inhibition of GSK-3 activity and phosphorylation or stabilisation of the GSK-3 substrates tau and β-catenin, respectively, in intact neurons [180]. These data provide clear pharmacological and biochemical evidence that selective inhibition of endogenous GSK-3 pools in primary neurons is sufficient to prevent death, implicating GSK-3 as a physiologically relevant principal regulatory target of the PI 3-kinase/Akt neuronal survival pathway.

Excessive glutamatergic transmission, particularly when mediated by the N-methyl-D-aspartic acid (NMDA) subtype of glutamate receptors, is thought to underlie neuronal cell death in several types of acute brain injury, including cerebral

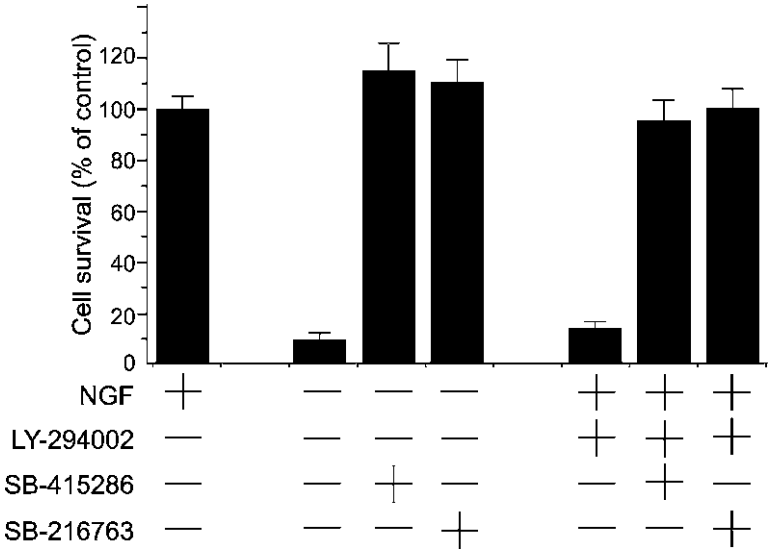

Fig. 6.4 The GSK-3 inhibitors SB-216763 and SB-415286 prevent death of chicken embryonic dorsal root ganglion neurons. Sensory neurons were cultured without NGF or with NGF (50 ng/ml) and 50 μM LY294002, in the absence or presence of SB-216763 (3 μM) or SB-415286 (30 μM). Surviving neurons were counted 48 h later. Data are expressed as a percentage of control cultures with NGF only (=100%) (± sem, n=3). [Reprinted from [180]. Copyright (2001) with permission from the International Society for Neurochemistry]

ischemia, hypoglycaemia, seizures and mechanical trauma [181]. Glutamate-induced neuronal cell death in discrete brain areas has also been implicated in chronic neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's chorea [182, 183]. Long-term treatment with SB-216763 protected CNS neurons against excitotoxic death mediated by both NMDA and non-NMDA receptors [184] (Table 6.1).

Interestingly, short pre-incubation or co-treatment alone with GSK-3 inhibitor was insufficient to render neurons resistant to excitotoxin injury [184], in contrast to cerebellar granule neuron death caused by pharmacological or physiological block of the PI 3-kinase/Akt cascade [180]. This suggests that distinct mechanisms may underlie these different death paradigms. The molecular basis for the neuroprotective effects of persistent treatment with GSK-3 inhibitors is not known but may reflect, in part, enhanced gene expression through reduced phosphorylation of c-Jun, causing disinhibition of the DNA binding activity of c-Jun [185].

In patients receiving radiation cancer therapy of the brain, learning disorders and memory deficits are a common consequence. A new study shows that GSK-3β is required for radiation-induced hippocampal neuronal apoptosis and subsequent neurocognitive decline. Inhibition of GSK-3β either by SB-216763 or SB-415286, or by ectopic expression of kinase-inactive GSK-3β before irradiation significantly attenuated radiation-induced apoptosis of hippocampal neurons [186]. GSK-3β inhibition also decreased apoptosis in the subgranular zone of the hippocampus in irradiated mice, leading to improved cognitive function in irradiated animals. Radiation led to increased accumulation of p53, whereas inhibition of the basal level of GSK-3β activity before radiation prevented p53 accumulation, suggesting a possible mechanism of cytoprotection by GSK-3 inhibitors [186].

Valproic acid is a mainstay drug alternative to lithium in treating bipolar mood disorder and has been widely used as an anticonvusant for seizure patients [187].

Table 6.1 Protracted GSK-3 inhibitor pre-treatment limits glutamate injury to hippocampal neurons

Treatment	LDH (% net release)
None (glutamate alone)	32.3 ± 5.5
SB-216763 (μM)	
1	25.4 ± 4.0
3	$21.3 \pm 3.7^{*}$
10	$18.3 \pm 2.7^{\#}$
SB-415286 (μM)	
3	29.4 ± 3.4
10	$22.5 \pm 2.6^{*}$
30	$13.1 \pm 0.6^{\dagger}$

Hippocampal cell cultures were incubated with SB-216763 or SB-415286 beginning at day 1. Cultures were exposed to glutamic acid (100 μM, 30 min) at day 9, in the absence of GSK-3 inhibitor. Cell viability was quantified by LDH release 24 h later. Values are means ± s.d. (n=3). $^{*}p<0.05$; $^{\#}p<0.02$; $^{\dagger}p<0.005$ vs. glutamate alone. [Reprinted from [184]. Copyright (2003), with permission from Lippincott Williams & Wilkins]

Both agents have frequently been used in combination to treat bipolar patients resistant to monotherapy with either drug. Lithium and VPA display neuroprotective properties in vivo and in vitro, with VPA action most likely associated with inhibition of histone deacetylase [188]. A recent study shows synergistic neuroprotective effects of lithium and VPA or other histone deacetylase inhibitors in cerebellar granule neurons treated with glutamate, while pre-treatment with lithium or VPA alone provided little or no neuroprotection [189]. Combined treatment with lithium and VPA potentiated serine phosphorylation of GSK-3α and GSK-3β and inhibition of GSK-3 enzyme activity, and the over-expression of wild-type GSK-3β suppressed the synergistic effects of these mood stabilisers [189]. A subsequent report from this group demonstrated that combined lithium and VPA treatment delayed disease onset, reduced neurological deficits and prolonged survival in an amyotrophic lateral sclerosis mouse model for motor neuron disease [190]. These and other studies suggest that neuroprotective and neurotrophic effects of mood stabilisers, most likely through GSK-3 inhibition, enhance cellular resilience and plasticity and, in turn, contribute to their clinical efficacy [191], although additional longitudinal clinical studies are required to firmly establish this linkage.

6.6 BDNF—Antidepressant Interactions

Antidepressant-induced neuroplastic changes, such as changes in synaptic plasticity, neurogenesis, and synaptogenesis, may at least partially explain the delayed-onset action of antidepressants [152, 192–195]. BDNF and its cognate receptor TrkB seem to be particularly relevant factors involved in both the development of mood disorders and the action of antidepressants [193–196]. BDNF and serotonin are two seemingly distinct signalling systems that play regulatory roles in many neuronal functions, including survival, neurogenesis, and synaptic plasticity. A common feature of the two systems is their ability to regulate the development and plasticity of neural circuits involved in mood disorders such as depression and anxiety [152, 193, 195]. BDNF promotes the survival and morphological differentiation of serotoninergic neurons both in culture and in vivo [197, 198], while BDNF levels are reduced in mood disorders and preclinical depression models [199, 200]. Conversely, long-term treatment with antidepressants, including selective serotonin reuptake inhibitors enhances BDNF gene expression [201–203] (Table 6.2).

A growing body of evidence suggests that BDNF-mediated TrkB signalling is both sufficient and necessary for antidepressant-like behaviours in rodents. Infusion of BDNF or over-expression of TrkB produces antidepressant-like behavioural responses in preclinical models of behavioural despair [204–206]. The behavioural effects of the antidepressants imipramine and fluoxetine were abolished in transgenic mice with reduced BDNF levels or inhibited TrkB signalling in brain [207] (Fig. 6.5); this observation was seen also in forebrain-specific *bdnf* null mice

Table 6.2 Regulation of BDNF and TrkB mRNA in hippocampus by antidepressant treatments

	BDNF mRNA		TrkB mRNA	
Treatment	4.4 kb	1.8 kb	9.0 kb	7.5 kb
Chronic antidepressant treatment				
Tranylcypromine	181 ± 28*	158 ± 29*	139 ± 9*	142 ± 7*
Mianserin	144 ± 18*	147 ± 18*	91 ± 14	85 ± 11
Sertraline	120 ± 6*	104 ± 11	136 ± 12*	135 ± 11*
Desipramine	126 ± 8*	109 ± 20	141 ± 14*	134 ± 13*
Acute antidepressant treatment				
Tranylcypromine	112 ± 14	102 ± 15	91 ± 13	93 ± 17
Mianserin	104 ± 7	101 ± 7	119 ± 17	116 ± 16
Sertraline	93 ± 6	75 ± 6	98 ± 6	100 ± 9
Desipramine	112 ± 13	110 ± 14	94 ± 12	92 ± 13
Chronic nonantidepressant treatment				
Morphine	181 ± 28*	158 ± 29*	139 ± 9*	142 ± 7*
Cocaine	144 ± 18*	147 ± 18*	91 ± 14	85 ± 11
Haloperidol	120 ± 6*	104 ± 11	136 ± 12*	135 ± 11*

Levels of BDNF and TrkB mRNA in hippocampus were determined 3 h after the last chronic or acute drug treatments by Northern blot analysis. Data are percentage of vehicle mean ± SEM. The indicated groups (*) were significantly different from the vehicle treated group by ANOVA and Fisher's post hoc test. See Ref. [201] for additional details. [Reprinted from [201]. Copyright (1995), with permission from The Society for Neuroscience]

by using desipramine [208, 209], which has a relatively high affinity for the norepinephrine transporter in comparison to imipramine and fluoxetine. The BDNF val66met polymorphism, which influences activity-dependent BDNF release [210] has been associated with mood disorders in humans [211, 212]. Taken together, these findings propose that activity-dependent and temporal activation of TrkB plays a critical role in the antidepressant response possibly through the formation and stabilisation of new functional synaptic connections.

The effect of antidepressants appears to be a general phenomenon, as pharmacologically diverse compounds, including inhibitors of monoamine transporters and metabolism, produce rapid alterations in BDNF mRNA levels [213] and activate TrkB [214] in the rodent anterior cingulate cortex and hippocampus. In addition, antidepressants block and accelerate the reversion of stress-induced downregulation in BDNF mRNA and protein, respectively [201, 215]. Furthermore, acute and long-term antidepressant treatments induced TrkB-mediated activation of PLC-Cγ1 and increased the phosphorylation of CREB, a major transcription factor mediating neuronal plasticity. Over-expression of CREB in the hippocampus induces antidepressant-like behavioural effects in rodents [216], and CREB is required for antidepressant-induced increases in BDNF mRNA levels [217]. Indeed, there is a tight link between TrkB activity and CREB phosphorylation. Modulation of the phosphorylation of TrkB Shc binding site, phosphorylation of mitogen-activated protein kinase or Akt by antidepressants was not observed [214]. Brain

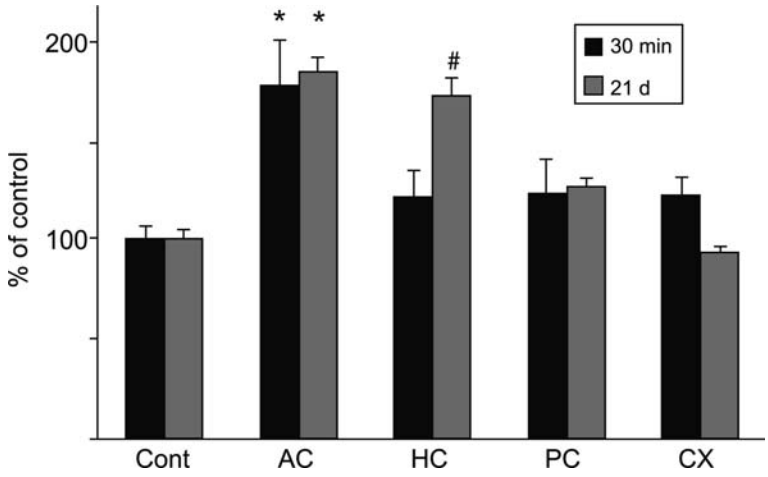

Fig. 6.5 Effects of acute or chronic anridepressant treatments on TrkB phosphorylation in different cortical areas. Mice were injected (i.p.) once or for 21 days with imipramine, tissue lysates precipitated with wheat germ agglutinin, and immunoblots probed with anti-phospho-trk 674/675 antibody. Values are means ± SEM; percentage of saline-treated control in the same cortical area shown (Cont) is saline control of the AC area. Anterior cingulate-prefrontal cortex (AC), hippocampus (HC), posterior cingulate cortex (PC), parietal cortex (CX). $^{*}p<0.05$; $^{\#}p=0.053$. See Ref. [207] for further details. [Reproduced from [207]. Copyright (2003), with permission from The Society for Neuroscience]

monoamines were crucial mediators of antidepressant-induced TrkB activation, as antidepressants did not produce TrkB activation in the brains of serotonin- or norepinephrine-depleted mice. Rapid activation of the TrkB neurotrophin receptor and PLC-Cγ1 signalling thus seems to be a common mechanism for all antidepressant drugs, and suggests that such molecules may serve as chemical templates for the design of TrkB modulators.

In this regard, small, dimeric peptides designed to mimic a pair of solvent-exposed loops important for the binding and activation of the BDNF receptor, TrkB, have been described [218]. The monomer components that make the dimers were based on a monocyclic monomeric peptide mimic of a single loop of BDNF (loop 2) shown previously to be an inhibitor of BDNF-mediated neuronal cell survival [219]. These bicyclic dimeric peptides behaved as partial agonists with respect to BDNF, promoting sensory neuron survival in vitro. A highly conformationally constrained tricyclic dimeric peptide, in which the monomeric subunits were linked by two dimerising linkages retained partial BDNF agonist action, while being 10-fold more potent in its neuronal survival effect than the best of the bicyclic dimers - making it ~2-fold less potent than BDNF itself. Partial agonistic behaviour could result from the compound being less efficient than BDNF in bringing about dimerisation of BDNF or because of inappropriate conformational flexibility of the dimerising linkages (although the retention of partial agonism by the highly constrained

tricyclic compound would argue against this). A consequence of reduced dimerisation efficiency could be a reduced ability to cause the autophosphorylation of the five tyrosine residues in TrkB (resulting in reduced activation of downstream signalling pathways). An alternative mechanism for the partial agonist behaviour of the dimeric peptides is that they act through TrkB only, even as full TrkB agonists, and not $p75^{NTR}$. A tandem repeat cyclic peptide agonist of TrkB has been described, as well [220, 221].

6.7 BDNF and the Glutamatergic System

The glutamatergic system has been implicated in the etiology and pathophysiology of schizophrenia [222]. NMDA receptor antagonists such as phencyclidine (PCP), MK-801 and ketamine cause schizophrenia-like symptoms in adult humans [223] and have been used in animals to model this disease [224]. Rats treated with PCP or ketamine during early postnatal development displayed schizophrenia-like behavioural changes during early adolescence or as young adults [225–227]. PCP treatment of developing rats induces apoptotic neurodegeneration in specific brain regions [228, 229] that is associated with schizophrenia-like alterations in sensorimotor gating and working memory later in life [225]. PCP-induced cortical degeneration is caspase-3 dependent and is also developmentally regulated [228–230].

Activation of synaptic NMDA receptors may enhance neuronal survival signalling cascades, including the PI 3-kinase/Akt pathway [231, 232]. PCP blockade prevents calcium influx through physiologically active NMDA receptors, which are mainly synaptic in location. Thus, PCP-induced neurotoxicity could result from a blockage of synaptic NMDA receptor signalling. Lei et al. [233] recently demonstrated that PCP inhibits the PI 3-kinase/Akt pathway and activates GSK-3β both in cortical cell culture and in neonatal rats. Activation of the PI 3-kinase/Akt pathway by enhancing NMDA receptor strength prevented PCP-induced neuronal cell apoptosis [233]. Consistent with other reports [234, 235], the anti-apoptotic property of the PI 3-kinase pathway was mediated by inhibiting GSK-3β, because GSK-3 small interfering RNA and lithium limited PCP-induced neuronal cell death [233]. A follow-up study from this group [236] showed that PCP also inhibits the MEK/ERK1/2 survival pathway in corticostriatal slices. Moreover, the neuroprotective effect of lithium against PCP-induced apoptosis in the cortical slices was mediated through co-stimulation of the PI 3-kinase/Akt and MEK/ERK pathways as well as through the indirect suppression of GSK-3β activity [236].

Akt-GSK-3β signalling may be impaired in schizophrenia. The *Akt1* gene is thought to be a potential susceptibility gene for schizophrenia [213, 237, 238]. Also, it has been shown that amphetamine-induced impairment of sensorimotor gating and working memory in prefrontal cortex is exaggerated in the Akt1-deficient mouse [239]. Moreover, antipsychotics, such as haloperidol and clozapine, reportedly enhance Akt-GSK-3β signalling [237].

The neuroprotective effect of low-level stimulation of NMDA receptors may also be linked to the regulation of neurotrophin production/signalling, namely BDNF/TrkB. Exposure of cerebellar granule neurons to a subtoxic concentration of NMDA (100 μM) elicited a time-dependent increase in BDNF mRNA, along with an accumulation of BDNF in the culture medium [240]. The increase of BDNF in the medium was followed by enhanced TrkB tyrosine phosphorylation, suggesting that NMDA increases the release of BDNF and therefore the activity of TrkB receptors. A soluble TrkB-IgG fusion protein, which is known to inhibit the activity of extracellular BDNF, and K252a, a tyrosine kinase/pan-Trk inhibitor, both blocked the NMDA-mediated TrkB tyrosine phosphorylation and subsequently its neuroprotective properties [240]. NMDA may thus activate TrkB via a BDNF autocrine loop, resulting in neuronal cell survival. More recently, Wu et al. [241] showed that activation of NMDA receptors increases exon 4-specific BDNF mRNA in a time-dependent manner in granule neurons. These authors discovered a nuclear factor-κB binding site in the 5′ flanking region of exon 4 of the *bdnf* gene and demonstrated that NF-κB plays an important role in NMDA receptor-mediated neuroprotection [242]. A similar BDNF autocrine loop was found with cultured hippocampal neurons, with a neuroprotective concentration of NMDA increasing BDNF [243], nuclear factor-κB and CREB [241].

6.8 Concluding Remarks

Initially discovered as target-derived survival factors, the neurotrophins are known to control cell fate, axon growth and guidance, dendrite structure and pruning, synapse formation, and synaptic plasticity. Considerable progress has been made in defining the nature of neurotrophin receptors and in characterising their signalling pathways. Determining the pathways activated by distinct factors provides insight into the molecular basis by which specific and biological effects can be elicited. The Ras-MAP kinase-ERK pathway is critical in neurotrophin-induced differentiation responses, particularly in neuritogenesis. This multicomponent pathway leads from the Trk receptors to nuclear transcription factors. Ras-independent pathways are less well understood, but appear to be critical in the effects of neurotrophins on survival. The PI 3-kinase signalling pathway has been implicated in intracellular targeting and the survival response, while activation of PLC-γ1 results in activation of Ca^{2+}-regulated pathways that promote synaptic plasticity. Perturbations in these signalling pathways may underlie neurodegenerative and neuropsychiatric disorders. Among the neurotrophins, BDNF appears to be a key player in these processes, whose synthesis and release are targeted by activation of glutamate receptors. A naturally occurring variation in humans, in the form of a common single-nucleotide polymorphism in the pro region of the polypeptide (Val66→Met), affects processing of the pro-BDNF protein and its activation-dependent release [244]. This variant is associated with differences in the volume of the hippocampal formation [245] and with anxiety and depression-related phenotypes [246, 247]. Moreover, convergent

findings supporting a role for BDNF in alterations to hippocampal structure and behaviour are found in a "humanised" BDNF transgenic mouse [246]. A more complete understanding of the influence of BDNF-mediated pathways in cell survival and plasticity will aid the development of novel approaches to restoring normal function in disease states, including those of a neuropsychiatric nature. These may include BDNF mimetics [220, 248] and small molecules like adenosine and pituitary adenylate cyclase-activating peptide that activate Trk receptors in the absence of neurotrophins, via transactivation of Trks through G-protein-coupled receptors [249, 250].

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Chapter 7
One-Compound-Multi-Targets at Amyloid β Cascade Offered By Bis(7)-Cognitin, a Novel Anti-Alzheimer's Dimer

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Abstract Alzheimer's disease (AD) is a progressive, degenerative disorder of the brain and the most common form of dementia among the elderly. The neuropathological hallmarks of AD are senile plaques, which are extracellular deposits predominantly composed of fibrillar amyloid β peptide (Aβ), and intracellular neurofibrillary tangles composed of filamentous aggregates called paired helical filaments of hyperphosphorylated tau protein. Aβ is proposed to play a key role in the pathogenesis of AD. Therefore, treatments targeting the biosynthesis, oligomerization/ aggregation, and toxicity of Aβ are likely to be the promising disease-modifying therapeutics. Bis(7)-Cognitin, one of our promising anti-Alzheimer's dimers, has previously been shown to possess potent acetylcholinesterase (AChE) inhibition, memory-enhancement, and neuroprotection against several stimuli that go beyond the inhibition of AChE. Our recent studies have further demonstrated that bis(7)-Cognitin exerts profound neuroprotective effects by targeting the multiple stages of the Aβ pathological cascade of AD, i.e. the biosynthesis, oligomerization/aggregation and toxicity of Aβ. These findings may offer not only a new and clinically significant modality as to how the agent exerts neuroprotective effects, but also a novel direction to rationally develop one-compound-multi-targets drugs for the prevention and treatment of AD, even of other neurodegenerative diseases.

Keywords Alzheimer's disease · Aβ · APP processing · Bis(7)-Cognitin · Disease modification

Abbreviations

AChE	acetylcholinesterase
AD	Alzheimer's disease
APP	amyloid precursor protein

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_7,

Aβ	amyloid β
BACE	beta-site APP cleaving enzyme
BuChE	butyrylcholinesterase
CTF	c-terminal fragment
LTP	long term potentiation
NMDA	N-methyl-D-aspartate
NOS	nitric oxide synthase
PAS	peripheral anionic site
PKC	protein kinase C
VDCC	voltage-dependent Ca^{2+} channel

7.1 Introduction

Alzheimer's disease (AD), the commonest cause of dementia in the elderly and affecting more than 30 million people worldwide, has emerged as one of the major public health problems [1]. The disease is characterized neuropathologically by the presence of amyloid plaques, neurofibrillary tangles, and synaptic and neuronal loss, with severe atrophy of the cortical and subcortical regions [2]. Amyloid β (Aβ), about 4 kDa in size, is formed through the amyloidogenic pathway in which amyloid precursor protein (APP) is sequentially cleaved by β- and γ-secretases [3]. Excessive Aβ may be produced through aberrant APP processing invoked by various AD-associated genetic and environmental factors [4]. Although the exact pathological mechanisms of AD are unclear yet, excessive Aβ has been proposed to play a key role in the pathogenesis of AD and may be the principal causative factor of AD [5, 6], which is strongly supported by increasing evidence that soluble oligomers of Aβ may be the nature of the molecular entity of dementia via causing synapse/neuron loss [7]. Interestingly, besides hydrolyzing acetylcholine, acetylcholinesterase (AChE) also functions as a promoter of Aβ oligomer and fibril formation, which is independent of its hydrolyzing activity and is associated with the peripheral binding site or peripheral anionic site (PAS) of AChE [8, 9]. Furthermore, most of the proposed mechanisms underlying pathogenesis of AD, such as cholinergic dysfunction, excitotoxicity, tau pathology and reactive oxygen species, are directly/indirectly associated with Aβ [10–12]. Therefore, the destructive potential now associated with Aβ suggests that the breakthroughs in the therapeutics for AD will emerge through directly targeting the pathological cascade of Aβ.

Disease-modifying therapies should interact with the disease process and lead to an arrest or slowing of neuronal loss and functional decline. Unfortunately, no disease-modifying therapies are available for AD [2, 3, 5]. Based on cholinergic deficit in the brains of AD patients and glutamate-induced excitotoxicity involved in the neurodegenerative process of AD, four AChE inhibitors and one glutamate receptor antagonist memantine are licensed for AD, respectively and have moderate symptomatic benefits [13]. Despite the fact that action mechanisms of some

AChE inhibitors and memantine may be suggestive of a disease-modifying potential, thus far these drugs have only been tested as symptomatic agents and do not have profound disease-modifying effects [2, 4].

Moreover, since multiple factors are closely indicated in pathogenesis of AD as well as in the pathological cascade of Aβ, AD will require multiple drug therapy to address the varied pathological aspects [14, 15]. Even if the strategy of combining drugs with different therapeutic targets is workable, the development of multi-functional compounds will obviate the challenge of administering multiple single drug entities with different degrees of bioavailability, pharmacokinetics, and metabolism [14]. Therefore, the new potential approaches have been developed expressly to target multiple sites in the brain with single molecular entities for the treatment of memory and cognition impairment by targeting multiple two or more of the following factors: AChE, glutamate toxicity, Aβ, tau protein, monoamine oxidase, metal ions and reactive oxygen species. Given that multiple factors are also implicated in the pathological cascade of Aβ, it has been proposed that drugs targeting the Aβ pathological cascade only at multiple steps would dramatically reduce the Aβ production and its toxicity, and offer clinically-significant therapy for AD [16].

Over the past decade, enormous efforts from our group have been directed towards the development of drugs which are more effective and potent than the currently drugs approved for the treatment of AD. Up to date, three novel series of tacrine/huperzine A analogs have been developed by linking tacrine or the fragment of huperzine A to itself or the fragment of huperzine A to tacrine [17, 18]. These dimeric drugs are much easier to produce and have been shown to be more potent than either tacrine or huperzine A alone, and represent a combination of the best of the Western and Chinese palliative drugs for AD. We have demonstrated that novel promising anti-Alzheimer's candidate bis(7)-Cognitin, one of our novel dimers, possesses multi-potencies including anti-AChE, -N-methyl-D-aspartate (NMDA) receptors and –nitric oxide synthase (NOS) signaling for synergistically combating AD [19]. Here we have further reviewed our most recent data that bis(7)-Cognitin exerts profound neuroprotective effects by targeting the multiple stages of the Aβ pathological cascade of AD, i.e. the biosynthesis, oligomerization/aggregation and toxicity of Aβ.

7.2 Multiple Profiles of Bis(7)-Cognitin on the *Aβ* Cascade

7.2.1 Bis(7)-Cognitin Reduces the Generation of Aβ Via Directly Inhibiting β-Secretase

APP processing represents a crucial and etiological step in the molecular cascade of AD pathogenesis [2, 5]. APP follows a complex trafficking pathway through secretory and endocytic compartments that determines processing into non-amyloidogenic and amyloidogenic products [20]. It is generally believed that en

route to the cell surface, most newly synthesized APP molecules are cleaved by α-secretase in a post-Golgi compartment or at the plasma membrane to produce soluble and neurotrophic sAPPα [21, 22]. AD-associated environmental factors such as cholesterol and hypoxia/stroke may induce overexpression of APP and/or APP trafficking from membrane to endosomes via endocytosis, leading to more APP entering amyloidogenic pathway [23, 24]. β-secretase, a rate-limiting enzyme, mediates the initial step of Aβ production by β-cleavage of APP; and the activity of β-secretase may be enhanced by AD-associated genetic and environmental factors through the increased expression [25, 26], lavished modifications such as phosphorylation/dimerization [27, 28], altered trafficking and/or reduced degradation [24, 29]. Furthermore, some AD-associated factors may increase the activity of γ-secretase via inducing expression, assembly/trafficking of the protease complex [30, 31], and/or decrease the activity of α-secretase via expression inhibition and altered trafficking to amyloidogenic compartments of neurons [32]. Collectively, more than two of the above proposed mechanisms may occur in the aberrant APP-Aβ processing of individual AD patient.

The aberrant processing of APP appears to be reversible and rectified by modulating abnormal trafficking and expression of APP, α-, β- and γ-secretases, and/or by regulating activities of these three proteases via targeting involved signal pathways [2, 4, 5]. Reduction of Aβ production by targeting aberrant APP processing may be one of the most promising strategies for disease-modification in AD, which could be more beneficial to AD patients than targeting Aβ and its downstream pathways. Our study shows that bis(7)-Cognitin (0.1–3 μM) substantially reduces the amounts of both secreted and intracellular Aβ in Neuro2a APPswe cells without altering the expression of APP. sAPPα and α-C-terminal fragment (CTFα) increased, while sAPPβ and CTFβ decrease significantly in Neuro2a APPswe cells following the treatment with bis(7)-Cognitin (Fig. 7.1a and b), indicating that this dimer may activate α-secretase and/or inhibit BACE-1 (BACE-1, i.e. β-secretase) activity.

It has been widely reported that the relationship between the cholinergic system and the processing of APP is inseparable. Although tacrine may inhibit the release of sAPPα and Aβ into culture media [33], many other AChE inhibitors such as donepezil have been found to activate the non-amyloidogenic pathway, and thus have the potential to preclude Aβ formation [34–36]. The underlying mechanism is that AChE inhibitors may alter aberrant APP processing by increasing activity of α-secretase via muscarinic receptors-protein kinase C (PKC) pathways [36–38]. In our study, we demonstrate that bis(7)-Cognitin significantly reduces the generation of both secreted $A\beta_{42}$ and $A\beta_{40}$ (Fig. 7.1a) in the concentrated media from N2a APPswe cells with concomitant increases in the α-secretase activity in cells without the direct activation of α-secretase activity [39], and increases in the amounts of sAPPα and CTFα, and a decrease in intracellular Aβ (Fig. 7.1b), indicating that bis(7)-Cognitin may activate the α-secretase to reduce the generation of Aβ. The alterations in the amounts of sAPPα and CTFα induced by bis(7)-Cognitin can almost be reversed by the α-secretase inhibitor TAPI-1 at 100 μM, however, at the same concentration, this inhibitor attenuates the reduction in Aβ generation by less than 10%, suggesting that activation of α-secretase may only play a minor or

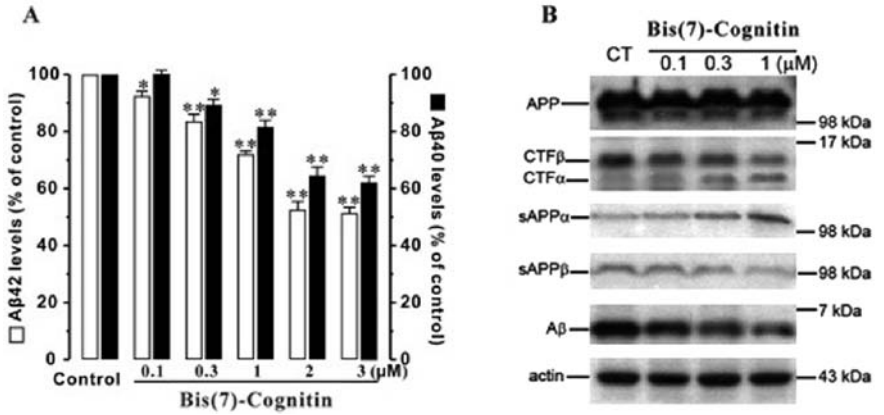

Fig. 7.1 Bis(7)-Cognitin reduces the secreted $A\beta_{42}$ and $A\beta_{40}$, and alters the amounts of APP cleavage products in N2a APPswe cells without affecting the expression levels of APP. (**a**) N2a APPswe cells are exposed to vehicle or bis(7)-Cognitin at the concentrations as indicated, for 24 h, and the levels of secreted $A\beta_{42}$ and $A\beta_{40}$ in the culture media are measured by ELISA. All the data, expressed as percentages of control (vehicle), are means ± SEM of three separate experiments; $^{*}p$ <0.05 and $^{**}p$ <0.01 versus control (ANOVA and SNK test). (**b**) The accumulation of sAPPα and sAPPβ in the concentrated media from N2a APPswe cells is analyzed by Western blotting using mouse 6E10 antibody and specific antibody for sAPPβ. The expression of APP and the accumulation of CTFα, CTFβ, and intracellular Aβ are also measured by western blotting using antibodies specific for APP (N-terminal region), APP (C-terminal region), and Aβ. Actin in the cell lysates is used as a loading control

partial role in the reduction of Aβ generation induced by bis(7)-Cognitin. Inhibition of BACE-1 and/or γ-secretase may also contribute to the effect of bis(7)-Cognitin on the biosynthesis of Aβ. Indeed, bis(7)-Cognitin significantly reduces the amounts of sAPPβ in culture media, and the level of intracellular CTFβ arising from APP cleaved by BACE-1 in N2a APPswe cells (Fig. 7.1b). Although there are very few reports that AChE inhibitors can inhibit BACE-1 activity, most AChE inhibitors activate the α-secretase cleavage pathway via the stimulation of M1- and M3-mAChR and subsequent activation of PKC [36, 38]. However, a recent study has suggested that BACE-1 expression can be suppressed by stimulation of M2-mAChR-mediated pathways [40], and activation of PKC has also been shown to modulate the activity of BACE-1 [41]. Therefore, we investigate whether the reductions in sAPPβ and CTFβ induced by bis(7)-Cognitin in N2a cells, which contain predominantly M2 receptors [42], are due to the inhibition of BACE-1 activity, or alternatively, to the activation of α-secretase.

To investigate the possible role of BACE-1 in the reduction of Aβ biosynthesis induced by bis(7)-Cognitin, N2a APPswe cells are treated with different concentrations of bis(7)-Cognitin or Z-VLL, a cell-permeable BACE-1 inhibitor, and the cellular BACE-1 activity was measured. Bis(7)-Cognitin, similar to Z-VLL, concentration-dependently inhibited intracellular BACE-1 activity (Fig. 7.2a). To further confirm the inhibition by bis(7)-Cognitin of BACE-1 activity in cells, the

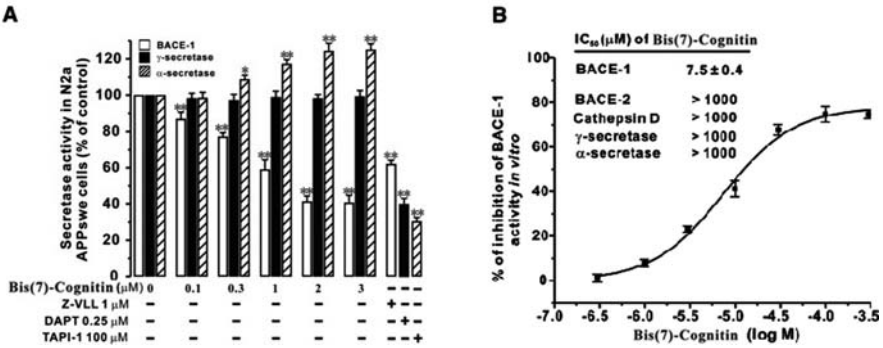

Fig. 7.2 Bis(7)-Cognitin inhibits BACE-1 activity in cultured cells and in vitro without affecting γ-secretase activity. (**a**) N2a APPswe cells are exposed to vehicle, bis(7)-Cognitin, Z-VLL, DAPT, or TAPI-1 at the concentrations as indicated, for 24 h, and the intracellular BACE-1, γ-secretase and α-secretase activities are measured using the fluorogenic BACE-1, γ- or α-secretase activity assay kits. The data, expressed as percentages of control (vehicle), are means ± SEM of three separate experiments; $^{*}p < 0.05$, $^{**}p < 0.01$ versus control (ANOVA and SNK test). (**b**) Recombinant human BACE-1 is incubated with vehicle or bis(7)-Cognitin at the concentrations indicated, and the fluorogenic BACE-1 substrate in vitro for 30 min. BACE-1 activity is measured. Recombinant mouse BACE-2, human Cathepsin D, crude γ-secretase, or human TACE are incubated with vehicle or different concentrations of bis(7)-Cognitin, and the specific fluorogenic substrate in vitro for 2 h, and their activities are measured

wild-type BACE-1 (BACE-1 wt) is transfected into normal N2a cells. It shows that BACE-1 activity increases dramatically when normal N2a cells are transfected with BACE-1 wt, and this increase is prevented by bis(7)-Cognitin. In addition, bis(7)-Cognitin significantly reduces the basal BACE-1 activity in normal N2a cells [39]. Results from in vitro experiments showed that bis(7)-Cognitin directly inhibited BACE-1 activity in a concentration-dependent manner (Fig. 7.2b). Because BACE-1 is a type 1 membrane-associated aspartyl protease and the coding sequences of BACE-1 and BACE-2 are 45% identical [43], the selectivity of bis(7)-Cognitin over BACE-1, BACE-2, and Cathepsin D, another main kind of aspartyl proteases, is further tested. We find that the IC50 of bis(7)-Cognitin for BACE-1 is 7.5 ± 0.4 μM, but bis(7)-Cognitin does not inhibit BACE-2 or Cathepsin D, even at 1 mM (Fig. 7.2b). Together, these findings suggest that bis(7)-Cognitin may be a selective BACE-1 inhibitor. In order to gain insight into the interaction mechanisms between bis(7)-Cognitin and β-secretase, computational molecular simulation is used to dock bis(7)-Cognitin to human β-secretase from the protein data bank database. The calculated binding free energy of bis(7)-Cognitin binding to β-secretase has the lowest value (-15.07 kcal/mol) when compared with those of tacrine (-7.12 kcal/mol) and E2020 (-8.49 kcal/mol), indicating a strong hydrophobic interaction between bis(7)-Cognitin and β-secretase (Fig. 7.3).

Given that cleavage of APP CTFβ by γ-secretase is the final step in the production of Aβ, and that the exact position of cleavage by γ-secretase is critical for the

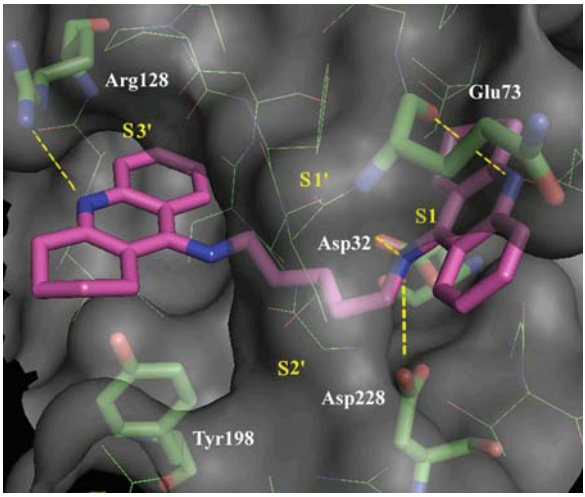

Fig. 7.3 Stereo presentation of interactions between bis(7)-Cognitin and β-secretase. In the bis(7)-Cognitin-β-secretase complex, one hydrogen atom in NH group in the tacrine moiety of bis(7)-Cognitin has close contacts with oxygen atoms in the catalytic aspartates Asp32 and Asp228, and the two tacrine moieties occupy the S1 and S3′ sites in the catalytic cavity, and make S1′ and S2′ sites unreachable for other molecules; and close contacts with β-secretase are also formed at the tacrine moieties with Glu73 and Arg128. Close contacts are shown in yellow dotted lines. The S1, S1′, S2′ and S3′ sites in the catalytic site are highlighted in *yellow*. Nitrogen and oxygen atoms are marked *blue* and *red*, respectively. The carbon atoms in bis(7)-Cognitin and β-secretase are colored in *magenta* and *green*, respectively

development of AD [44], the involvement of γ-secretase in the reduction of Aβ production induced by bis(7)-Cognitin is examined. We find that bis(7)-Cognitin does not inhibit γ-secretase activity either in living N2a APPswe cells (Fig. 7.2a), or in vitro, using crude γ-secretase extracted from normal N2a cells (Fig. 7.2b). Taken together, our results not only suggest that bis(7)-Cognitin may reduce the biosynthesis of Aβ mainly by directly inhibiting BACE-1 activity, but also provide new insights into the rational design of novel anti-Alzheimer's agents that might have disease-modifying properties.

7.2.2 Bis(7)-Cognitin Inhibits the Oligomerization and Aggregation of Aβ

Aβ exists in several different physical states, including monomers, oligomers, or fibrils. Evidence from in vitro studies demonstrates that synthetic Aβ monomers aggregate in a time-dependent fashion to form oligomers, which may eventually form fibrils [16]. In vitro and in vivo experimental evidence points to soluble Aβ oligomers, also referred to as Aβ-derived diffusible ligands, as the predominant neurotoxic species for neurons [45]. In this regard, in vitro, Aβ oligomers

are very potent toxic species, as even nanomolar concentrations have been shown to kill mature neurons in hippocampal slices [46, 47]. Moreover, Aβ oligomers appear to interfere with many critical neuronal activities, including inhibiting long term potentiation (LTP) in organotypic hippocampal slices, calcium dysregulation and membrane disruption [48–51]. The toxicity of Aβ oligomers has also been shown in vivo. In particular, intracerebroventricular injection of oligomers inhibits LTP and specifically disrupts cognitive function [52]. Importantly, the concomitant injection of the anti-Aβ antibody 6E10 with Aβ oligomers neutralizes the oligomer-induced LTP dysfunction [53]. In addition, Aβ oligomers may play a role in the induction of tau pathology [54]. These data strongly support the idea that oligomers represent a fundamental species responsible for mediating Aβ toxicity in AD, making the inference of Aβ oligomerization a valid therapeutic target.

Amyloid targeted therapeutic approaches aimed at blocking the neurotoxic activity of Aβ are presently pursued for the inhibition of Aβ production by inhibiting the enzyme cleaving APP, immunizing against AD, and inhibition of amyloid oligomerization or aggregation. An attractive therapeutic strategy is to inhibit peptide oligomerization or aggregation itself, which appears to be the first step in the pathogenic process of amyloidosis and not associated with natural biological function [46]. On the other hand, on the basis of the three-dimensional structure of AChE, much evidence has been provided about the existence of two distinct binding sites for substrate and inhibitors: a catalytic and an allosteric site, or peripheral binding site, localized at the bottom and the entrance of the active-site gorge, respectively [55]. The link between the amyloid and the cholinergic hypotheses has been provided by the discovery of Inestrosa and co-workers that AChE is able to promote the aggregation of Aβ [8]. Several studies on this topic have been carried out following the first report, whose most interesting outcomes are that the PAS of AChE is involved in this action and AChE inhibitors targeted at the PAS can prevent it [9, 56], and the Aβ proaggregating effect of AChE is operating also in vivo, as recently shown by experiments carried out on transgenic mice [57, 58].

Based on the assumption that the interference of Aβ oligomerization/ aggregation is a valid therapeutic targets, the drugs that prevent the automatic or AChE-induced oligomerization/aggregation of Aβ may be the promising candidates for the treatment of AD. We design and synthesize new compounds with dual action of effective anti-AChE and -Aβ peptide aggregation inhibition [59]. Specifically, we focus our efforts on tacrine derivatives binding with a catalytic and a peripheral site of AChE, and having inhibitory potency against Aβ oligomerization/aggregation. Soluble oligomeric $A\beta_{42}$ prepared as described previously [60] is incubated with vehicle or bis(7)-Cognitin/tacrine/curcumin at concentrations indicated for 4 h at 4°C. The samples are centrifuged at 15,000 g for 10 min at 4°C and the supernatant is mixed with an equal part of tricine sample buffer without reducing agents. Samples are separated by a 15% Tris-Tricine SDS gel and immunoblotted with rabbit anti-Aβ antibody. It is found that bis(7)-Cognitin concentration-dependently reduces the amounts of both small molecular weight and high molecular weight $A\beta_{42}$ oliogmers, while 50 μM tacrine only has the similar effect to that of 3 μM bis(7)-Cognitin. The

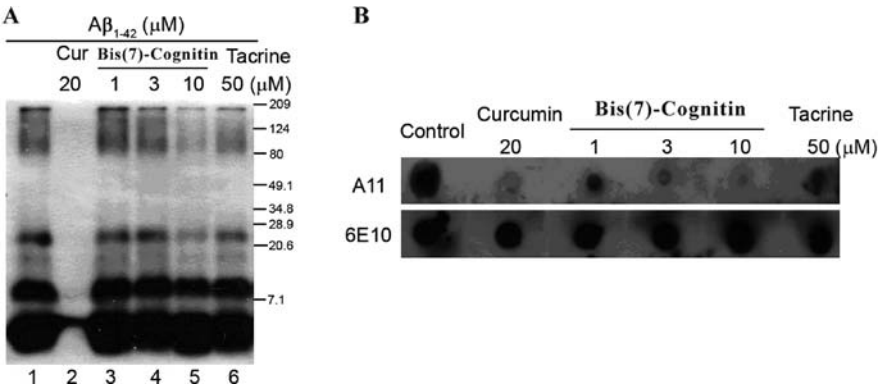

Fig. 7.4 Bis(7)-Cognitin inhibits the oligomerization of $A\beta_{42}$. (**a**) Soluble oligomeric $A\beta_{42}$ is prepared, and samples are electrophoresed at 100 V on a 15% Tris-Tricine SDS gel and probed with rabbit anti-Aβ antibody followed by goat anti-rabbit horseradish peroxidase, and developed with ECL chemiluminescence kit. (**b**) Samples are spotted onto the membrane with air-dry. The membrane is blocked in 10% non-fat dry milk TBST solution at 4°C overnight. Then the membrane is immunoblotted with rabbit anti-oligomer antibody (A11) and goat anti-rabbit horseradish peroxidase, and developed with ECL chemiluminescence kit. The same membrane is stripped and immuno- detected with mouse 6E10 antibody (Aβ 1–17) as described above

positive control, 20 μM curcumin almost blocks the formation of $A\beta_{42}$ oliogmers (Fig. 7.4a).

To further confirm the inhibitory effect of bis(7)-Cognitin on the oligomerization of Aβ, we also use a specific anti-oligomer antibody (A11) to carry out the dot blot assay, which is designed to detect the formation of Aβ oligomers. The results show that similar to the positive control (20 μM curcumin), bis(7)-Cognitin significantly reduces the amounts of Aβ oligomers in a concentration-dependent manner, whereas tacrine has a much weaker effect on reducing Aβ oligomers than does bis(7)-Cognitin. At the same time, we find that the total Aβ detected by 6E10 antibody does not have any significant difference among different groups (Fig. 7.4b).

Interestingly, besides hydrolyzing acetylcholine, AChE also functions as a promoter of Aβ oligomer and fibril formation, which is independent of its hydrolyzing activity and is associated with the PAS of AChE [8]. To facilitate rational drug design for AChE inhibitors based on the hypothesis of dual binding that the ligand targets the two sites of AChE concurrently, we employ an automated computer docking system to devise synthesis of tacrine analogs with superior therapeutic potential [59]. This system can estimate the potential energetically favorable binding sites in the AChE active site gorge by rotating the incoming substrate-tacrine, and match each available conformation of these two counterparts. On the other hand, with this docking system, the existence of the peripheral site near the opening of the enzyme gorge is further confirmed. This is reasonable, since strong binding at the PAS may hinder the entering of the substrate into the lower portion of the enzyme

gorge, which in turn lowers the efficacy of hydrolysis. Through structural modification of tacrine, a dimeric tacrine analog linked by a 7-carbon alkylene chain (i.e. bis(7)-Cognitin) offers a much stronger potency and selectivity towards AChE, where bis(7)-Cognitin simultaneously binds at both the catalytic and the peripheral sites. Since the peripheral site is absent in butyrylcholinesterase (BuChE), the facilitated binding offered by this dimeric analog provides a better selectivity towards AChE over BuChE in terms of its binding property [59]. More importantly, bis(7)-Cognitin can bind the PAS of AChE. Therefore, we propose that bis(7)-Cognitin would prevent the AChE-induced aggregation of Aβ.

According to the related reports [8, 61], we setup a system for testing the aggregation of Aβ induced by AChE. For coincubation experiments, aliquots of AChE to a final molar ratio of 100:1 are added. To quantitate amyloid formation, we use a thioflavine-T fluorescence method as described [8, 56]. Thioflavine-T binds specifically to amyloid and this binding produces a shift in its emission spectrum and a fluorescent signal proportional to the amount of amyloid formed. Fluorescence is monitored at excitation 446 nm and emission 482 nm using a Hitachi F-2000 pectrofluorometer. We find that the fluorescence density of human AChE (2.3 μM)-induced $A\beta_{40}$ (230 μM) aggregation is about 6.3 ± 0.27 times higher than that of automatic aggregation of Aβ at 24 h after the incubation, and then compared with the inhibitor of AChE peripheral site propidium at 100 μM (the inhibition rate: 83.4%) and the inhibitor of AChE active site tacrine at 100 μM (the inhibition rate: 7.8%) as controls, bis(7)-Cognitin (0.1–10.0 μM) inhibits the aggregation of Aβ induced by AChE in a concentration-dependent manner by 33.4–81.7% fibrillogenesis inhibition (Fig. 7.5).

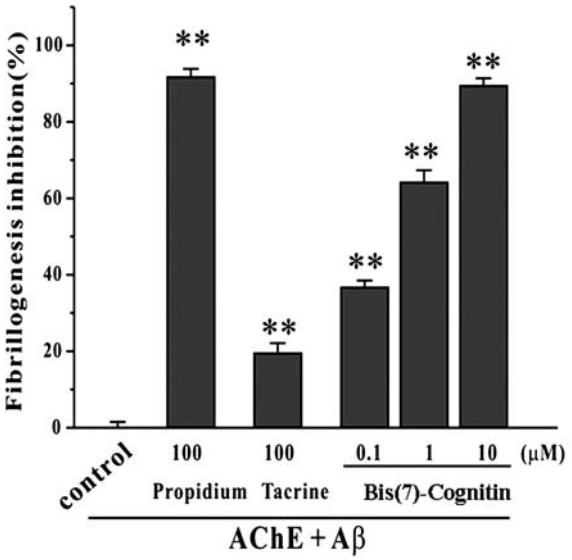

Fig. 7.5 Bis(7)-Cognitin inhibits the AChE-induced aggregation of Aβ. The data are expressed as 100-($IF_i/IF_0 \times 100$) from three independent experiments (n=6 for each group) where IF_i and IF_0 are the fluorescence intensities at 485 nm obtained from Aβ plus AChE with/without inhibitors; $**p<0.01$ versus control

7.2.3 Bis(7)-Cognitin Attenuates the Neurotoxicity Caused by Aβ

Both fibrillar and soluble oligomeric forms of Aβ are considered to be important factors contributing to AD, although it remains controversial whether fibrillar or soluble oligomeric forms of Aβ are the active species of the peptide that ultimately cause the synaptic loss and dementia associated with AD as well as their roles in the pathogenesis of AD [10, 62]. Both Aβ forms have been shown to be very toxic in several cell models [63, 64]. Increasing evidence has indicated that the disruption of Ca^{2+} homeostasis plays a very important role in the neurotoxicity induced by both the fibrillar and soluble oligomeric forms of Aβ [62, 65]; and the increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) by fibrillar Aβ has been shown to be mediated via L-type voltage-dependent Ca^{2+} channels (VDCCs) [66]. Besides the destabilization of intracellular Ca^{2+} homeostasis both in neurons and in glial cells, Aβ neurotoxicity is also associated with oxidative stress and the reduction of endogenous antioxidants [65, 67, 68], mitochondrial damage [69], membrane disruption [65] and several signaling pathways [70]. However, the exact mechanisms of fibrillar and soluble oligomeric forms of Aβ-induced neurotoxicity remain elusive.

The use of AChE inhibitors, such as tacrine, donepezil, rivastigmine, galanthamine and huperizine A for treating AD is based on the link between cholinergic dysfunction and AD severity [71]. Neuronal apoptosis and synaptic loss in the cerebral cortex and hippocampus are recently suggested to be the major reasons for cognitive decline in AD [72], while fibrillar and soluble oligomeric form Aβ may directly contribute to the apoptosis in AD [73–75]. Therefore, AChE inhibitors with anti-apoptosis may be more beneficial than those merely inhibiting AChE for the treatment and prevention of AD [76, 77]. Bis(7)-Cognitin has been reported by us as a promising therapeutic agent for AD on the basis of its superior AChE inhibition [59] and memory-enhancement [78] and neuroprotection against several apoptotic model in nerve cells [79, 80]. Therefore, we wonder to know whether this dimer prevents both fibrillar and soluble oligomeric forms of Aβ-induced neurotoxicity and the relevant mechanisms.

Both forms of Aβ are found to cause neurotoxicity and induce apoptosis in our primary cultured cortical neurons, which is demonstrated by condensation of cell bodies, blebbing and distraction of neurites, chromatin condensation and DNA fragmentation (Fig. 7.6). Furthermore, $A\beta_{25–35}$, the functional part mediating the toxicity of fibrillar $A\beta_{1–42}$, has displayed a similar neurotoxicity to that of fibrillar $A\beta_{1–42}$, and oligomeric $A\beta_{1–42}$ is about ten times toxic than fibrillar Aβ [60]. These results are consistent with the previous findings [63, 73]. When cortical neurons are pretreated with bis(7)-Cognitin at 1–500 nM, Aβ-induced neurotoxicity is significantly attenuated as measured by MTT reduction ($ID_{50} = 12 \pm 1.04$ nM). Furthermore, 100 nM bis(7)-Cognitin significantly reverses the morphological changes and chromatin condensation, prevents the DNA fragmentation and reduces the apoptotic rates (Fig. 7.6). Our results strongly suggest that bis(7)-Cognitin protects cortical neurons from both forms of Aβ-induced apoptosis.

The protective mechanisms of bis(7)-Cognitin against Aβ neurotoxicity are subsequently investigated. Bis(7)-Cognitin has been shown to be a selective and potent

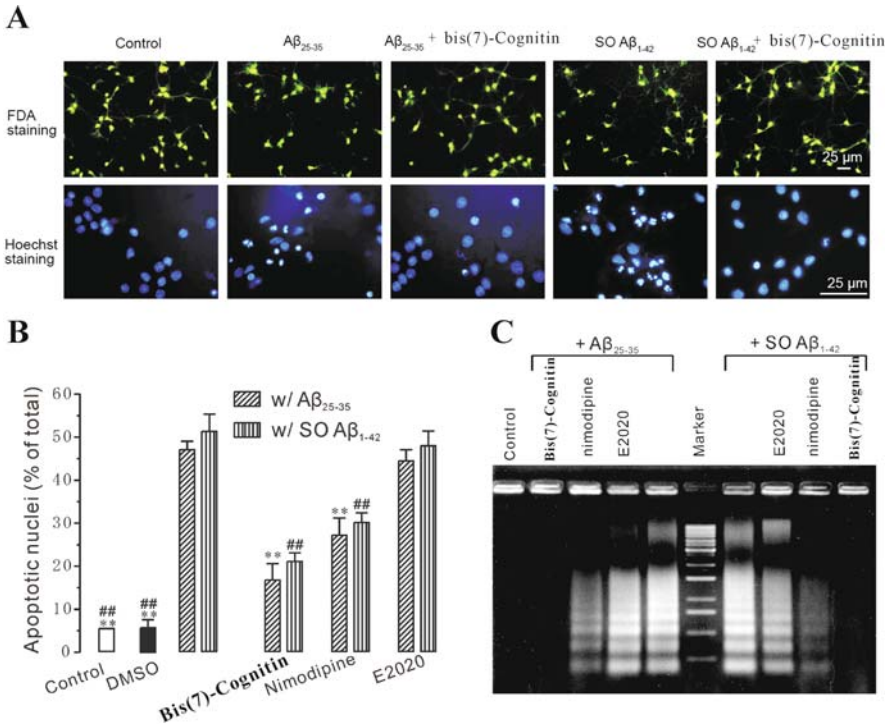

Fig. 7.6 Bis(7)-Cognitin attenuates the hallmarks of apoptosis induced by Aβ in cortical neurons. (**a**) At 6 DIV, cortical neurons are preincubated with or without 100 nM bis(7)-Cognitin/10 μM nimodipine/10 μM E2020 and exposed to 20 μM $A\beta_{25–35}$ or 1 μM SO $A\beta_{1–42}$ 2 h later. At 48 h after Aβ challenge, the cortical neurons are observed by either FDA or Hoechst 33342 staining assays. (**b**) The apoptotic nuclei stained by Hoechst 33342 are counted. The data, expressed as the percentage of total nuclei, are the means ± SEM of three independent experiments ($n = 500$ nuclei in each group); $^{**}p < 0.01$ versus $A\beta_{25–35}$; $^{\#\#}p < 0.01$ versus SO $A\beta_{1–42}$ group (one-way ANOVA and SNK test). (**c**) Cortical neurons are preincubated with or without 100 nM bis(7)-Cognitin /10 μM nimodipine/10 μM E2020 and exposed to 20 μM $A\beta_{25–35}$ or 1 μM SO $A\beta_{1–42}$ 2 h later. At 48 h after Aβ challenge, the DNA is extracted from the cortical neurons, and then agarose gel electrophoresis and ethidium bromide staining are used to visualize DNA fragmentations extracted from the above samples. *Note*: FDA, fluorescein diacetate; SO Aβ, soluble oligomeric Aβ

AChE inhibitor [59], however, it is not known whether bis(7)-Cognitin attenuates Aβ-induced neurotoxicity by inhibition of AChE. Our in vitro study show that bis(7)-Cognitin attenuates Aβ-induced neurotoxicity in cortical neurons possibly not via the inhibition of the AChE activity since other AChE inhibitors (tacrine and donepezil) hardly show any neuroprotective properties on neuronal death induced by Aβ in cortical neurons although donepezil and tacrine at 10 μM possess similar effects on inhibiting the activity of AChE to bis(7)-Cognitin at 100 nM [60, 80]. However, we do not find that Aβ peptides can stimulate AChE expression or activity in our model, which is different from some other group work [67, 81–83]. The possible reason is that the time point we choose to measure AChE activity is

very early (2 h) after Aβ challenge, therefore, it may not be measured in our system even though the expression of AChE may be upregulated by Aβ. On the other hand, the activation of cholinergic receptors is able to prevent apoptosis induced by several apoptotic inducers such as Aβ in cortical neurons or low potassium in cerebellar granule neurons [84]. However, the neuroprotective properties of bis(7)-Cognitin are not affected by atropine or dihydro-β-erythroidine in our model. Therefore, bis(7)-Cognitin prevents Aβ-induced neurotoxicity independent of inhibiting the activity of AChE and cholinergic transmission.

Increasing evidence shows that a rise of $[Ca^{2+}]_i$ and its activated downstream signaling pathways have been suggested to be responsible for both fibrillar [62, 70] and soluble oligomeric Aβ-induced apoptosis [65, 74, 75]. In our system, both forms of Aβ also induce a significant increase of $[Ca^{2+}]_i$in cortical neurons, which begin immediately after the exposure of Aβ and last for up to 1 h. Furthermore, EGTA nearly prevents Aβ-increased $[Ca^{2+}]_i$, however, neither thapsigargin nor FCCP cannot reduce this event. These findings suggest that neuronal $[Ca^{2+}]_i$ increase triggered by Aβ is dependent on extracellular Ca^{2+} but not ER and/or mitochondria Ca^{2+}. It has been shown that the activation of L-type VDCCs and ionotropic glutamate receptors may be the possible reasons of $[Ca^{2+}]_i$ increase triggered by Aβ [66, 85]. Therefore, we wonder to know which one contributes the Ca^{2+} influx and apoptosis induced by Aβ in our system. Nimodipine, a L-type VDCCs inhibitor, can almost abolish the Aβ-induced $[Ca^{2+}]_i$ increase and attenuate Aβ-induced neurotoxicity in cortical neurons [60]. However, the responses are not significantly affected by other types of VDCCs inhibitors or ionotropic glutamate receptor antagonists, which is consistent with the work from another group [66]. Together, we propose that blockade of L-type VDCCs may prevent neuronal cell death induced by Aβ.

Subsequently, we determine how bis(7)-Cognitin interferes with the $[Ca^{2+}]_i$ increase triggered by Aβ in cortical neurons. With the confocal microscopy, it is found that bis(7)-Cognitin inhibits the rise of $[Ca^{2+}]_i$ caused by both fibrillar and soluble oligomeric form Aβ to the near basal level, but donepezil does not inhibit such event. These effects are exactly matched with their neuroprotective properties. On the other hand, we have shown that the activation of L-type VDCCs is the major route of Aβ-induced $[Ca^{2+}]_i$ influx. With the whole-cell patch clamp assay, the current is elicited by voltage step from –60 to 0 mV and blocked by cadmium chloride, indicating that this current is caused by the activation of VDCCs [86]. Furthermore, Aβ can enhance the amplitude of calcium currents. Bis(7)-Cognitin, similar to nimodipine, significantly reduces both currents elicited by the voltages and enhanced by Aβ in neurons, while donepezil and tacrine at about several times higher than therapeutic concentrations cannot yet (Fig. 7.7). Collectively, the findings suggest that bis(7)-Cognitin may reduce Aβ-induced $[Ca^{2+}]_i$ influx via blocking L-type VDCCs.

In conclusion, we demonstrate in this part that bis(7)-Cognitin attenuates Aβ-induced neuronal apoptosis by inhibiting the $[Ca^{2+}]_i$ increase through regulating L-type VDCCs instead of AChE inhibition and cholinergic transmission, which offers a novel modality as to how the drug provides neuroprotective effects.

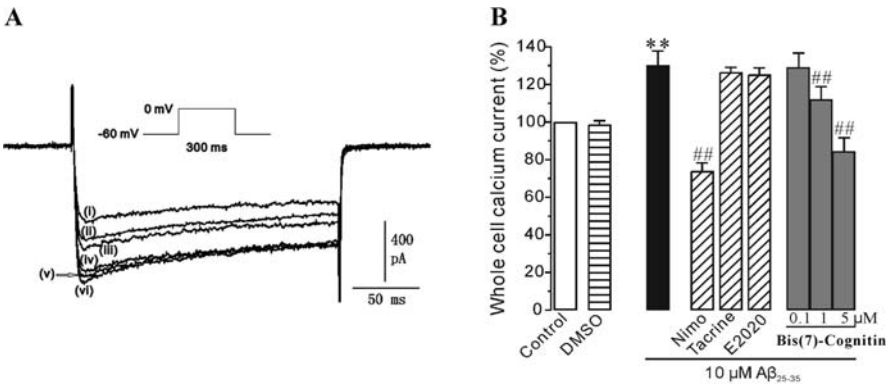

Fig. 7.7 Bis(7)-Cognitin inhibits the augment of L-type VDCCs calcium currents triggered by $A\beta_{25–35}$ in rat hippocampal pyramidal neurons. (**a**) Representative whole cell current traces recorded from the same neuron are elicited by stepping membrane potentials from -60 mV to 0 mV. The voltage steps are applied for 300 ms at intervals of 10 s. (**i**)–(**vi**) Representing the neuron is applied with vehicle/10 μM $A\beta_{25–35}$ plus 10 μM nimodipine/ 10 μM $A\beta_{25–35}$ plus 5 μM bis(7)-Cognitin/10 μM $A\beta_{25–35}$ plus 10 μM E2020/10 μM $A\beta_{25–35}$ plus 10 μM tacrine/10 μM $A\beta_{25–35}$ alone, respectively. The arrow points to group (**v**). (**b**) The effects of the above treatments on the $A\beta_{25–35}$-evoked augument of whole cell VDCCs calcium currents. The data are expressed as the means ± SEM of the ratios of normalized currents of three separate experiments ($n = 10$ for each group); $^{**}p < 0.01$ versus control, $^{\#\#}p < 0.01$ versus $A\beta_{25–35}$ alone group (one-way ANOVA and SNK test). *Note*: Nimo, nimodipine

Additionally, we have also demonstrated that bis(7)-Cognitin prevents $A\beta_{1–40}$-indcued neurotoxicity partially via neuronal NOS inhibition [87].

7.3 Conclusions and Future Directions

AD represents one of the most serious socio-medical problems in aging populations. No effective pharmacotherapy exists. Current disease-modifying approaches for AD mainly focus on targeting specific aspects of Aβ-associated pathology. By rectifying the AD-associated pathological cascade of Aβ, our multifunctional dimer bis(7)-Cognitin represents a highly promising novel agent that marks significant progress in the fight against AD. More encouragingly, we have found that bis(7)-Cognitin can also enhance high frequency stimulation-induced LTP and reverse the inhibition of LTP by soluble oligomeric Aβ in rat hippocampus slices [19]. Collectively, our findings strongly suggest that bis(7)-Cognitin may modify the pathogenesis of AD by targeting the multiple steps of Aβ pathological cascade (Fig. 7.8), which offer a new and clinically significant modality as to how the dimer exerts substantial neuroprotective activities.

Existing therapeutic pharmacological approaches with one-drug-one-target are limited in their ability to modify the course of the disease. Novel therapeutic strategies comprise multi-functional compounds designed specifically to act on multiple

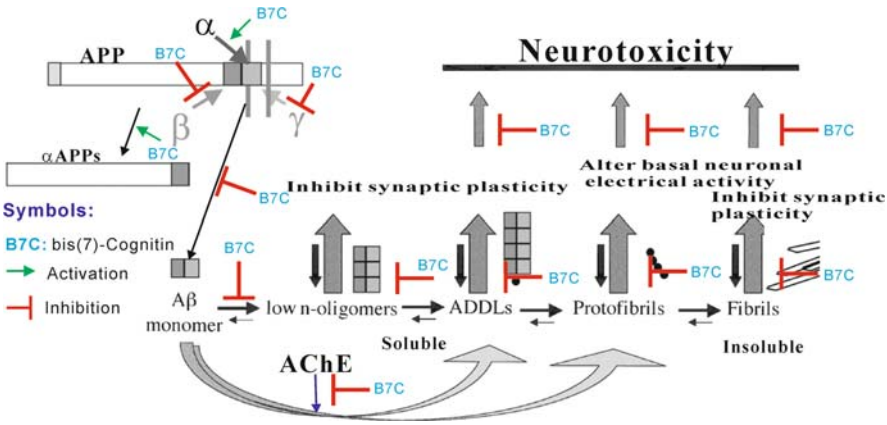

Fig. 7.8 Proposed schematic model for blocking the pathological cascade of Aβ by bis(7)-Cognitin. The cascade of Aβ: Aβ is generated by sequential proteolysis of APP first by β-secretase and then γ-secretase; and above a critical concentration Aβ monomer can self-associate to form dimers, trimers and other low-n oligomers, which automatically or under AChE induction form ADDLs, protofibrils and fibrils, all of which cause AD-associated pathological changes as indicated in figure. On the other hand, bis(7)-Cognitin inhibits the pathological cascade of Aβ at the multiple steps, including inhibition of BACE-1, activation of α-secretase, inhibition of automatic oligomerization and AChE-induced aggregation of Aβ and reduction of Aβ-induced neurotoxicity. *Note*: ADDLs, Aβ-derived diffusible ligands; B7C, bis(7)-Cognitin

neural and biochemical targets at multi-factorial etiopathogenesis of AD thereby providing greater pharmacological efficacy for AD therapy. Therefore, the synergism between anti-AChE, -NMDA receptor, -NOS and -Aβ cascade might serve as one of the most effective therapeutic strategies to prevent and slowdown the neurodegeneration in addition to improving the cognitive functions for AD.

Acknowledgements This research is supported by grants from the Research Grants Council of Hong Kong (PolyU6441/06 M, 6608/07 M, 5609/09 M; N_PolyU618/07 and AoE/B15/01), Hong Kong PolyU (G-YX96, G-U439), the MOST of China (2008CB517411), and the Shenzhen Shuangbai Scheme 08. The authors appreciate Mr. Marvin MAK and Mr. Wei CUI for their kind assistances during the processing of this paper.

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Chapter 8
Common Pathways to Neurodegeneration and Co-morbid Depression

Darcy Litteljohn, Emily Mangano, and Shawn Hayley

Abstract Depression is highly co-morbid with a number of neurodegenerative conditions, including Parkinson's disease (PD), Alzheimer's disease (AD), stroke and multiple sclerosis. Although psychosocial stress and impairment play a substantial role, accumulating evidence suggests that co-morbid depressive illness may emerge from alterations in processes related to the primary neurodegenerative condition. For instance, depression in PD may occur long before motor disability and in many cases is likely related to early degeneration in brainstem or limbic regions. We posit that pro-inflammatory cytokines and their associated inflammatory signaling pathways (e.g., JAK-STAT, NFκB, and MAP kinases), as well as other immuno-inflammatory factors such as the microglial inducible enzyme, cyclooxygenase-2 (COX-2), may play a primary role in modulating the emergence of co-morbid depression. In this regard, neuroprotective/neurotrophic anti-inflammatory factors may have important antidepressant properties. The present review will cover the evidence concerning the mechanisms through which depression might emerge in PD and other neurodegenerative disorders. Secondly, we will focus on the important cytokines, inflammatory co-factors and intracellular signaling proteins that could be targeted to potentially provide therapeutic benefit for depression as well as the primary neurodegenerative condition.

Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
5-HTTLPR	serotonin-transporter-linked polymorphic region
6-OHDA	6-hydroxydopamine
AA	arachidonic acid
AD	Alzheimer's disease
BBB	blood brain barrier

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_8, © Springer Science+Business Media B.V. 2010

Bcl-2	B-cell lymphoma-2
BDNF	brain-derived neurotrophic factor
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
COX-2	cyclooxygenase-2
CREB	cAMP response element-binding protein
CRH	corticotrophin-releasing hormone
CSF	cerebrospinal fluid
DA	dopamine
DHA	docosahexaenoic acid
GDNF	glial cell line-derived neurotrophic factor
IDO	indoleamine 2,3-dioxygenase
ICE	interleukin-converting enzyme
i.c.v.	intracerebroventricular
IFN	interferon
IGF	insulin-like growth factor
IκB	inhibitor of kappaB
IL	interleukin
iNOS	inducible nitric oxide synthase
JAK	janus kinase
JNK	c-Jun N-terminal kinase
LRKK2	leucine rich repeat kinase 2
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCAO	middle cerebral artery occlusion
MHC	major histocompatibility complex
MnSOD	manganese superoxide dismutase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine
NFκB	nuclear factor kappaB
NGF	nerve growth factor
NMDA	N-methyl D-aspartate
NO	nitric oxide
NSAID	nonsteroidal anti-inflammatory drug
PD	Parkinson's disease
PG	prostaglandin
ROS	reactive oxygen species
SNc	substantia nigra pars compacta
STAT	signal transducer and activator of transcription
TGF	transforming growth factor
Th1	T helper type-1
TNF-α	tumor necrosis factor-alpha

8.1 Introduction

Depression is a common co-morbid condition present in a number of neurological disorders, including Parkinson's disease (PD), Alzheimer's disease (AD) and cerebral stroke [1–3]. As well, recent evidence indicates that depression is surprisingly common following traumatic head injury or concussion [4]. Not surprisingly, the co-occurrence of depression may affect not only quality of life, but also the progression of the primary condition [5–7]. Indeed, several reports indicate that chronic depression can exacerbate neurological symptoms, as well as even contribute to neuronal injury over time [8–12]. Of course, the converse may also be true, such that the progressing severity of the primary neurological condition may enhance the magnitude of depressive episodes [13, 14]. Hence, a bidirectional relationship likely exists between the co-morbid states, and it has been our contention that common biological processes are involved [15, 16]. In this regard, we posit that neuroinflammatory mechanisms involving microglial cells, inflammatory enzymes [e.g., cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)], and pro-inflammatory cytokines [e.g., interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α)], may be one such common pathway to co-morbidity (see Fig. 8.1). Indeed, as we discuss the current evidence concerning the relationship between depression and PD, and to a lesser extent other neurodegenerative conditions (AD and stroke), it will become apparent that neuroinflammatory factors play an integral role in the onset and/or progression of each of these illnesses.

Depression may be viewed as a spectrum disorder which varies in severity and symptom profile as a function of its etiological origins [17, 18]. Although depression resulting from medical conditions, such as the aforementioned neurological

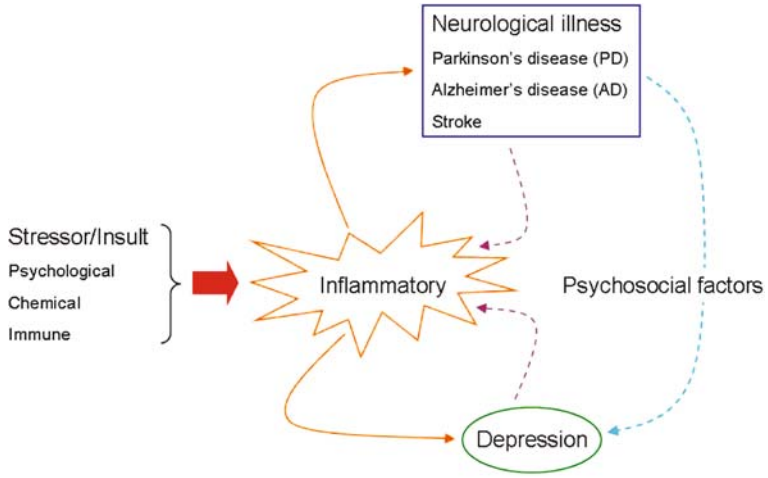

Fig. 8.1 Neuroinflammatory links between environmental stressor exposure and the development of neurological illness with co-morbid depression

diseases, may share many of the core features of depression without such co-morbidity (e.g., anhedonia and melancholia), there also may be several important differences [19, 20]. Not surprisingly, cognitive impairment (e.g., concentration, memory and attentional deficits) and psychomotor retardation are much more prevalent in neurological patients with co-morbid depression than among those in which the depression was more associated with psychosocial stresses or problematic developmental stages (e.g., adolescence) [21–23]. In effect, the variations of symptoms exhibited in depression may reflect the nature of the combination of risk factors that ultimately come to shape the evolution of the illness. Indeed, it has been our perspective that psychological-chemical-immune insults may all act in concert, upon the backdrop of genetic and developmental factors, to influence the onset or progression of co-morbid depression and neurological illness; and that these agents may, in a sense, be considered three separate categories of environmental "toxins" that share common mechanisms of action [24, 25].

Accumulating evidence suggests that cytokines (glycoprotein messengers of the immune system) may be one such common signaling mechanism subserving the impact of seemingly disparate classes of environmental toxins upon central nervous system (CNS) functioning [26]. Although cytokines normally function to convey messages between immune cells, alerting them to the presence of pathogens or injury, these messengers also act to provide input to the CNS regarding the status of the body following encounters with environmental insults [27, 28]. For instance, chemical (e.g., pesticides) and immune (e.g., viral and bacterial pathogens) toxins have been shown to increase the levels of circulating cytokines; and these immunotransmitters, in turn, act upon the brain to modulate a number of functionally-relevant processes (e.g., immunological surveillance, fever, cellular differentiation/proliferation, memory and learning) and behaviours (e.g., feeding, sexuality and aggression) [29–34]. Recently, the brain's own specialized immune cells, called microglia, have also been shown to produce de novo cytokines within the brain, which at high concentrations can have deleterious effects on neuronal functioning and survival [35–38]. Indeed, microglial- and- cytokine-dependent processes have been implicated in each of the neurological disorders discussed herein (i.e., PD, AD, and stroke) [39–42]; and evidence is accumulating to suggest a similar role for cytokines in depression and associated mood and anxiety disorders [43–46].

It is important to mention from the outset that, in some instances, the development of co-morbid depression that occurs in conjunction with a neurological pathology might be directly related to the distress or disability (e.g., loss of function) or the psychosocial stress attributable to the disease (see Fig. 8.1) [47–51]. For instance, in PD patients, job loss, marital strain and bleak outlook on the future owing to the progressive nature of the disease have all been correlated with increased risk of co-morbid depression in these individuals [52]. In such cases, the stress associated with the neurological impairment would be expected to provoke biological changes that might not only contribute to secondary depressive-like pathology, but may also exacerbate the primary illness. Indeed, stressor-provoked elevations of hormones, particularly glucocorticoids, were reported to induce hippocampal neuronal damage [53, 54], as well as inhibit neurogenesis [55, 56]; and

as such, could contribute to both depression and primary chronic neurodegenerative processes. Likewise, stressor-induced alterations of glutamate and dopamine (DA) activity within the basal ganglia were suggested to have neurotoxic consequences that might be relevant for PD [57–59].

Yet, it should also be considered that depression may sometimes *precede* the manifestation of the primary disease pathology (e.g., motor impairment in the case of PD) or diagnosis, making it unlikely that such symptoms are uniformly secondary to psychological distress [60–64]. Thus, in such cases it is reasonable to posit that underlying common biological processes (not necessarily tied to psychological distress) may contribute to both co-morbid states, and that inflammatory immune factors (e.g., microglia, cytokines, COX-2) may be of particular importance in this regard. Whatever the case, the present review will focus on inter-related factors that might account for depression being so commonly co-morbid with illnesses involving a prominent neurodegenerative component.

8.2 Inflammatory Processes in Relation to Central Nervous System Functioning

Until relatively recently, the brain was seen as an immune-privileged organ that, by virtue of tight junctions formed between cerebral microvascular endothelial cells (i.e., the blood-brain barrier, BBB), was impenetrable to noxious, blood-borne molecules and circulating immune cells in the periphery [65]. However, it has become apparent that the microenvironment of the brain is, in fact, capable of both mounting and sustaining an inflammatory immune response. In this regard, a short-lived neuroinflammatory response to transient CNS insult might very well be expected to restrict damage to and promote healing of viable brain tissue (e.g., removal of cellular debris, release of trophic factors) in a manner analogous to a short-lived immune response in the periphery [66, 67]. However, unchecked CNS inflammation of a chronic (i.e., genetic mutation or prolonged exposure to environmental toxin) or sustained/self-perpetuating nature is, indeed, capable of mediating profound neuropathologic effects [68, 69]. Indeed, as will be discussed in ensuing sections, accumulating evidence suggests the involvement of protracted immuno-inflammatory processes in a host of CNS disorders, including neurological disease-states (e.g., PD, AD, stroke) and depression.

8.2.1 Cytokine Influences on the Central Nervous System

The inflammatory immune system functions primarily to protect the host organism from bacterial and viral insults. Foreign particles ordinarily stimulate an orchestrated immune response against specific antigens (foreign particles), and immunological factors develop a memory for them. Thus, upon subsequent encounters with these antigens, a rapid and robust immune response is mounted. Immune cells

(T and B lymphocytes, macrophages, granulocytes, dendritic cells and endothelial cells) synthesize and secrete signaling molecules, referred to as cytokines, which also elicit growth and differentiation of lymphocytes. Thus, these cytokines are fundamental for the organism to mount an effective immune response against invading pathogens [26].

The list of polypeptides that comprise the rapidly increasing family of immunotransmitters (cytokines) includes the interferons (IFN), interleukins (IL), tumor necrosis factors (TNF), chemokines (subclass of chemoattractant cytokines), and growth and cell stimulating factors [70–72]. Historically, the classification of cytokines has been based upon their molecular structure, as well as common physiological actions they possess, including the production of inflammation (i.e., swelling and irritation resulting from leukocyte infiltration) or fever (pyrogenicity). IL-1β, TNF-α and IL-6, which are all released from activated macrophages, are potent pro-inflammatory cytokines, whereas IL-4 and IL-10, which are released from T-cells, have anti-inflammatory actions. As well, IL-1β and IFN-γ provoke febrile responses and hence are examples of pyrogens; conversely, IL-2 is a non-pyrogenic cytokine [70]. Interestingly, although TNF-α and IL-6 are also often referred to as pyrogens, under certain conditions they may actually reduce the fever provoked by IL-1β, thus acting in a cryogenic fashion [73].

Given that the BBB is relatively impermeable to cytokines, owing to their rather large size (up to ~70 kDa) and hydrophilicity, the mechanism by which circulating cytokines affect brain function is still a matter of debate. However, cytokines may gain entry to the brain through sites where the BBB is somewhat compromised (i.e., areas bereft of tight junctions), namely at circumventricular organs such as the median eminence, subfornical organ, area postrema, and organum vasculosum [74]. As well, saturable carrier-mediated transport mechanisms capable of moving IL-1β and TNF-α may allow for limited penetration of cytokines into the brain [75–77]. In addition to routes dependent upon the circulatory system, cytokines may also influence brain processes by way of afferent projection fibres ascending from the periphery to the CNS (i.e., without actually entering the brain parenchyma). Indeed, TNF-α and IL-1β stimulate visceral branches of the vagus nerve to modulate many neuroendocrine and neurochemical states, and hence behaviour [78, 79].

It is worth noting that stressful events or certain immunologic challenges may also compromise BBB integrity, thereby affording greater cytokine action within the CNS [80]. In this regard, it has been proposed that corticotrophin-releasing hormone (CRH) release promotes brain mast cell activation, which in turn increases the release of IL-6, IL-8 and vascular endothelial growth factor [81, 82]. Likewise, pro-inflammatory cytokines themselves enhance permeability of the BBB through the upregulation of various endothelial cell adhesion molecules, such as intercellular adhesion molecule-1 [83], and hence up-regulate their own production at vascular sites while augmenting T cell trafficking across the BBB [84–86]. Furthermore, it seems that chemokines (chemo-attractant cytokines) may also disrupt BBB functioning, promoting the formation of vasogenic brain edema that is associated with various neuropathological conditions, including brain trauma, ischemia, CNS infection, presence of brain tumors, and recurrent MS flares [87–89].

Once cytokines gain entry to the brain, they interact with receptors on cells lining the BBB, around the meninges, as well as at vascular areas of the brain [90]. Through volume diffusion, infiltrating cytokines may ultimately penetrate deep within the brain parenchyma, where they interact with receptors located at hypothalamic, amygdaloid and brain stem nuclei [91–95]. In this respect, it seems that cytokines may actually have several functions similar to those of classical neurotransmitters by modulating neuronal Ca^{2+} channels, activation of intracellular second messenger systems, and stimulation of the MAP kinase pathways [96]. Indeed, induction of secondary mediators, such as COX-2-derived prostaglandins (PGs), may also be responsible for some of the central neurochemical effects of cytokines. Evidence supporting this proposition comes from studies showing that pretreatment with the non-specific COX inhibitor, indomethacin (inhibiting COX-mediated metabolism of arachidonic acid into PGs), attenuated at least some of the central actions of IL-1β, such as the altered NE variations observed within the hypothalamus [97].

Beyond their actions on CNS processes, cytokines and their receptors are endogenously expressed in the brain, likely reflecting de novo synthesis by glia (i.e., astrocytes and microglia, as well as oligodendroglia, radial glia and ependymocytes) and possibly by neurons [91, 98]. Moreover, pro- and anti-inflammatory cytokine levels are markedly increased by traumatic insults, [99–101], endotoxin treatments, [102, 103], as well as by neurogenic and psychogenic stressors [104–106]. In this regard, endothelial cells that line the interior surface of blood vessels and that of the ventricles produce IL-1β and IL-6, and infection or injury augments their concentrations [107]. Further, microglia, which serve as the brain's own specialized immune cells, are primary cytokine producers, and the synthesis of these cytokines were augmented by head injury, stroke and by neurotoxins [108–110].

8.2.2 Cyclooxygenase-2 and Central Nervous System Functioning

Cyclooxygenase, present in the CNS as COX-1, COX-2 and COX-3 isoforms, is an integral heme-containing membrane glycoprotein critically involved in the production of PG species [111]. Indeed, COX (otherwise known as PGH_2 synthase) is a rate-limiting enzyme in PG synthesis from arachidonic acid (AA). However, the first step in PG biosynthesis involves the conversion of membrane-bound glycerophospholipid into free arachidonate by phospholipase A_2(PLA_2), which is ubiquitously present in all brain tissues and whose expression is up-regulated by infection or injury. Thereafter, AA is metabolized by COX into the PG precursor, PGH_2(via the PGG_2 intermediate), which is further metabolized by terminal synthases into synthase-specific PGs (e.g., prostaglandin E_2, PGE_2), thromboxanes (e.g., TXA_2), and prostacyclin (PGI_2) [111]. In addition to COX, AA is also a substrate for the lipoxygenase and cytochrome P450 epoxygenase enzymes, all of which generate a number of biologically active lipid mediators, known collectively as eicosanoids [112]. Although a thorough consideration of these pathways, moreover of the effects

accorded to their enzymatic products (e.g., leukotrienes and epoxyeicosatrienoates, respectively), falls beyond the scope of the present discussion, it ought to be underscored that many of the eicosanoids are pleiotropic by nature and thus serve diverse, yet overlapping functions in both health and disease [112].

Within the CNS, all three COX isozymes are constitutively and heterogenously expressed in several discrete cellular populations (i.e., glia, neurons, endothelia), although they serve rather disparate, yet multiple (and therefore somewhat intersecting) functions under normal physiological conditions. Nonetheless, COX-1 may be generally described as a housekeeping enzyme, providing PGs at physiological levels relevant for the regulation of myriad homeostatic brain processes (e.g., cerebral blood flow) [113]. Although much less is known regarding the functions of COX-3, which is, in effect, a splice variant of COX-1, the recently discovered COX isozyme has been implicated in febrile responses and the processing of painful stimuli [114, 115]. Contrastingly, under normal physiological conditions, COX-2 partakes in dynamically regulated response-related activities, including synaptic plasticity and signaling, neurotransmission, memory consolidation during rapid eye movement (REM) sleep, membrane excitability, and gene expression [116–118]. Indeed, the COX-2 gene (but not COX-1) is flanked on its 5′-end with several regulatory/enhancer elements [e.g., for glucocorticoids, cytokines, nuclear factor-kappaB (NF-κB), and cyclic adenosine monophosphate (cAMP)] [112, 119]. Thus, COX-2 expression is dynamically regulated by factors that either potentiate (e.g., reactive oxygen species, ROS) or inhibit (e.g., IL-10) the promoter activities of relevant transcription factors, most notably the redox-sensitive NF-κB and members of the activating transcription factor (ATF)/cAMP response element-binding protein (CREB) family [112].

It should come as no surprise, then, that COX-2 but not COX-1 (or COX-3), is subject to induction by a variety of inflammatory stimuli, many of which (e.g., cytokines, ROS) have been implicated in the generalized activation of microglial cells in response to and as a corollary of brain injury and infection [68, 119]. Indeed, immunogenic challenge with the gram-negative bacterial endotoxin, lipopolysaccharide (LPS), elicited a marked increase in microglial COX-2 expression without provoking a commensurate increase in the expression of neuronal (i.e., constitutive) COX-2 (or COX-1) [120–122]. To this end, COX-2 (or its induction by inflammatory mediators) has long been considered an integral facet of immuno-inflammatory responding in the CNS and a growing body of evidence suggests an important role for the enzyme in the pathogenesis of both neurodegenerative disorders [123, 124] and depression [125].

8.3 Parkinson's Disease: Background

Parkinson's disease (PD) is one of the most common and debilitating age-related neurodegenerative disorders, affecting nearly 4 million people worldwide. The disorder is particularly prevalent in the elderly population, with a typical clinical onset

after 60–65 years of age [126]. Notwithstanding the rare familial forms of PD that appear to have a strong genetic component, the vast majority of PD cases (upwards of 95%) are idiopathic in nature [127]. The primary neuronal component that has received the most attention is the degeneration of dopamine (DA) neurons that occurs in the substantia nigra pars compacta (SNc) region of the midbrain in PD patients, resulting in the diminished monoamine release at downstream neural terminals within the striatum. Clinically, the parkinsonian syndrome, which typically becomes manifest following 50–60% SNc DA neuron loss, comprises a constellation of well-defined motor symptoms including cogwheel rigidity, resting tremor, postural instability and bradykinesia/hypokinesia [128, 129]. However, various autonomic [130] and olfactory [131] symptoms, as well as cognitive [132] and affective [64] disturbances have also been described in the clinical setting, although the pathological mechanisms underlying these relatively heterogeneous non-motor symptoms are not well understood.

Not surprisingly, symptoms often vary widely between PD patients, with a substantial number displaying psychiatric symptoms in addition to the motor disturbances that are evident in all patients. Of the co-morbid psychiatric symptoms, depression is particularly common, with 40–50% of PD patients showing clinically significant depression [64]. Of course, depression is also an important co-morbid feature of other neurological states, including stroke, multiple sclerosis and AD [1–3], as well as heart disease [133] and diabetes [134].

Notably, PD belongs to the spectrum of CNS α-synucleopathies, which have in common the histopathological finding of cytosolic α-synuclein-containing inclusions known as Lewy bodies, and of which AD, dementia, and diffuse Lewy body disease are also members [135, 136]. In sporadic PD, Lewy bodies are widely disseminated throughout the CNS, most notably within the SNc but also in the locus coeruleus, hypothalamus, cortex, and a number of limbic brain regions [137]. Although the findings are not wholly incontrovertible, mounting evidence implicates the abnormal ubiquitination, folding and aggregation of α-synuclein protein in the pathogenesis of PD, ostensibly via a targeted disruption in cellular Rab homeostasis and consequently, endoplasmic-to-Golgi vesicular trafficking [138–141].

Although familial early-onset forms of PD are relatively rare, certain genetic mutations have been reported to enhance susceptibly to environmental insults and hence, might contribute to the more common late-onset idiopathic cases of the disease. In fact, a recent study revealed that individuals who possessed a combination of mutations of the dopamine transporter (DAT), and who had substantial life-long pesticide exposure were at greater risk for developing PD than did individuals with either the genetic factor or pesticide exposure alone [142]. Moreover, the recent findings that polymorphism within certain environment responsive genes encoding effector proteins critical for cellular detoxification and xenobiotic metabolism, including cytochrome P450 debrisoquine 4-hydroxylase (CYP2D64) and glutathione S-transferase T1 and P1 (GSTT1 and GSTP1, respectively), modified the risk of developing PD, suggests that environmental toxicants might contribute to PD in genetically vulnerable individuals [143, 144]. Yet, another recent report indicated

that pesticide exposure was a significant predictor of PD incidence even among individuals with a negative family history versus those with a positive family history of the disease [145]. The latter finding suggests that pesticides (and other agents such as manganese) [146] might increase susceptibility to variants of parkinsonism. However, it is likely that the role of genetics depends upon the particular "sub-type" of PD. Indeed, PD appears to be a highly heterogeneous disorder with corresponding heterogeneity in etiological origins. Whereas autosomal dominant/recessive early-onset familial forms of PD (e.g., LRRK2, DJ-1) appear to be at one end of the spectrum, purely environmental "toxic exposure" cases may represent the other end. Hence, the bulk of "idiopathic" PD cases falls in the middle and will likely involve a mix of genetic and environmental influences. For instance, there is a very low penetrance of LRRK2 heterozygotic carriers that actually express the PD phenotype; yet, currently a significant proportion of idiopathic late-onset PD patients carry a LRRK2 mutation, suggesting that such genes can clearly be seen as susceptibility factors.

Several compelling lines of evidence suggest that environmental events may act as causative agents in PD. Indeed, although genetic vulnerability may act as a backdrop for disease provocation, accumulating evidence suggests that environmental agents, including commonly used pesticides, may act as triggers for the development of PD [147–150]. In fact, a progressively greater odds ratio for developing PD was associated with pesticide exposure [151], and several other epidemiological studies have implicated specific pesticides, including rotenone (an organic insecticide) and paraquat (a chemical herbicide still widely used throughout the world), in neurological pathology [152–156]. Indeed, a sharp increase of PD incidence was seen in agricultural areas that use these pesticides [157–160]. In particular, the non-selective herbicide, paraquat (N,N′-dimethyl-4,4′-bipyridylium ion), has been shown to dose-dependently increase the risk of developing PD as a function of cumulative pesticide exposure [161].

Animal studies also demonstrate that paraquat and rotenone, which are chemically similar to the widely validated DA neurotoxin, MPTP, can reliably induce PD-like pathology; and hence, are becoming widely used to produce a parkinsonian syndrome (e.g. bradykinesia, loss of DA neurons) in rodents and primates [162–167]. For instance, systemic exposure to paraquat provoked a dose-dependent loss of DA neurons in the SNc [168, 169], coupled with a reduction in the density of striatal DA fibres expressing tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis [170]. Moreover, the pesticide was shown to recapitulate certain PD-like bradykinesia symptoms and loss of striatal DA concentration in mice [169–171].

In addition to having greater ecological validity than the MPTP model of PD, paraquat and rotenone have been shown to provoke histopathological changes that more closely resemble the disease, particularly the aberrant fibrillization and aggregation of ubiquinated α-synuclein containing Lewy body inclusions (as occurs in PD patients) [172–175]. Moreover, it was reported that exposure to certain combinations of heavy metals and pesticides may synergistically provoke conformational changes in α-synuclein favoring the development of PD pathology [176]. In fact, a recent study revealed that exposure to a combination of iron and paraquat

synergistically increased α-synuclein aggregation and fibrillization and augmented the extent of oxidative stress-induced neurodegeneration [177]. Similarly, although the dithiocarbamate pesticide, maneb, had no effect on SNc DA neurons alone, when co-administered with paraquat, it synergistically enhanced nigrostriatal damage and associated glial reactivity [178, 179].

Pesticides can adversely affect neuronal survival by impairing mitochondrial functioning and over-stimulating microglial cells, causing an accumulation of oxidative free radicals (e.g. superoxide) and inflammatory factors [180–183]. Indeed, as will be discussed in ensuing sections, we and others showed that paraquat and/or rotenone enhanced the expression of pro-inflammatory cytokines and elicited oxidative stress through activation of the microglial inflammatory enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) [184–188]. Similarly, accumulating evidence suggests that the PG synthase, COX-2, may contribute to the neurodegenerative effects of numerous DA toxins, including paraquat [171, 189, 190].

8.4 Parkinson's Disease and Co-morbid Depression

In addition to the hallmark motor disturbances (e.g., bradykinesia, resting tremor) that are evident in all PD cases, a substantial number of PD patients also display prominent psychiatric symptoms. Indeed, major depression occurs in 40–50% of PD patients [64]. While co-morbid depression, anxiety and other psychiatric symptoms often negatively influence the quality of life for PD patients (just as the case in cerebral stroke, multiple sclerosis and other illnesses of which depression is often co-morbid), they may also come to affect the course of the primary illness itself. In fact, PD patients with a high degree of depressive co-morbidity often perform more poorly on a variety of motor tasks and tests of executive cognitive functioning [21, 191, 192]. Moreover, these individuals are distinguishable from their non-depressed counterparts, displaying decreased metabolism within the frontal cortex-basal ganglia-thalamic loop and impaired neuronal activity of motor regulatory elements of the basal ganglia (e.g., subthalamic nucleus) [193–197]. As well, electrical stimulation of the subthalamic nucleus, which is clinically implemented to relieve essential tremor, provoked depressive symptoms in PD patients and in animal models of the disease [198]. In fact, a reduction of serotonin (5-HT) activity within brainstem raphe nuclei was observed following subthalamic stimulation, suggesting the possibility that changes in basal ganglia inputs to brainstem nuclei might dysregulate 5-HT circuits, thereby contributing to depression.

As already alluded to, co-morbid depression and neurological illness might be due to the stress or other psychological aspects of the primary disease or its diagnosis [49, 64]. Yet, retrospective studies revealed that co-morbid psychiatric pathology, including anxiety and/or depression, occurred in PD patients prior to the onset of motor symptoms [60–64]. Moreover, given the temporal relations between these disease states, depression has even been suggested as a risk for factor PD development [62]. However, it is probably more likely that antecedent depressive symptoms

reflect early stages of the neurodegenerative process of neuroinflammatory cascades provoked at emotional regulatory limbic or brainstem regions (likely resulting in disturbances of multi-neurotransmitter systems). Interestingly, depression, along with olfactory and sleep disturbances, have even been posited to reflect early biomarkers indicative of a vulnerability for PD [199].

In support of the contention that depressive symptoms evolve from underlying neuropathological processes occurring in PD, histological pathology and signs of neuronal degeneration occur in several limbic and other brain regions important for emotional and cognitive processes. Indeed, long before the appearance of PD motor disturbances, neuropathology may occur in brain regions (e.g., hippocampus, locus coeruleus, prefrontal cortex) that have important emotional, arousal and cognitive functions [200–204]. In fact, post-mortem analyses of PD tissue revealed substantial degeneration of the nucleus basalis of mynert and the locus coeruleus [205, 206]. Moreover, early histological signs of PD may involve accumulation of Lewy bodies within the olfactory tract, locus coeruleus and several other brainstem regions [207].

Several experimental studies indicated that toxins thought to induce PD-like nigrostriatal and motor pathology also provoke co-morbid behavioural changes and target "non-motor" stressor-sensitive brain regions [208–213]. For instance, our work revealed that the herbicide, paraquat, in addition to provoking the loss of midbrain DA neurons, had neurotoxic effects on non-DA cells within the locus coeruleus, hippocampus and prefrontal cortex, and promoted depressive-like behavioural responses to the open field test (D. Litteljohn and S. Hayley, unpublished findings) [171]. Likewise, others have reported that pesticides, many of which have been epidemiologically linked to PD, as well as the commonly used experimental DA toxin, MPTP, caused a marked loss of neurons within both the locus coeruleus and SNc [214, 215]. Interestingly, it was even proposed that an early loss of norepinephrine (NE)-producing neurons of the locus coeruleus augmented the neurodegenerative impact of MPTP upon SNc DA neurons [216–219]. Hence, early brainstem pathology might place nigrostriatal neurons in a vulnerable state, possibly owing to a loss of growth factor and/or antioxidant support or a heightened pro-death or pro-inflammatory environment. Indeed, the fact that locus coeruleus NE neurons degenerate to an even greater degree than midbrain DA neurons (assessed in post-mortem PD brains) [220, 221] is consistent with the view that early pathology may first occur outside the nigrostriatal system (or that these NE neurons are hit harder over the course of the illness).

In addition to chemical toxins, heavy metals such as lead and iron have recently been shown to provoke SNc dopaminergic cell loss, together with neurochemical disturbances akin to those observed in stressor-related conditions [222–224]. For instance, manganese exposure promoted neuronal damage to the globus pallidus region of the motor regulatory basal ganglia [225], just as methyl mercury provoked anxiety-like symptoms (i.e., reduced exploratory behaviour) and activated c-Fos expression in stressor-reactive regions, including the amygdala, hippocampus and locus coeruleus [226]. Moreover, welders chronically exposed to heavy metals also displayed signs of depression, anxiety, irritability, and slowness of movement [227–229].

Notably, the neurodevelopmental time of toxin exposure might have particularly important long-term implications for CNS functioning. Indeed, several pesticides administered during periods of critical neonatal brain development engendered developmental behavioural pathology characterized by impaired habituation and cognitive functioning [230] and PD-like pathology in adulthood [231]. Neonatal exposure to the organophosrphorus pesticide, chlorpyrifos (at doses below the threshold for systemic toxicity), also had protracted consequences upon 5-HT functioning, and promoted a depressive-like phenotype in adulthood [232]. Moreover, inflammatory (bacterial endotoxin) and chemical (organophosphate pesticides) toxins themselves can cause impaired BBB functioning [233–237], and even psychological stress can enhance the penetration of neurotoxins into the brain parenchyma, thereby magnifying the damaging neuronal consequences of these agents [80, 238, 239].

The fact that emerging evidence suggests that treatments which prevent stressor-induced behavioural and neurochemical pathology might also benefit neurodegenerative processes, provides a further link between neurological illness and co-morbid psychiatric pathology. In this respect, stressor-induced alterations of basal ganglia glutamate and DA activity may have neurotoxic consequences relevant for PD [59]; and conversely, antidepressants may have neuroprotective actions in PD [14, 24]. Indeed, the tricyclic antidepressant, imiprimine, had anti-apoptotic effects upon hippocampal neurons and promoted the survival of neural stem cells through the up-regulation of the protective factors, brain-derived neurotrophic factor (BDNF) and B-cell lymphoma-2 (Bcl-2) [240]. Similarly, antidepressant treatment (with the serotonin reuptake accelerator, tianeptine) reduced hippocampal and cortical apoptosis induced by chronic psychosocial stressor exposure [241]. Finally, in several cases antidepressants were demonstrated to impart neuroprotection by down-regulating inflammatory processes, just as anti-inflammatory drugs may have anti-depressant properties [242–245].

Interestingly, non-pharmacological manipulations that positively influence mood (e.g., aerobic exercise) also had neuroprotective consequences through the activation of the growth factor, glial cell line-derived neurotrophic factor (GDNF), which has protective effects in animal models of PD [246]. Indeed, there have been several reports suggesting the general utility of targeting growth factors for promoting both anti-depressant and neuroprotective responses [247–251]. The opposite also appears to be true, namely that neuroprotective treatments, including a range of anti-inflammatory, anti-oxidant and anti-apoptotic agents, may impart positive effects upon emotional processes [245, 252]. For instance, the anti-inflammatory agent, minocycline, had both neuroprotective consequences for nigrostriatal DA neurons (against endotoxin exposure) [253], as well as antidepressant-like behavioural effects similar to traditional antidepressants, such as fluoxetine [254, 255]. Moreover, it was suggested that minocycline might be particularly effective in treating depressed patients with co-morbid cognitive disability, as well as those with organic brain pathology [245]. Clearly, close links exist between processes associated with mood and with neurodegeneration and we posit that inflammatory processes might be a common mechanistic link between these two seemingly disparate types of pathology.

8.5 Neuroinflammatory Mechanisms of PD and Depression

Infectious and inflammatory challenges influence both neurodegenerative and depressive-like pathology. For instance, humans, other primates and rodents exposed to LPS display marked signs of depressive behaviour (e.g., anhedonia, disturbed sleep and feeding) and neuro-hormonal changes (e.g., elevated glucocorticoids) similar to those observed following stressor exposure [26]. At the same time, central infusions of high doses of LPS have been shown to provoke degeneration of SNc DA neurons [256, 257]. Interestingly, SNc neurons were found to be far more vulnerable to LPS-induced damage than hippocampal, cortical and thalamic neurons; and it was suggested that such an effect stemmed from the high concentration of highly reactive microglia found in this brain region [257]. As well, clinical administration of immunotherapeutic immune proteins, particularly interferon cytokines used in the treatment of various cancers and hepatitis C, have in some instances been linked to the emergence of depressive symptoms, as well as demyelinating neuropathy and loss of cortical neuronal fiber density [258–260]. Similarly, other pro-inflammatory cytokines, particularly IL-1β and TNF-α, have been reported to induce depressive-like behavioural and neurochemical changes, [261, 262], and also have been shown to contribute to the neurodegenerative process in models of PD [263]. Hence, separate lines of evidence have implicated neuroinflammatory mechanisms in both the degeneration of DA neurons and the modulation of emotional processes.

Systemic infection may interact with environmental insults to induce exaggerated neuroinflammatory, degenerative and behavioural changes in neurological patients [110, 264, 265]. Indeed, exposure to pathogens or cytokines stemming from such exposure might have especially marked CNS consequences when encountered in the context of concomitant chemical toxin, traumatic head injury, or psychological stressor exposure, each of which can contribute to a breakdown of the BBB, hence favoring entry of peripheral immune pathogens into the CNS. In this regard, the bacterial endotoxin, LPS, synergistically augmented DA loss in midbrain-microglia co-cultures exposed to pesticides, such as rotenone [184, 266]; and these effects may be related to enhanced NADPH oxidase-mediated release of the superoxide radical [256, 267]. Our work has similarly shown that a low dose of LPS enhanced the neurotoxic effects of the herbicide, paraquat, such that a substantial number of DA-producing neurons were destroyed and PD-like symptoms emerged [188]. The augmented neurodegenerative response was observed when paraquat administration occurred at a time of maximal LPS-induced microglial activation (after 2 days), suggesting that the inflammatory priming sensitized microglial responding, thereby contributing to the degenerative effects of later paraquat exposure. Importantly, although relatively high concentrations of LPS alone had neurodegenerative consequences on DA neurons [257, 268], our studies involved concentrations of the endotoxin that alone activated microglia but had no effect upon DA neuronal survival.

Furthermore, the possibility exists that environmental or inflammatory toxins might promote a sensitization of neuronal processes across the lifespan, such that

exposure to an immune/chemical toxin at one point in life enhances vulnerability to the behavioural and neurodestructive effects of these challenges when subsequently encountered months or even years later. In particular, at in utero and early life stages when neuronal migration and synaptic pruning are occurring, neurons are especially sensitive to perturbations caused by environmental agents. At the same time, biological detoxification systems involved in metabolism and clearance of toxic substances are not fully developed in fetuses, infants and young children. Indeed, prenatal exposure to LPS induced a relatively permanent elevation of inflammatory factors within the nigrostriatal system and reduced the number of mature DA neurons in adulthood [269, 270]. Exposure to LPS during critical developmental times was also found to have protracted consequences that involve a dramatic long-term sensitization of the inflammatory immune response, such that the neuroinflammatory and neurodegenerative actions of pesticides applied during adulthood were greatly enhanced [271, 272]. As well, bacterial vaginosis, a common infection during pregnancy, has been linked to both the development of neurological disorders, including cerebral hemorrhage and cerebral palsy, and with enhanced levels of several pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α in adulthood [273–275]. Consistent with these findings, our contention has been that early immunogenic exposure may provoke mild neuroinflammation that, over time, renders neurons vulnerable to the effects of normally low-grade insults later in life. It may also be that early toxin exposure causes modest neuronal damage (or a silent lesion) that only becomes "un-masked" upon later multiple toxin exposures, again resulting in some threshold of neuronal vulnerability eventually being breached.

Pointing towards an inter-relationship between inflammatory processes, depression and neurodegeneration, antidepressant drugs, such as rolipram and buproprion, were reported to enhance the production of the anti-inflammatory cytokine, IL-10, while reducing levels of the pro-inflammatory cytokines, TNF-α and IFN-γ [276, 277]. At the same time, the tricyclic antidepressant, imiprimine, protected hippocampal neurons from the apoptotic consequences of high doses of the immunogenic agent, LPS, and these effects were related to the anti-inflammatory actions of the antidepressant [240]. Conversely, as already mentioned, anti-inflammatory treatments that have well documented neuroprotective actions in models of PD, particularly the tetracycline antibiotic, minocyline, also had antidepressant-like actions [255, 278]. Moroever, glatiramer acetate (otherwise known as Copaxone or Copolymer-1), a synthetic polypeptide that was neuroprotective in an MPTP model of PD [279] and that is used to treat multiple sclerosis, was hypothesized to have antidepressant effects through its stimulatory actions on the anti-inflammatory cytokine, IL-10, as well as on the nerve growth factor, BDNF [280]. As well, cytokine inhibitors used in treating inflammatory disease diminished co-morbid depressive symptoms [281]. Finally, consistent with the notion that inflammatory processes are fundamental to both depression and neurodegeneration, a growing number of studies have indicated a possible role of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, in preventing, delaying, and/or treating depression and neurological disease-states, including PD [282–284].

8.5.1 Role of Pro-inflammatory Cytokines in PD and Depression

Cytokines primarily act through activation of either of three molecular pathways, involving activation of: (1) NFκB; (2) c-Jun N terminal kinase (JNK); or (3) janus kinase (JAK) and signal transducer and activator of transcription (STAT). The latter two pathways involve the sequential phosphorylation of a series of intracellular proteins following administration of several cytokines, including IL-6, IL-10 and IFN-γ, resulting in the production of factors important for inflammatory and neuronal processes [26]. Similarly, the production of immune and CNS factors, including the inflammatory enzyme, COX-2, occurs following NFκB activation. In particular, IL-1β and TNF-α trigger the phosphorylation and degradation of the inhibitory factor, IκB, which normally holds NFκB in an inactive state, resulting in its translocation to the nucleus where it influences (inflammatory) gene expression. Indeed, we found that COX-2 deletion markedly influenced the production of cytokines following stressor and endotoxin exposure [285].

Increasingly, cytokines have been implicated in acute and chronic neuronal demise [15, 286]. Indeed, clinical studies revealed increased levels of the pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IFN-γ) in postmortem brain as well as in the blood of patients with stroke, head injury, multiple sclerosis, AD and PD [263, 287–290]. Although these findings have been recapitulated in animal models, it is still uncertain whether these cytokines primarily play a neuroprotective or neurodestructive role. It may be that relatively low endogenous cytokine levels act in a protective capacity to buffer against damage related to death processes, whereas relatively high levels of these factors may contribute to neuronal damage [291]. Indeed, low levels of cytokines can provoke the release of potentially beneficial trophic factors and free radical scavengers, but elevated levels may activate inflammatory cascades or even induce apoptotic death (self destructive programmed death mechanism). For instance, mice genetically lacking TNF-α receptors (thereby removing the influence of endogenous TNF-α) were more susceptible to ischemic injury; yet, administration of exogenous TNF-α at the time of ischemia exacerbated neuronal death [291]. Likewise, administration of the endogenous IL-1 antagonist, IL-1ra, reduced infarct size in response to middle cerebral artery occlusion (MCAO) and prevented the accumulation of inflammatory infiltrates within the area of damage [292], suggesting a prominent destructive role for IL-1 in acute cerebrovascular insults. In effect, the concentration as well as timing of cytokine exposure likely determines whether primarily protective or deleterious consequences arise from these immunotransmitters.

Cytokines may influence the development of depressive pathology by affecting processes aligned with neuroplasticity, such as neurogenesis [293]. Indeed, IL-6 over-expressing transgenic mice displayed greatly reduced hippocampal neurogenesis [294]. As well, chronic IFN-α administration (in a schedule mimicking an immunotherapeutic protocol) similarly reduced hippocampal neurogenesis, and this effect was attenuated by inhibition of IL-1β [295]. A further link between stressors, cytokines and neurological processes is suggested by other studies showing that psychogenic stressors may promote the demise of cortical neurons through

apoptotic mechanisms, and that IL-1β as well as the TNF-α family member, Fas, were involved [296]. Conversely, antidepressants elevated neurogenesis and prevented the effects engendered by chronic stressors [297]. Furthermore, attenuating the inflammatory response through administration of the anti-inflammatory treatments, minocycline and indomethacin, restored neurogenesis otherwise reduced by irradiation, endotoxin or IL-6 treatment [294, 298]. As such, these findings raise the possibility that cytokine actions on depressive states stem from effects on neuroplasticity. Taken together, the available data suggest that cytokines are critical "general" mediators of the CNS consequences of immune challenges, stressors and environmental toxins; and hence, are likely important contributors to the pathogenesis of depression and neurological disease.

Interferons in depression and PD: Interferons (IFNs) are broadly divided into either type I IFNs, including the IFN-α and IFN-β isoforms, which originated from a common ancestral gene, or the structurally unrelated yet functionally similar type II form, IFN-γ [299]. The α and β isoforms of IFN bind to a 66 kDa receptor chain, whereas a distinct 90 kDa receptor binds IFN-γ; however, both require the presence of an accessory protein to be functionally active [300, 301]. The main signaling pathways utilized by the IFNs involve the phosphorylation of STATs by intracellular JAK protein kinases [302]. IFN-γ is secreted predominantly from type 1 helper T lymphocytes (Th1) and from natural killer (NK) cells [303, 304]; yet, recent reports indicate that IFN-γ may also be synthesized de novo within the brain by activated microglia [305]. In contrast, the production of IFN-α and IFN-β does not appear to be under the control of specific cell types, and indeed, most cells appear to be able to secrete these cytokines in response to viral insult.

The IFNs play an important role in both early host defense against infection and cancer (i.e., antiviral and antiproliferative activities) and later adaptive responses to chronic infection (i.e., immunoregulatory activities) [306]. Indeed, before immune mechanisms come into play, IFN-α/β are released to provide the first line of defense against viral infection. They interfere with viral replication early during infection, while IFN-γ is only released after T cells have become sensitized to the viral antigens [299]. In addition to their antiviral actions, IFN-α and IFN-γ are particularly adept at fighting intracellular bacterial infections (e.g., *Listeria monocytogenes*). As well, IFN-γ has potent anti-tumor properties largely stemming from its ability to stimulate tumoricidal activity of macrophages. Indeed, IFN-γ induces macrophages to produce TNF-α together with reactive oxygen intermediates that may contribute to the natural resistance to cancer through the selective lysis of tumor cells [307]. Although IFNs were originally believed to be exclusively antiviral substances, it has become apparent that the cytokine family is involved in a broad array of functions which may either inhibit or promote disease states within the periphery or CNS [308]. Indeed, accumulating evidence suggests a particularly important role for IFN-α and IFN-γ in both depression and PD, thus raising the possibility that IFN-associated inflammatory processes constitute a significant pathway to co-morbidity.

In the case of depression, recent studies reported that systemic IFN-α treatment promoted neuronal signs of oxidative stress and reduced the density of cortical

neuronal terminals [295]. Moreover, IFN-α, which is often used as a therapeutic treatment for certain cancers and hepatitis C, has been reported to cause a depressive syndrome amenable to antidepressant treatment [259, 260, 309]. Similarly, rodents treated with IFN-α displayed marked signs of anhedonia, impaired hippocampal neurogenesis and a reduction of cortical neuronal fiber density [295, 310]. As well, we have shown that systemic IFN-α administration provoked neurochemical changes implicated in depression (5-HT and NE) in several stressor-sensitive brain regions [311]. As mentioned previously, many antidepressants, including fluoxetine, trimipramine and reboxetine, either suppress IFN-γ production (independent of monoamine reuptake blockade) or reduce the IFN-γ/IL-10 ratio [276, 312]. Consistent with these findings, antidepressant treatment (with the SSRI, fluoxetine) was recently shown to normalize the otherwise high levels of IFN-γ message observed among depressed patients [313]. Similarly, genetic ablation of the IFN-γ receptor (thereby nullifying the effects of endogenous IFN-γ) protected against the depressogenic effects of Bacillus Calmette-Guerin infection in mice (a model of immune-mediated depression), [314] and preliminary work from our laboratory suggests a role for IFN-γ in mediating chronic stress-induced changes in hippocampal neurogenesis, pro-inflammatory cytokine expression, limbic neurotransmission (NE, 5-HT, DA), and affective behaviour (e.g., exploration of a novel environment, consumption of a palatable substance) (D. Litteljohn and S. Hayley, unpublished findings).

Many of the depressive effects of IFN-α and more recently, IFN-γ, were suggested to stem from the ability of these cytokines to affect the enzyme, indoleamine 2,3-dioxygenase (IDO), which is responsible for 5-HT metabolism as well as the catabolism of tryptophan to kynurenine [315]. Specifically, IFN-γ induced microglial IDO in vitro [314], just as in vivo experiments demonstrated that IFN-α elevated IDO, resulting in diminished tryptophan availability and consequently, reduced 5-HT levels [316, 317]. However, the depression provoked by IFN-α was also accompanied by elevated levels of the potentially toxic metabolic by-product, kynurenine [317, 318]. As indicated by Wichers and Maes [318], kynurenine metabolites, such as 3-hydroxy-kynurenine and quinolinic acid, through their effects on ROS or stimulation of hippocampal N-methyl D-aspartate (NMDA) receptors, may engender neuronal cell loss. Hence, the IDO pathway may be one common route through which IFNs, and pro-inflammatory cytokines more generally, could promote both depressive and neurodegenerative pathology.

Besides depressive symptoms, cancer patients receiving IFN-α immunotherapy have also been observed in many instances to develop a number of PD-like symptoms, including tremors, muscle rigidity and a generalized paucity of movement [319]. Similarly, recent data suggests an important role for IFN-γ in MPTP and paraquat models of PD [320–322]. In corroboration of these results, IFN-γ levels are markedly elevated in the blood [320, 323] and postmortem SNc brain tissue of PD patients [324, 325]; and a polymorphism in the gene coding for IFN-γ differentially modified the risk of developing early- or late-onset PD [326]. In addition, the viricidal interferon-inducible GTPase, MxA, localizes to nigrostriatal Lewy bodies in PD brain [327], and serum and cerebrospinal fluid (CSF) neopterin (pteridine

derivative associated with IFN-γ signaling) levels are elevated among PD patients, reflecting enhanced IFN-γ/Th1-dependent immune system activation [328]. Indeed, PD patients exhibit fewer infectious episodes and malignancies [329], possibly stemming from enhanced pro-inflammatory IFN signaling.

As will be recalled, IFN-γ (and IFN-α/β) acts primarily through the JAK/STAT signaling pathway. Indeed, following ligand binding, intracellular JAK protein kinases phosphorylate homodimeric STAT DNA-binding complexes (otherwise known as γ-IFN activation factors), which then translocate to the nucleus and induce the transcription of myriad IFN-γ-responsive genes [302]. Interestingly, a recent study indicated that IFN-γ may be capable of inflicting direct excitotoxic neuronal damage (e.g., dendritic beading) via the interaction of its receptor with the AMPA receptor GluR1 subunit to form a unique, Ca^{2+}-permeable receptor complex [330]. However, most available evidence suggests that IFN-γ likely influences neuronal survival and functioning through its actions on microglia.

To this end, while the microglial gene network subject to regulatory control by IFN-γ is at once both extensive and diverse (reflecting the pleiotropic nature of IFN-γ and cytokines in general), a number of positively regulated IFN-γ-responsive genes encode proteins implicated in immuno-inflammatory processes (either directly or indirectly) [331]; and as such, may be of particular relevance for neurological disorders, such as PD, and affective disturbances (depression) that involve a prominent neuroinflammatory component. For instance, IFN-γ-associated microglial JAK/STAT signaling mediates either the up-regulated or de novo synthesis of several proteins critical for antigen presentation to lymphocytes (e.g., MHC class I/II, proteasome subunits LMP-2/7), recruitment and activation of T cells (e.g., chemokines and adhesion molecules), and classical pathway-dependent complement deposition (i.e., opsonization of antigens) [332, 333]. Importantly, many of these proteins have been localized to microglia in the SNc of post-mortem PD brains [35, 334] or animals exposed to DA neurotoxins [188, 335], suggesting that IFN-γ may be a critical determinant of prospective adaptive immune responses in PD (although the functional relevance of adaptive immune activation in PD remains controversial) [67].

In addition, the genes encoding iNOS, key subunits of NADPH oxidase, as well as the PKR gene (IFN-inducible dsRNA-activated serine/threonine kinase), are all governed by microglial JAK/STAT signaling [336–338]. NADPH oxidase and iNOS, via the production of ROS and nitric oxide (NO), respectively, are important mediators of nitritive/oxidative stress; and PKR, through its actions on the NF-κB signaling pathway, is capable of inducing microglial COX-2 (i.e., via NF-κB transactivation of the COX-2 promoter) [339]. Indeed, pretreatment with the indole hormone, melatonin, attenuated microglial COX-2 induction by IFN-γ, and this effect was attributed to the inhibition of NF-κB activation [340]. Accordingly, IFN-γ may impact neuronal survival by way of its downstream effects on key microglial enzymes implicated in the elaboration of deleterious inflammatory factors (i.e., NO, ROS, prostanoids).

Likewise, the pro-inflammatory cytokines, IL-12, IL-18 and IL-1β, are all up-regulated by IFN-γ-associated JAK/STAT signaling, suggesting that IFN-γ may be

an early mover of pro-inflammatory cytokine cascades [333]. Further, each of these cytokines (including IFN-γ itself) can skew $CD4^+$ T cell development towards a Th1/pro-inflammatory phenotype, which is, in fact, typical of PD [341]. In addition, IFN-γ stimulates microglial TNF-α production, presumably through the sensitization of these cells to antigens (e.g., LPS) and, potentially, xenobiotic agents (e.g., pesticides) [342–344].

Lastly, IFN-γ-associated JAK/STAT signaling may also drive the down-regulation of several ostensibly neuroprotective species in microglial cells. For instance, IFN-γ dampened microglial expression of osteopontin [331], a secretory phosphoprotein with anti-apoptotic properties that can attenuate the neurodegenerative consequences of stroke and various neurotoxins [345–347]. Whatever the case, a large body of evidence suggests a potentially central role for IFNs in both PD and depression, and hence, identifies these cytokines as prime candidates for mediating the co-morbid presentations of PD.

Interleukins and tumor necrosis factor-α in depression and PD: The two structurally related proteins, IL-1α and IL-1β, were the first of the IL-1 family of inflammatory cytokines discovered. The protease, interleukin-converting enzyme (ICE), cleaves the 31–33 kDa precursor, pro-IL-1, to form the mature and biologically active IL-1α and IL-1β cytokines [348]. Some of the synthesized IL-1 is secreted in a soluble form, but a proportion is retained within the cell membrane [349]. Both the soluble and membrane-bound forms of IL-1 are biologically active, particularly with respect to lymphocyte activation [349]. Although IL-1 is a potent pro-inflammatory agent, it also has many other actions, including the formation and remodeling of bone, appetite regulation, insulin secretion, fever regulation and neuronal phenotype development [350]. It appears that IL-1 signaling is dependent upon an interaction between its type I receptor and the IL-1 receptor accessory protein, which is located on an adjacent portion of the cell membrane [70].

Based upon its involvement in the wasting syndrome (cachexia) often seen in cancer patients, the pro-inflammatory cytokine, TNF-α, was previously referred to as cachectin. Interestingly, this cytokine was at one point considered to be a promising treatment for some types of cancer (e.g., melanoma) [351]. Much like IL-1β, TNF-α is a pleiotropic cytokine, which exerts a wide array of actions on numerous cell types. For instance, it has physiological actions on bone osteoclasts (important for rheumatoid arthritis), mononuclear and polymorphonuclear blood cells, fibroblasts, skin keratinocytes, insulin sensitive adipocytes, as well as brain neurons and glial cells [352]. Like other cytokines, TNF-α typically acts locally at the site of generation; however, small amounts of the cytokine are found circulating in the bloodstream.

Like the case for IFNs, mounting evidence suggests a role for ILs and TNF-α in both PD and depression. Specifically, postmortem analyses of PD brain tissue revealed increased expression of TNF-α and its related Fas receptor, as well as the cytokines IFN-γ, IL-1β and IL-6 [353]. Likewise, in animals, MPTP induced alterations of pro-inflammatory cytokine genes, including those encoding IL-1β and

TNF-α; and the DA neurotoxin, 6-OHDA, increased levels of these cytokines within the SNc and striatum [354–356]. The few studies assessing cytokine manipulations on PD-like pathology generally indicated a neuroprotective role for IL-1β and IL-6, but a more destructive role for TNF-α. Indeed, infusion of IL-1β protected DA neurons from 6-OHDA and MPTP toxicity and induced dendritic branching from residual neurons following SNc lesion [357, 358]. Correspondingly, IL-6 knock-out mice displayed enhanced degeneration of SNc neurons and striatal terminals following MPTP, suggesting enhanced sensitivity in the absence of endogenous levels of the cytokine [359]. Involvement of TNF-α in PD is more controversial, with two conflicting reports indicating that TNF-α deletion either protected striatal terminals and normalized dopamine levels in MPTP-treated mice [360, 361] or increased DA metabolism, without necessarily affecting neuronal survival [362]. Interestingly, in one study there was no effect of intra-SNc infusion of TNF-α or IL-1β either alone or together upon neuronal survival [268], but the source for this outcome is uncertain.

Exogenously administered IL-1β and TNF-α induce an array of behavioural symptoms collectively referred to as "sickness behaviour", which are mediated, in part, by central mechanisms. For instance, cytokines administered systemically or directly into brain elicit soporific effects, anorexia, fever, fatigue, reduced motor activity, curled body posture and reduced sexual behaviour [363]. Although these behavioural changes are of potential adaptive significance, acting to minimize energy expenditure and sustain body temperature in the face of cytokine challenge [364], it will be recognized that these behaviours are in many ways reminiscent of the neurovegetative symptoms that characterize depressive disorder. In addition, IL-1β was shown to engender anhedonic-like effects (e.g., responding for rewarding brain stimulation, responding for sucrose reward on a progressive ratio schedule) [365, 366], together with disturbed social interaction [367] and impaired cognitive performance (e.g., in a Morris water-maze) [368]. Although these effects may be secondary to the sickness, this does not exclude the possibility that cytokines, and in particular IL-1β, affect motivational, anxiety or depressogenic processes involving CNS mechanisms [16].

The cytokines IL-1β and TNF-α typically influence central processes through NFκB, a transcription factor that plays a critical role in the regulation of innate and adaptive immune reactions, including the mobilization of inflammatory chemokines and lymphocyte proliferative responses following infection or traumatic injury [369, 370]. Indeed, NFκB signaling occurs ubiquitously throughout the brain, and IL-1β infusion into the lateral ventricles induced the translocation of NFκB to the nucleus at several brain regions distal to the site of infusion, including the choroid plexus, ependymal cells, cerebral vasculature and meninges, as well as at the basolateral amygdala [371]. NFκB is composed of five subunits, together with a nuclear localization signal, which are normally held in an inactive state by an endogenous inhibitory factor, IκB. However, exposure to inflammatory stimuli triggers the phosphorylation and consequent degradation of IκB, resulting in the translocation of NFκB to the nucleus where it promotes gene expression [369]. Immunological

insults may initiate this NFκB cascade through the provocation of cytokines, particularly IL-1β and TNF-α, which, after binding to their cell surface receptors, stimulate kinases that target IκB for ubiquitination and subsequent proteasomal degradation [369]. As well, these cytokines may also affect CNS processes by stimulating NFκB signaling cascades.

To this end, NFκB appears to have potent effects upon CNS processes important for neuronal survival and plasticity. In terms of functional outputs, NFκB is readily activated in response to excitatory neurotransmission and is believed to play an important role in learning and memory [372]. In particular, NFκB expression within the hippocampus is increased during activation of neuroplastic processes, such as the induction of long term potentiation; and the factor is involved in promoting neuronal survival following hippocampal injury [373, 374]. The transcription factor may have a neuroprotective role through the induction of anti-apoptotic proteins, such as Bcl-2 and the antioxidant enzyme, manganese superoxide dismutase (MnSOD) [372]. Yet, NFκB signaling may also result in the synthesis or upregulation of inflammatory cytokines and enzymes, ROS, and excitotoxins that can contribute to neurodegeneration [375, 376]. For instance, iNOS expression within microglia and astrocytes is readily provoked by NFκB activation following exposure to cytokines, such as IL-1β or IL-12 [376, 377]. Similarly, stressor exposure may contribute to neurological pathology by affecting NFκB-mediated production of oxidative radicals given that restraint stress was shown to promote neuronal excitotoxicity in rats that was associated with enhanced TNF-α release and NFκB mediated activation of iNOS and COX-2 [378]. In the case of HIV-1 infection, NFκB was found to be important for the production of chemokines by glial cells and the influx of peripheral inflammatory cells into the CNS [379]. Ultimately, a host of factors, including the chronicity and type of inducing stimulus, likely influence whether NFκB activation has protective or detrimental effects upon neuronal survival or functioning.

Besides its role in neurodegenerative responses, NFκB signaling might play a role in the evolution of psychiatric pathology, such as anxiety and depression. Indeed, NFκB is constitutively expressed in dorsal raphe brainstem 5-HT neurons and its activity at these neurons was shown to be readily influenced by several steroidal hormones that are often affected in depression, including estrogen, progesterone and glucocorticoids [380]. Moreover, inhibition of NFκB signaling attenuated the social withdrawal and reduction in food intake promoted by IL-1β, and dramatically reduced IL-1β-induced hypothalamic and amygdaloid c-Fos expression [381]. Similarly, a recent report indicated that mice genetically lacking the p50 subunit of NFκB (resulting in impaired NFκB activity) displayed reduced stressor-induced anxiety, suggesting that endogenous activity of the transcription factor was required for the manifestation of anxiety [382]. As well, elevated NFκB levels were observed in lymphocytes obtained from bipolar patients currently experiencing depressive symptoms [383] and in women experiencing the emotional stress of having a breast biopsy [384]. Accordingly, NFκB may be an important down-stream signaling factor for cytokines and stressors that may contribute to neurodegenerative as well as depressive pathologies [385].

8.5.2 Cyclooxygenase-2 in Relation to Parkinson's Disease and Depression

As will be recalled, cyclooxygenase-2 (COX-2) catalyzes the conversion of AA into PGH_2, which is the precursor of synthase-specific prostanoids (i.e., PGs, thromboxanes, prostacyclin). Within the CNS, prostanoids exert their actions on various cell types by binding to PG-specific transmembrane receptors coupled to second messenger cascades [111]. Although not all the enzyme's central effects are mediated by PGs (e.g., COX-2 also catalyzes the synthesis of docosanoids from docosahexaenoic acid, DHA) [386], prostanoid receptor signaling constitutes the primary mechanism by which COX-2 influences neuronal functioning and survival [387]. In this regard, not only is PGE_2 one of the most abundant prostanoids in the brain, the vasoconstrictive prostanoid has long been considered an important mediator of CNS inflammatory responses (e.g., pyrexia) and hence, may be a critical player in inflammatory brain pathology [112, 388].

Indeed, as is the case for pro-inflammatory cytokines, substantial evidence indicates that COX-2 may play an important role in PD and, likely, depression. In this regard, COX-2 expression was up-regulated in activated microglial cells within the SNc of post-mortem PD brain [389]. Likewise, MPTP-intoxicated mice displayed augmented nigrostriatal COX-2 immunoreactivity [390], and pharmacologic inhibition or genetic ablation of COX-2 prevented the loss of DA neurons following exposure to MPTP or 6-OHDA [391–394]. Similarly, we found that mice genetically lacking COX-2 were resistant to the PD-like neurological (nigrostriatal DA transmission) and behavioural (bradykinesia) effects of the herbicide, paraquat [171]. Also, several epidemiological reports indicated that certain NSAIDs, which act primarily to inhibit COX-2 enzymatic activity [395], may either prevent or delay PD onset [282–284].

As previously indicated, COX-2 influences neuronal survival primarily through PG-coupled signal transduction cascades, and in particular those mediated by PGE_2 and its receptors ($EP_{1–4}$). For instance, PGE_2-EP_1/EP_3receptor signaling elicits a marked increase in intracellular Ca^{2+} concentration, thereby leading to a disruption in cellular Ca^{2+} homeostasis and, potentially, excitotoxicity [396]. Indeed, EP_1/EP_3receptor blockade (with selective antagonists) completely prevented DA neuron loss following 6-OHDA treatment [397]. Further, PGE_2-EP receptor signaling has downstream effects on cAMP/protein kinase A (PKA) pathway activation (via changes in cAMP second messenger levels), which, among other things, may lead to the instigation of caspase-dependent pro-apoptotic events [398, 399] and the production of potentially damaging factors, such as NO [400].

Interestingly, emerging evidence indicates that PGE_2 may also act in an autocrine or paracrine (i.e., PGE_2 derived from damaged neurons or other microglial cells) manner to augment the COX-2-dependent glial production of further prostanoid species [401]. To this end, PGE_2 was recently shown to promote the inherent transcriptional activities of NF-κB [402, 403], which, as it will be recalled, exerts transactivational control over the COX-2 gene promoter and as such, may mediate the positive feedback regulation of COX-2 by its own PG products [404]. Indeed,

NF-κB immunoreactivity was markedly increased (70-fold) within the SNc of PD patients relative to age-matched controls [405], and as already mentioned, post-mortem PD brain displayed increased SNc COX-2 staining [389]. Whatever the case, it appears likely that bidirectional neuron-microglial interactions afforded by prostanoid signaling contribute significantly to the pathogenesis of PD. Indeed, it has been suggested that PG signaling between neural cells might serve in the recruitment of otherwise quiescent microglia (i.e., acting to propagate microgliosis) and augment the synthesis and release of inflammatory mediators, including further PG species, from heretofore activated microglial cells (i.e., acting to reinforce microgliosis) [388, 393]. This, in turn, would of course be expected to exacerbate ongoing DAergic cell death.

COX-2 enzymatic activity also generates highly potent ROS, including the superoxide anion radical and several tyrosyl- and carbon-centered free radical species, all of which promote oxidative damage to neurons [112, 406]. In addition, while acting as a co-substrate for the COX-2 peroxidase-catalyzed reduction of PGG_2 into PGH_2, cytosolic DA may become oxidized to the highly reactive DA-quinone species [407], which in turn, can compromise the integrity and functioning of myriad proteins through its binding to sulfhydryl-containing cysteine residues [408]. Indeed, COX-2 deficiency protected against MPTP-induced DA cell loss and the formation of protein-bound cysteinyl-DA adducts (surrogate biomarkers for COX-2-mediated DA oxidation) [390].

It is worth noting, however, that COX-2, in addition to mediating potentially deleterious pro-inflammatory responses, appears also to promote inflammatory immune resolution both in the CNS and the periphery [111]. Consistent with this notion, even as PGE_2 signaling through EP_1/EP_3 receptors is implicated in COX-2-mediated neurotoxic events, EP_2/EP_4 receptor signaling often mediates pro-survival responses. For instance, stimulation of EP_2 and/or EP_4 receptors prevented neuronal cell death following excitotoxic insult (NMDA) [409] or beta-amyloid exposure [410]. More generally, multiple COX-2-derived prostanoids seem able to promote central immunosuppressive/anti-inflammatory responses by directing a reduction in pro-inflammatory factors (e.g., TNF-α, NO) or an increase in anti-inflammatory ones (e.g., IL-10, BDNF) [411–413]. Further, other COX-2 enzymatic products, namely the recently discovered DHA-derived docosanoids, appear to antagonize the pro-inflammatory effects of PGs and curb inflammatory CNS responses more generally (e.g., up-regulate anti-apoptotic Bcl-2 protein expression, down-regulate COX-2 expression) [414–416].

In addition to the important role COX-2 plays in PD, several lines of evidence have indicated that COX-2-associated inflammatory processes may also contribute to the pathogenesis of depression. In this regard, clinically depressed patients had elevated PGE_2 levels in saliva, blood and CSF [417–419], and displayed increased mitogen-stimulated production of PGE_2 (by whole blood) relative to non-depressed individuals [420]. Further, the selective COX-2 inhibitor, rofecoxib, was effective in the treatment of depression co-morbid with osteoarthritis [421], just as celecoxib (another selective COX-2 inhibitor) was reported to enhance the antidepressant actions of the noradrenaline reuptake inhibitor, reboxetine [242], and to

alleviate depressive symptoms in bipolar disease patients [422]. Consistent with these findings, pharmacologic studies showed that COX-2-mediated PGE_2synthesis was down-regulated secondary to antidepressant (tricyclics and selective serotonin reuptake inhibitors) inhibition of pro-inflammatory cytokines [423, 424].

Also, several animal studies have suggested that COX-2 might play a fundamental role in the anxiety- and depressive-like pathology associated with immune challenge or exposure to stressors [425, 426]. For instance, COX-2 antagonists reduced the hippocampal 5-HT variations [427], as well as the hormonal and cytokine variations associated with an inflammatory insult [428, 429]. Likewise, inhibition of COX-2 attenuated the anxiety, locomotor and cognitive disturbances, as well as the oxidative damage provoked by an immobilization stressor [430–432]. Moreover, treatment with the selective COX-2 inhibitor, celecoxib, prevented the depressive-like behavioural effects induced by chronic unpredictable stress in rats and dose-dependently lowered brain COX-2 expression and PGE_2 levels in these animals [433]. In fact, naïve (i.e., non-stressed) animals also displayed improved emotional functioning following celecoxib application, and this effect again correlated with decreased central COX-2 activity [433]. Taken together, these findings indicate that COX-2 likely plays an important role in both PD and depression, and as such, may be one common biological determinant of co-morbid pathology.

8.6 Other Neurodegenerative Conditions with Co-morbid Depression

8.6.1 Depression Co-morbid with Alzheimer's Disease (AD)

As is the case for PD, depression is highly co-morbid with Alzheimer's disease (AD) [3], and a recent meta-analysis indicated that depression may be a significant risk factor for AD [434]. Yet, once again, as is true for PD, these early signs of depression might reflect the initial stages of AD disease onset. Specifically, modest degeneration, neuroinflammatory or other pathological processes may be occurring in emotional regulatory brain regions (e.g., prefrontal cortex, amygdala, hippocampus, and brainstem). Alternatively, damage to the basal forebrain, which is a core histopathological feature of AD, may effectively disconnect or "short-circuit" the proper neurotransmission between circuits important for mood.

Irrespective of their origins, the presence of anxiety and depressive symptoms among individuals with mild cognitive impairment (i.e., pre-clinical state) enhanced the likelihood that such persons would later develop AD [435]. Similarly, co-morbid anxiety and depression additively increased the severity of cognitive decline in AD patients [436]. In general, however, elderly individuals that experience depressive illness appear to be particularly sensitive to the cognitive aspects of the disorder, such that it may be difficult to disentangle the affective disturbances from symptoms

of AD or dementia. In fact, underlying clinical depression might be masked by a primary AD syndrome, hence resulting in under-diagnosis of affective disturbances in the elderly [7].

In support of a link between depressive illness and AD, an elevated number of hypothalamic CRH neurons were reported in both AD and depression, and it was suggested that hyperactivity of the hypothalamic pituitary adrenal (HPA) axis may be characteristic of both depressive and AD pathology [437]. However, it may also be the case that variations of CRH signaling that occur in AD may stem from a loss of a sub-population of these neurons, which may then promote disturbances of affect [438]. Indeed, the widespread neurodegeneration observed in AD was associated with monoamine deficiencies in several brain regions (e.g., hippocampus, brainstem raphe) that have been linked to depressive symptoms [439]. Accordingly, treatments that influence common neurochemical processes may be beneficial for both depressive and AD co-morbid states, and may even be used in a prophylactic capacity. For instance, the antidepressant, paroxetine, improved cognitive performance and reduced the accumulation of β-amyloid and abnormal tau protein in a transgenic rodent model of AD [440]. As well, the proposition was advanced that new therapies aimed at targeting cytokines and growth factors (e.g., BDNF), together with their downstream effectors, might be important for the treatment of AD and co-morbid depression [441].

One common theme of this chapter is that inflammatory processes likely contribute to the co-evolution of depression with neurodegenerative conditions. For instance, inflammatory factors, such as oxidative radicals and COX-2 products, might act as triggers for the co-emergence of depression and AD [442, 443]. In fact, COX-2 activation was shown to enhance β-amyloid toxicity and, as in the case of depression, inhibition of COX-2 signaling attenuated pathology in an animal model of AD [443, 444]. Similarly, pretreatment with COX-2 inhibitors, such as naproxen, rofecoxib and valdecoxib, attenuated not only the anxiety effects of an immobilization stressor, but also the cognitive deficits and oxidative damage provoked by this insult [432]. There has even been some clinical evidence to suggest that the COX-2 inhibitor, celecoxib, improved cognition in schizophrenic, as well as depressed patients [445]. Hence, although not exclusively linked to AD, the COX-2 pathway might have general importance for changes in neural pathways involved in cognition that occur in several CNS conditions.

Several pro-inflammatory cytokines have been implicated in the formation of senile plaques, which represent a defining histological feature of AD. In particular, IL-1β and IL-6 have been associated with β-amyloid enriched neuritic plaques and neurofibrillary tangles [446, 447], and activated microglia expressing IL-1β, IL-6 and TNF-α have been found in high levels around neuritic plaques [448]. These cytokines may interact with one another or with endogenous β-amyloid to influence neurodegeneration. In this regard, TNF-α, IL-1β and IFN-γ synergistically enhanced the release of the free radical, NO, from astrocytes, an effect that was further amplified by addition of soluble β-amyloid fragments [449]. As well, neuritic plaque-associated microglia expressing IL-1α were more likely to be in an activated phagocytic state than those not associated with plaques [450].

Not only are cytokines associated with the presence of neuritic plaques, they may also contribute to plaque maturation. Although neuritic plaque density in AD patients correlates with clinical dementia, diffuse (i.e., less mature) plaques do not correlate well with cognitive decline [451]. One intriguing hypothesis that has been offered is that cytokines, in fact, drive the evolution of the plaques into their more mature dense-core forms [452]. Thus, IL-1 and IL-6 may contribute to AD pathology by increasing β-amyloid deposition and dystrophic neurite formation [453]. Evidence in favor of this hypothesis is supported by reports indicating that IL-1β released from microglia may provoke the release of the neurotrophin, S100b, from astrocytes, which then contributes to pathological alterations in neuronal elements associated with the plaques [452]. Other cytokines, including TNF-α and IFN-γ, may also contribute to β-amyloid deposition. Both of these cytokines enhanced β-amyloid production from cultured glial cells, whereas inhibition of IFN-γ reduced β-amyloid accumulation in an animal model of AD involving mutation of the β-amyloid precursor protein [454]. Cytokines may also promote AD pathology by interfering with the production of growth factors critical for the proper maintenance of neurons. For instance, IL-1β inhibited BDNF signaling in cultured neurons by blocking activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAP) pathway [455]. Taken together, these data suggest that cytokines, through their actions on β-amyloid and growth factor functioning, may influence the course of AD.

Besides acting in a neurodegenerative capacity in AD models, cytokines may also function in a neuroprotective manner. For instance, TNF-α pretreatment of neuronal cultures reduced β-amyloid induced hippocampal neuronal death and hyper-phosphorylation of tau protein [456]. However, as already mentioned TNF-α, together with IL-1β and other pro-inflammatory cytokines, served to enhance β-amyloid accumulation and augmented its neurodegenerative effects [449]. In fact, it was reported that activation of the TNF-R1 receptor by β-amyloid induced apoptotic protease-activating factor (APAF) and subsequent neuronal death [457]. Indeed, TNF-R1 has well known pro-death effects that are mediated by its intracellular death domain and associated apoptotic caspases. Conversely, TNF-R2 mainly has pro-survival and proliferative functions [458, 459]. Indeed, TNF-R2 activation typically stimulates several intra-cellular adapter proteins that influence the production of a number of neuroprotective proteins by NFκB [460]. As well, the degree of activation of microglial cells may influence the neurodegenerative effects of TNF-α. Indeed, this cytokine had neurotoxic effects in neuronal cultures exposed to β-amyloid in the presence of microglia, but not in those treated with the glial suppressor, Ara-C [456, 461]. These findings suggest that the TNF-α exposed glia may be releasing toxic factors that counteract any effects that the cytokine may have on protective neuronal pathways.

Variations of circulating cytokines and associated inflammatory factors may also contribute to AD, as well as the evolution of co-morbid depression. In this regard, altered cytokine (TNF-α, IL-1β) and histamine serum levels were correlated with an increased incidence of depression among AD patients [462]. Moreover, cytokines might contribute to the progression from pre-clinical states, characterized by mild

cognitive impairment, to the AD syndrome. Indeed, elevated IL-6, IL-8 and IL-10 expression in peripheral immune cells has been associated with mild cognitive impairment [463]. Similarly, circulating TNF-α was increased in AD, as well as among those individuals experiencing mild cognitive impairment; and levels of this cytokine were negatively correlated with concentration of the protective growth factor, IGF-1 [464].

In some instances, changes in peripheral cytokines and clinical symptoms might stem from genetic alterations in AD patients. In fact, polymorphisms of the IL-10 promoter region were found to predict increased risk of AD [465], and a polymorphism of the IL-18 gene was associated with a faster rate of cognitive decline in AD patients [466]. It was also reported that co-morbid AD and depression were associated with common polymorphism of the promoter region of IL-1β (-511 variant) [467]. Ultimately, it may be that certain genetic factors engender inflammatory responses to stressful or neurotoxic insults that may favor the evolution of AD pathology and could contribute to secondary depression by virtue of widespread variations of cytokine and COX-2 signaling.

8.6.2 Post-Stroke Depression

Of the conditions linked to major depression, particular attention has been devoted to the depressive condition that often follows an acute cerebrovascular stroke, which is often referred to as post-stroke depression. Indeed, substantial evidence indicates that depression regularly occurs following ischemic or hemorrhagic cerebral stroke [1]. It was recently reported that one third of cerebral stroke survivors exhibit post-stroke depression and that stroke survivors have more than a six-fold higher risk of developing depression after two years following the stroke episode [468]. Depression occurring relatively early following stroke is typically characterized by substantial anxiety and guilt, coupled with loss of libido, however, later-onset depression is more closely aligned with symptoms of social isolation and diurnal variations of mood [469]. Although post-stroke depression may be treated with several antidepressants, treatment resistance is quite common compared to individuals experiencing depression in the absence of cerebral insult [470]. It is also not surprising that anxiety often co-occurs in stroke patients, resulting in a complex combination of co-existing psychiatric and neurologic pathologies. For instance, generalized anxiety disorder was co-morbid with depression in 20% of patients assessed several months after stroke [471]. Importantly, besides enhancing mood, antidepressants significantly diminished anxiety and augmented overall patient functioning [472].

Several common biological processes could play a role in cerebral stroke and co-morbid depression, including neuronal lesions, alterations of neuroplasticity, as well as changes in central monoamines and pro-inflammatory cytokines [473, 474]. Indeed, reports have indicated that post-stroke depression was associated with cortical lesions, including those of the prefrontal, left anterior cingulate and right

occipital cortices [475]. Alternatively, ischemic lesion might disrupt ascending brainstem NE and 5-HT projections from the locus coeruleus and raphe, resulting in diminished monoaminergic input to limbic and cortical regions [476]. Indeed, diminished CSF concentrations of the 5-HT metabolite, 5-HIAA, were found in post-stroke depression [477]. At the same time, reductions of growth factors such as BDNF, which have been reported following stroke, [478], might not only result in pro-degenerative processes, but also enhance susceptibly for depression. Indeed, BDNF has well established positive effects on neuroplasticity that have been supposed to promote antidepressant consequences [479, 480]. In fact, reductions of hippocampal and frontal cortical volumes in depression likely stem from disturbances in growth factor-regulated neuroplasticity [481].

Activation of neuroinflammatory processes occur in both stroke and depression, and as such, inflammatory signaling pathways involving cytokines, such as IL-1β, TNF-α and IL-6, are likely important in this regard. Indeed, these cytokines are substantially altered within the brain and periphery following cerebral stroke, as well as in response to a variety of psychological stressors [363, 482]. In fact, stroke-induced elevations of IL-1β, TNF-α and IL-18 have been suggested to increase the activity of various enzymes, particularly IDO, which subsequently deplete 5-HT levels in emotional regulatory limbic brain regions [483]. Curiously, however, a robust elevation of anti-inflammatory cytokines was also observed following stroke [468]. Conversely, stressors that normally provoke depressive-like pathology have been shown to augment the deleterious impact of ischemia and this effect was thought to result from the actions of pro-inflammatory cytokines. In particular, immobilization enhanced infarct size caused by cerebral stroke, whereas inhibition of IL-1β or TNF-α prevented this effect [484]. These data support the contention that psychological and cerebrovasular insults might interactively promote depressive and neurodegenerative pathology by stimulating pro-inflammatory cytokine signaling.

Animal models of ischemia involving occlusion of middle cerebral carotid arteries (MCAO) result in time-dependent increases of gene expression for several cytokines, including IL-1 and its endogenous receptor antagonist, IL-1ra [485]. Interestingly, direct infusion of IL-1 into the brain exacerbates the neuronal damage provoked by ischemic insult [71]; and conversely, inhibition of IL-1 (e.g., by an IL-1 antibody or by the IL-1 converting enzyme) reduced ischemic infarct size [485]. As expected, IL-1ra reduced ischemic damage within the striatum and prefrontal cortex [71]. Although elevated IL-1 and IL-1ra expression is associated with the area of damage around the cortical and striatal infarcts, the cytokines may also be increased at distant brain regions that do not show neuronal damage, such as the amygdalae [71]. Indeed, mRNA for CRH has also been found to be upregulated in the amygdala of rats 1 h following focal cerebral ischemia induced by MCAO [486]. Moreover, after administration of CRH antagonists or lesioning of the amygdala, ischemic damage was inhibited [486]. These findings are particularly intriguing in light of the plethora of evidence implicating this area in the modulation of behavioural responses to stress and cytokine treatment. Although the mechanism by which CRH influences MCAO-induced damage remains to be elucidated, it was

posited that amygdaloid CRH may act as a diffusible factor that serves as part of a regulatory loop, hence influencing cortical and striatal ischemic infarct damage [71].

As already alluded to, cytokines are not invariably damaging to the CNS. In fact, emerging evidence suggests that cytokine or cytokine receptor antagonists may be involved in the development of ischemic tolerance [26]. For instance, animals initially exposed to a mild temporary MCAO insult display fewer behavioural and histopathological consequences upon later exposure to a more severe form of MCAO [291]. Ischemic tolerance may result from the provocation of various protective factors, including cytokines or their endogenous receptor antagonists. As well, the effects of a particular cytokine may be influenced by the experimental paradigm and the particular dose employed. For example, in contrast to its neurodestructive role in vivo, application of relatively low picomolar doses of IL-1β protected neuronal cultures from subsequent damage related to excitotoxic or other pharmacological manipulations [487]. Very high concentrations (in the nanomolar range), which exceed that seen even in the injured brain, were toxic to neural cultures, but such an effect was probably not applicable to typical in vivo conditions [71]. However, when the cytokine was added to mixed glial and neuron cultures, IL-1 was found to be toxic at picomolar concentrations [488, 489]. It was suggested that the neuroprotective actions of IL-1 in neuronal cultures may be related to neuronal production of nerve growth factor (NGF) induced by the cytokine, while in mixed cultures cytotoxic effects may have stemmed from the production of NO and other glial-derived superoxides [490]. It seems likely that the determination of either protective or destructive actions of IL-1 depends upon a critical balance between competing systems activated by the cytokine and the numerous secondary mediators that are induced. Neuroprotective effects of IL-1 may stem from production of NGF, elevated gamma-aminobutyric acid (GABA) activity or inhibition of Ca^{2+} entry into neurons [491]. Conversely, neurotoxic actions may be attributable to induction of other pro-inflammatory cytokines, eicosanoids, cellular adhesion molecules, CRH, acute phase proteins, activation of complement pathways and disruption of the BBB [71].

As well as IL-1, other cytokines such as TNF-α may play an important role in ischemia. However, whether the cytokine is involved in the provocation of cellular damage or acts in a protective capacity remains controversial. Evidence in favor of the former comes from studies showing that mRNA for the cytokine is up-regulated after ischemic insults or hippocampal damage [492], and that application of TNF-α to neuronal cultures has neurotoxic consequences and promotes neuronal degeneration [489]. Moreover, TNF-α is a potent activator of microglia and induces the production of neurotoxic substances, such as NO and excitotoxins [70]. In vivo i.c.v. (intracerebroventricular) adminstration of TNF-α to spontaneously hypertensive rats before temporary or permanent MCAO increased infarct volume and exacerbated forelimb deficits [493]. Further, when endogenous TNF-α was neutralized by intravenous or intracortical application of antibodies to the cytokine, a reduction of cortical infarct size was evident after MCAO [494].

Evidence in favor of a neuroprotective role for TNF-α in ischemia is derived largely from studies involving normal and genetically altered mice. In contrast to

the reports using hypertensive rats, murine models of ischemia indicated that pre-treatment with TNF-α shortly before MCAO reduced cortical infarct size in mice [495]. Furthermore, mice lacking both of the TNF-α receptors displayed increased infarct volume 24-h following MCAO [292]. Other genetic studies revealed that a specific TNF-α receptor subtype might be important in mitigating against the neuronal injury subsequent to MCAO or excitotoxins. Specifically, mice deficient in the TNF-R1 (but not the TNF-R2 receptor) were susceptible to both ischemic as well as excitotoxic neuronal damage [496]. Mice lacking the TNF-R1 receptor also displayed increased degeneration of CA3 hippocampal neurons in response to infusion of the excitotoxin, kainic acid, into this brain region [496]. Although it remains to be elucidated precisely how TNF-α may exert neuroprotective actions, several mechanisms may account for such effects. For example, TNF-α acts upon neurons to increase their resistance to oxidative stress by reducing intracellular accumulation of Ca^{2+} and ROS [497, 498]. Indeed, TNF-α has also been reported to stabilize calcium homeostasis [499], thereby potentially protecting neurons from the over-stimulation that occurs after ischemic or excitoxic events. Likewise, the cytokine may also have neuroprotective properties owing to its ability to induce the release of the free radical scavenger, MnSOD [500, 501]. Summarizing briefly, it appears that the neuroprotective or neurotoxic actions of TNF-α may be differentially influenced by the two receptor subtypes for the cytokine. Whether such effects are also dependent upon the animal species examined remains to be determined.

Although limited data are available concerning the role of IL-6 in ischemia, the available evidence suggests that the cytokine may be primarily neuroprotective. In response to an ischemic challenge, IL-6 protein and mRNA were increased in the hippocampus of gerbils, as well as in astrocyte cell cultures [502]. It was suggested that astrocyte-derived IL-6 may play a neurotrophic role by acting in a paracrine fashion to promote neuronal survival after an ischemic event [502]. Moreover, i.c.v. IL-6 administration reduced infarct size after MCAO [503]. Time-course studies indicated that neuronal and glial bioactivity for cortical IL-6 is increased in a multimodal fashion after MCAO, with peaks occurring 2, 8 and 24 h after the insult [503]. In clinical stroke patients, serum IL-6 levels increased progressively during the first 24 h following the insult; however, control levels were still exceeded 3 and 7 days later [504], suggesting that the brain insult may have protracted effects on peripheral immune or inflammatory processes. Likewise, elevated IL-6 levels within the CSF can be detected in stroke victims within the first 3 days following the insult and concentrations of the cytokine were correlated with the extent of brain damage, as assessed 2–3 months later [505].

It is presently unclear as to the exact behavioural consequences that cytokines might have following cerebral stroke. However, one recent study did report that anhedonia (assessed by a reduction in sucrose solution consumption) was induced following MCAO and that this effect was dependent upon IL-1β activity [506]. Similarly, it was suggested that the well documented elevation of IL-1β and TNF-α following cerebral stroke serves to induce depression by increased activity of the aforementioned IDO enzyme at multiple brain regions, thereby promoting widespread 5-HT reductions [483]. It is also noteworthy that genetic variations of

the 5-HT transporter have been linked to post-stroke depression, with the short allele of the serotonin-transporter-linked polymorphic region (5-HTTLPR) being associated with the illness [507]. Importantly, both IL-1β and TNF-α have been reported to affect 5-HT transporter activity [508].

Overall, it is clear that cytokines are induced following stroke and it is expected that these inflammatory messengers affect the functioning of multiple pathways in several brain regions. Hence, it is highly likely that emotional, cognitive and motor functioning would come to be affected by cytokines in the days and months following stroke. In this regard, temporal changes in the balance between pro- (IL-1, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) that occurs over time might, as occurs in the case of multiple sclerosis, come to either enhance or deter certain recovery processes. As well, the same cytokines might even have different effects over the course of the recovery process following stroke. Indeed, although IL-1β has been shown to enhance infarct volume under some conditions, this same cytokine has also been reported to promote striatal neuroplasticity and behavioural recovery following lesion [71]. Along these lines, neuroplastic processes, such as neurogenesis and dendritic branching, have well established importance in neuronal and behavioural recovery [509]. Given recent evidence that several cytokines, including IL-1β TNF-α, IL-6 and IFN-α, can differentially affect neurogenesis and synaptic activity [510], these cytokines might come to secondarily affect depressive-like behaviours by modulating neuroplastic processes that become engaged following stroke. Indeed, our own recent evidence suggests an importance for IFN-γ in the regulation of several signaling factors known to have important consequences upon behaviour and neuroplasticity, including CREB and p70S6 kinase (D. Litteljohn, E.N. Mangano and S. Hayley, unpublished findings). In particular, we posit that these signaling factors underlie striatal plasticity and behavioural recovery following toxin-induced injury.

8.7 Conclusions and Future Directions

As presently described, there are ample data to suggest an important role for cytokines and inflammatory processes in both acute (stroke) and chronic (AD, PD) neurodegenerative states. Owing to the high degree of co-morbid depression evident in these neurological conditions, we have begun to assess the possibility that common inflammatory mechanisms (e.g., cytokines, COX-2) might promote both the primary neurodegenerative state, as well as the co-morbid depression. Indeed, there is little doubt that cytokines and other inflammatory mediators are involved in at least some of the behavioural (particularly neurovegetative symptoms) and neurochemical pathology evident in depression. Owing to the multi-system pleiotropic effects of cytokines, these immunotransmitters are in an ideal position to influence a range of pathology by affecting endocrine, immune, vascular and neuronal systems. In fact, besides their role in promoting CNS co-morbidity, cytokines likely also influence the onset of co-morbid peripheral pathology, such

as diabetes, heart disease, obesity and autoimmunity, which in turn, affect CNS functioning.

The findings discussed in this chapter provide support for our contention that anti-inflammatory treatments might have general clinical utility in treating neurodegenerative and depressive conditions. Given the complexities of the inflammatory response, future efforts would be wise to focus on developing more selective anti-inflammatory agents that target specific cytokines (and other inflammatory mediators) at certain stages of the illness. This is not to say that such anti-inflammatory agents should replace conventional treatments, rather these novel drugs might be useful as adjuncts or "add-ons" to existing therapies. Indeed, owing to the multifaceted nature of and likely multiple mechanisms involved in CNS illnesses, a multi-pronged drug approach seems reasonable. Along these lines, using antidepressants in neurodegenerative conditions, such as PD, has recently proven beneficial for the co-morbid mood and anxiety symptoms, as well as the underlying primary condition. In summary, a better understanding of the basic mechanisms that give rise to the individual conditions and their co-emergence is required to ultimately devise novel therapeutic approaches.

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Chapter 9
Breakdown of the Blood-Brain Barrier in Stress Alters Cognitive Dysfunction and Induces Brain Pathology: New Perspectives for Neuroprotective Strategies

Hari Shanker Sharma and Aruna Sharma

Abstract Emotional, psychological or environmental stress (e.g., heat or nanoparticles) influences brain function. However, the detailed mechanisms of stress induced brain dysfunction are not well known. Research carried out in our laboratory since last 20 years show that various kinds of stressors depending on their magnitude and durations alter the blood-brain barrier (BBB) permeability to proteins leading to brain pathology. These stressed animals also show marked behavioral and cognitive deficits at the time of the BBB leakage. Entry of several restricted elements from the blood to the brain compartment after breakdown of the BBB results in immunological, biochemical and pathological reaction causing brain edema formation and cell injury. Blockade of several neurochemical receptors, e.g., serotonin, prostaglandin or opioids as well as neutralization of key neurodestructive elements, i.e., neuronal nitric oxide synthase (nNOS), Tumor necrosis factor-alpha (TNF-α), dynorphin A or hemeoxygenase-2 (HO-2) using specific drugs or antibodies against these factors reduces BBB disturbances, cognitive and behavioral dysfunction, and brain pathology. Based on these new evidences, it appears that the BBB is the *gateway* to neuropsychiatric diseases. Thus, efforts should be made to maintain a healthy BBB in various brain diseases to achieve neuroprotection. The possible mechanisns of BBB breakdown and brain pathology in stress in relation to altered cognitive and sensory-motor functions is discussed in this review.

Abbreviations

BBB	Blood-brain barrier
bbb	brain-blood-barrier
nNOS	neuronal nitric oxide synthase

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_9,

TNF-α	Tumor necrosis factor alpha
HO-2	Hemeoxygenase-2
IPS	Information processing system
GAS	General adaptation syndrome
CRF	Corticotrophin releasing factor
ACTH	Adrenocorticotrophic hormone
HPA	Hypothalamus pituitary adrenal axis
TRH	Thyrotrophin releasing hormone
PVN	Para ventricular nucleaus
DHEAS	dehydroepiandrosterone sulfate
WN-25	West Nile virus
SFV-A7	Semliki Forest virus
EBA	Evans blue albumin
PS	Paradoxical sleep
SWS	Slow wave sleep
ROS	Reactive oxygen species
WBH	whole body hyperthermia
BSCB	Blood-spinal cord barrier
Dyn	Dynorphin A
5-HT	5-hydroxytryptamine
CNS	Central nervous system
p-CPA	para-Cholorophenylalanine
H1	Histamine H1 receptor
H2	Histamine H2 receptor
5,7-DHT	5,7-dihydroxytryptamine
6-OHDA	6-hydroxydopamine

9.1 Introduction

Blood-brain barrier (BBB) was first recognized by Paul Ehrlich in Germany about 125 years ago [1]. However, its role in various neurological and psychiatric diseases is still not well known. About 30 years ago, our laboratory was the first to demonstrate that the BBB is compromised to protein tracers under different stressful situations [2–4]. This opening of the BBB under stressful situations is somehow related with neurochemical metabolism and appears to be mediated via specific neurotransmitter receptors for serotonin [5, 6], prostaglandin [7], histamine [8, 9] and opioids [4, 10–14].

However, our knowledge regarding the functional significance of BBB breakdown in stressful situations is still unclear (see [13]). Thus, we still do not know how this information is useful in interpreting neurological or neurodegenerative diseases? Whether the BBB breakdown is crucial for cognitive or sensory-motor impairment? Is brain pathology always associated with BBB breakdown? Or, increased BBB permeability in disease situations reflects some kind of compensatory mechanisms? This review is focused on these pertinent questions that are

largely based on our own investigations and in the light of the recently available new data in the field. In addition, the new results obtained in other stressful situations caused by psychostimulants or nanoparticles on BBB functions are also discussed. Furthermore, the functional significance of BBB breakdown in relation to brain pathologies and its modulation by various neuroprotective agents to enhance neurorepair are presented.

9.2 Blood-Brain Barrier: A Dynamic Anatomical Barrier

BBB is a physiologically dynamic barrier whose permeability properties are very similar to that of an extended plasma membrane [4, 14, 15]. Anatomically, the BBB mainly resides within the endothelial cells of the cerebral microvessels, which are connected with tight junctions [16], a feature that is not found in non-cerebral capillaries (Fig. 9.1). The other important feature of cerebral capillaries includes a very low level or almost absence of vesicular transport, a phenomena that is very common in other vascular beds [4, 11, 17]. Furthermore, endothelial cells of the cerebral capillaries are almost covered with glial end-feet that encircle the microvessels (about 85–90%) at the one end and the other end is connected to the nerve cells or to the dendrites [18]. It is still uncertain whether glial end-feet contribute to the BBB phenomena; however, it has been established that their presence is needed to induce BBB properties within the endothelial cells during development [15, 19].

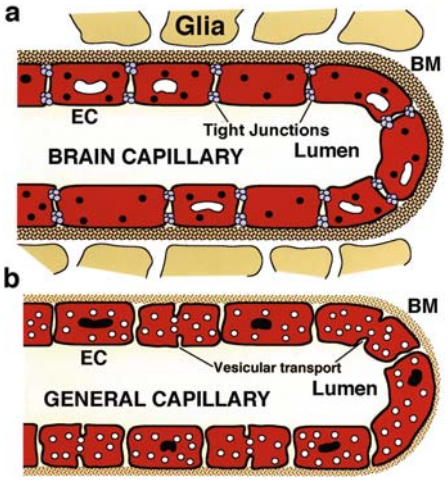

Fig. 9.1 Anatomical site of the blood-brain barrier (BBB). Schematic drawing of the ultratstructural aspects of one brain capillary (**a**) and one general capillary (**b**). The endothelial cells (EC) of cerebral capillaries are connected with tight junctions and normally do not contain microvesicles for vesicular transport as compared to the non cerebral capillary. The endothelial cells of the cerebral capillaries are also covered with a thick layer of basement membrane (BM) compared to the general capillary. Modified after [11–14]

The basal lamina, which is also known as basement membrane, is quite thick in cerebral microvessels; however, its role in BBB function is still not well known. Available evidences mainly points out that the basal lamina, per se, has no role in restricting tracer transport from vascular compartment to the neural compartment when the endothelial cell barrier is compromised (see [11, 12]).

9.2.1 BBB: A Physiological Regulatory Barrier

The BBB strictly regulates the composition of the extracellular fluid microenvironment of the brain in which neurons bath. Thus, the BBB works as exchange medium for essential nutrients from blood to brain as well as excretory medium for metabolic products from brain to blood [12, 13]. Many substances are transported actively from blood to brain via active transport (Fig. 9.2). Recently, presence of several transporter genes has been found for many essential substrates or neurochemicals [13–15]. Among which glucose transporter, 5-HT transporters are widely examined. Although, the role of transporters are still not clear, there are evidences that these transporters alter in several neurological diseases, indicating that altered transport properties of the BBB either reflect disease processes or in disease conditions, abnormalities in the BBB transport occurs [4, 9, 12, 13–15].

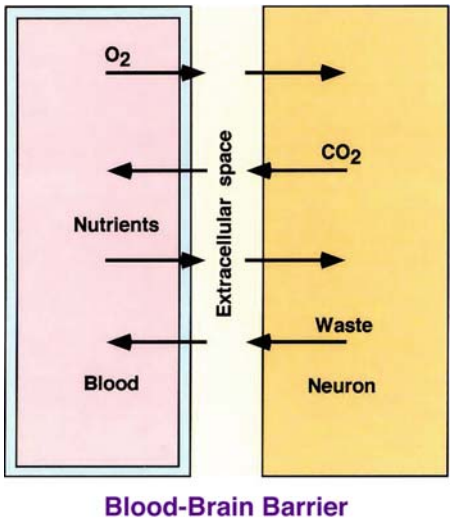

Fig. 9.2 Blood-brain barrier (BBB) is a physiological dynamic barrier. The BBB effectively regulates exchange of substances between brain fluid microenvironment and the vascular system. Thus, nutrients and waste products are easily exchanged between the neuronal and microvascular endothelial cell membrane interface through the extracellular space. Modified after [12]

9.2.2 BBB: A Chemical Barrier System

The endothelial cells of the cerebral microvessels possess several enzymes, which are capable to degrade various neurotransmitters while their passage from blood to brain [12–15] (Table 9.1). Thus many neurotransmitters substances, that as such

Table 9.1 Enzymatic aspects of the BBB function

Enzymes	Transporters/receptors	Antigens
γ-glutamyl transpeptidase	glucose transporter	EBA
aminopeptidase-N	aminoacid transporters	HT7/OX-47/Basigin
alkaline phosphatase	transferrin receptor	PC-1
butyryl cholinesterase	LDL receptor	Thrombomodulin
monoamine oxidase	insulin receptor	Meca 32
diphosphopyridine nucleotide diaphorase	Na^+-K^+-ATPase	ApoA1
lactate dehydrogenase	ion channels	
malate, α-glycerophosphate		
glutamate and glucose 6-phosphate dehydrogenase		
Succinic dehydrogenase		
β-hydroxylbutyrate and ethanol dehydrogenase		
acid phosphatases, ATPase		
DOPA-decarboxylase		
Inosine diphosphatase		
acetylcholinesterase		
α-ketoglutarate transaminase		
carboxyl esterases[#]		

[#]foetal rat capillaries only
Modified after [11–14]

cannot pass the endothelial cell barrier are eliminated due to degradation within the endothelial cells [12, 15, 21]. On the other hand, precursor of most of the neurotransmitters gain easy access within the brain compared to their prototypes. Blockade of endothelia cell enzymes using pharmacological agent is thus one suitable approach to increase the concentration of the neurotransmitters, if needed. This aspect is used to enhance the penetration of L-DOPA in the brain after inhibiting the carbonic anhydrase enzyme using methyl-DOPA [12–15, 20, 21].

Apart from neurotransmitter metabolizing enzymes, several other enzymes are found in the cerebral endothelial cells whose functional significance is yet to be established [12, 13, 20]. Thus recently described nitric oxide synthase enzyme responsible for generation of nitric oxide is present in cerebral endothelial cells (see [22, 23]). Likewise, hemeoxygenase enzyme, responsible for carbon monoxide production in vivo is also found to be present in endothelial cells [21, 24]. These observations suggest an active role of endothelial cells in maintaining the fluid microenvironment of the brain in a very strict fashion.

9.3 The Brain-Blood Barrier (*bbb*)

Very little is known about the barrier between brain and blood [12, 20, 24, 26]. It is believed, that brain-blood barrier (*bbb*) is also very tight as blood-brain barrier [27, 28] (Fig. 9.3). The assumption for this comes from the idea that in brain several

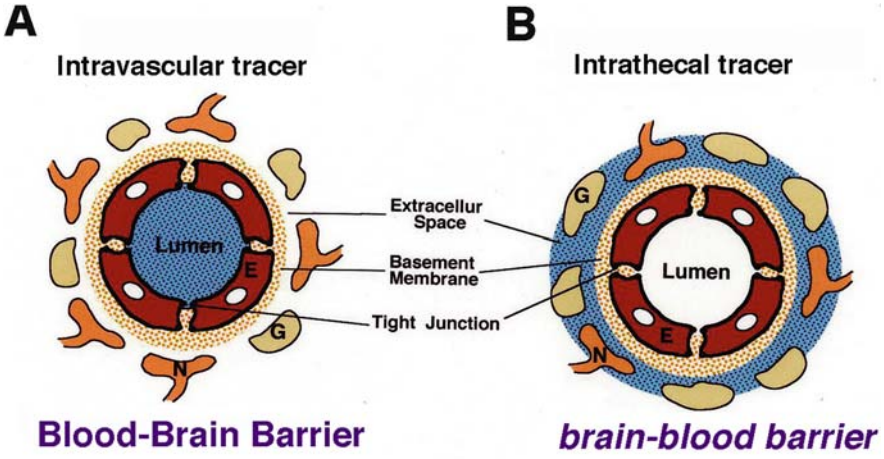

Fig. 9.3 The brain-blood barrier (*bbb*) is equally effective as the Blood-Brain Barrier (BBB). A. Schematic diagram showing bbb (**B**) and BBB (**A**). The endothelial cells (E) in the brain microvessels are connected with tight junction and are surrounded by a thick basement membrane. The glial cells (G) and nerve cells (N) around the cerebral endothelial cell are clearly seen. Intravascular tracer is normally stopped at the tight junction and donot penetrate the luminal endothelial cell membrane to reach extracellular space indicating a very tight BBB (**A**). Likewise, intrathecal tracer does not normally pass the abluminal endothelial cell membrane and/or the tight junctions to reach the vascular compartment (**B**) suggesting that the bbb like the BBB also effectively regulates exchange of substances between brain microenvironment and the vascular system (**B**). It is still not known whether BBB leakage is also accompanied with bbb disruption in stress or in brain diseases. Modified after [11–14]

neurotransmitters are released due to altered demand or stimulation of particular pathways and/or due to altered chemical sensation in different neuronal pathways responsible for pain or stress [21, 22, 25, 26]. In such circumstances, the brain could produce large quantity of neurochemicals that are released in the brain or in spinal cord for functional purposes. In most of the cases, the release of neurochemicals will be prolonged and reabsorption will occur very slowly. If these neurochemicals can gain access into the systemic circulation due to a defect in the *brain-blood barrier*, then rebound phenomena may amplify the effects of these vasoactive neurochemicals by many folds into the brain [12, 24–28]. However, in normal circumstances this does not occur and the peripheral circulation is kept largely aloof from changes in the neurochemical transmission in the brain and *vice versa* (see [23–28]). The main reason behind these regulatory phenomena is a strong *brain-blood barrier* function.

However, there are reasons to believe that under stressful conditions or following brain diseases the brain-blood barrier (*bbb*) is also altered [12, 27, 28]. Although, information about breakdown of the *bbb* in disease conditions are still lacking, it remains to be seen whether drugs influencing various neurochemical receptors could also modified the *bbb* [28].

9.4 BBB and Brain Pathology

An increased permeability of the BBB is quite common in almost all the diseases afflicting the brain or spinal cord in which marked alterations in the neural, glial and endothelial cells are seen (see [11–13, 20]). This suggests BBB dysfunction is crucial for brain diseases. However, the functional significance of BBB opening in these pathological conditions is still not well understood. Thus, it is not yet clear whether increased BBB permeability is the result of the pathological alterations in the brain or a breakdown of the BBB permeability has resulted in all the pathological changes seen under these clinical and experimental brain disease situations.

9.4.1 BBB "Opening", "Leakage", "Dysfunction", "Breakdown" or "Increased Permeability"?

The BBB opening or breakdown under brain disease conditions is quite common-[12–15, 21]. In the literature, the term "opening", "leakage" and "breakdown" are used most often interchangeably. However, some caution must be made while using these terms. The opening of the BBB can be a physiological demand for increased transfer of some molecules across the cerebral endothelium. It seems reasonable to assume that this opening is most likely a temporary one. Thus, the term "breakdown" should be reserved for long-term opening associated with often-irreversible phenomena, which cannot be controlled any longer [12–16]. However, in the literature, these terms are often used interchangeably in almost all experimental or disease states. Moreover, in a clinical situation, it is hard to find whether the "leakage" of the BBB is reversible or irreversible [20]. Thus, both terms are misleading. Further understanding on the mechanisms of BBB permeability will probably determine the real nature of the leakage that can fall in the category of BBB breakdown or opening [11–13]. In this review, both terms are used to identify leakage of tracers across the BBB as opening and breakdown. However, the term "breakdown" is used quite sparingly in this review to denote pathology of the endothelial cells as we believe that "dysfunction", "leakage" or "increased permeability" of the BBB is more appropriate to describe acute disturbances in the BBB function that can be restored or repaired eventually over time or by drugs [11–13, 21].

9.5 BBB in Hypertension

One of the earliest literature describing a comprise of the BBB in clinical condition is hypertension [14, 15, 29]. This subject is mainly developed by the pioneer works or Johansson from Sweden [30–33]. Her results clearly show that hypertension associated with chemical means, or by clipping of renal arteries are associated with focal leakage of protein tracers in the brain [34]. These early findings show that cerebrovascular diseases associated with hypertension, or encephalopathy following long-term hypertensive crisis are

somehow associated with the damage of the BBB [30–34]. Later works from her group clearly show that hypertensive opening of the BBB plays major role in brain dysfunction leading to long-term consequences and brain pathology (see [34]).

Using hypertension as a model, it was seen further that it is the magnitude and degree of abrupt hypertensive insult to the cerebral vessels that will produce a mechanical disruption of the BBB, probably by increasing intramural pressure of the vascular wall [12–15, 30–34]. Under such circumstances, mechanical widening of the tight junctions is possible [14, 15]. These works are further supported from the laboratory of several workers (see [12–15]). It was found that a slow development of hypertension is not associated with the breakdown of the BBB function [11–16, 20–25, 30–34]. This was evident from the fact that cerebral vessels have the capacity to autoregulate and thus could sustain any increase in the intramural pressure if applied within 90–120 sec [14, 15]. However, if an abrupt increase in the transmural pressure occurs within 90 sec, the BBB disruption occurs [14, 30–33]. However, increase in arterial pressure beyond that time limit will allow the cerebral microvessels to resist the hypertension induced BBB leakage [14, 15, 30–33].

9.5.1 Widening of the Tight Junctions Vs. Increased Vesicular Transport

The probable mechanisms of increased BBB permeability under hypertension are still controversial. Previously, it was believed that widening of the tight junctions could be largely responsible for BBB disruption in hypertension [14, 15]. However, further research revealed that apart from a widening of the tight junctions in hypertension there also occurs an increase in vesicular transport [30–33]. Although, the magnitude of tracer transport by vesicular transport across the BBB in hypertension is still controversial [11–13]. This aspect was addressed in details by researchers from Lund, Sweden. Thus, Larsson and his co-workers for the first time administered Vinca alkaloids [4–8, 23, 29, 33, 35, 36], Vincristine as a pretreatment before chemical induced hypertension in rats [35, 36]. Their results demonstrated that vincristine pretreatment, which is mainly an inhibitor of vesicular transport, attenuated the tracer transport across the BBB [36]. Since the BBB opening was not prevented, these authors concluded that vesicular transport plays major role in arterial hypertension induced breakdown of the BBB permeability [35, 36].

Additional studies to demonstrate tracer transport using lanthanum across the cerebral microvessels in hypertension were carried out by Nag et al. [37–42]. The studies of Nag and her co-workers demonstrated that exudation of the electron dense tracer takes place in different regions of the brain across the microvessels following hypertensive insults to the brain [40–42]. Thus, the lanthanum was found within the endothelial cell cytoplasm as well as in the vesicular profiles of large vessels. The lanthanum was also seen within the tight junctions demonstrating that, the ion can pass the junctional barrier probably due to widening of the gaps between the endothelial tight junctions [40–42].

Apart from simple widening of the tight junction, it appears that several other factors are also operating in hypertension induced increased BBB permeability. This is evident with the fact that pretreatment with hydrocortisone attenuated the BBB permeability in hypertension without reducing the intensity of arterial pressure increase [14, 15, 30–34, 37–42]. Thus, pressure induced triggering of secondary injury mechanisms seems to play important role. However, this is a new subject in hypertension and requires further study.

9.6 BBB in Disease Conditions

The BBB permeability is compromised in almost all neurological and psychiatric diseases as mentioned above [4, 11–15, 20–23] (Table 9.2). It may be that leakage of BBB to endogenous serum proteins in neurological diseases could lead to abnormal mental functions precipitating neuropsychiatric diseases [11–15, 20, 21]. This idea is supported by the fact that extravasation of serum proteins can be found in the brain extracellular space and in some cases in the intracellular space in several psychiatric diseases [12, 14, 15, 21, 29]. The CSF protein concentration also increased markedly in these psychiatric diseases, as normal protein concentration in the CSF is almost negligible [14, 21, 43–45]. These observations clearly indicate that the permeability properties of the BBB are different in neurological disease conditions. Recent research show that breakdown of the BBB also occurs in Alzheimer's diseases [21], Parkinson's disease as well as in Huntingdon's Chorea [12, 13–15]. The other psychiatric diseases, which reflect an increased BBB permeability to wide variety of tracers, include dementia, autism and depression [21, 29]. Although, demonstration of increased BBB permeability or disturbed barrier function is known in such neurological or psychiatric diseases several decades ago, the function significance of such findings are still obscure.

Table 9.2 Summary of various experimental and disease conditions in which the BBB is disrupted to various tracers

Disease/conditions	BBB breakdown	Possible mechanisms
A. Neurodegeneration		
Alzheimer's disease, brain tumors, neoplasms, Schizophrenia, Dementia, ischemia, infarction peripheral nerve lesion, leukemia	Serum proteins HRP microperoxidase Lanthanum radiotracers	vesicular transport endothelial cell permeability
B. Trauma		
mechanical, hypoxia, hyperoxia, ischemia,	HRP, radiotracers Evans blue	vesicular transport widening of tight junctions[a]

Table 9.2 (continued)

Disease/conditions	BBB breakdown	Possible mechanisms
metabolic insults,	Trypan blue	endothelial cell permeability
incision[b]	Lanthanum	
stab wounds, concussion,		
cryogenic lesions, thrmocoagulations		
C. Influence of Chemicals		
serotonin[b], histamine	HRP, Evans blue	vesicular transport
protamine, norepinephrine,	Lanthanum,	widening of tight junctions[a]
5-HTP[b], bradykinin,	microperoxidase	endothelial cell permeability
prostaglandins, leukotrienes,	radiotracers	
glutamate, L-NAME, chemical induced		
convulsions, cAMP, dibutyric cAMP,		
adrenaline, 6-OHDA, indomethacin,		
bicuculline, angiotensin, amphetamine,		
matrimonial, pentylenetetrazol		
D. Hyperosmotic solutions		
infusion of various electrolytes,	radiotracers, HRP	widening of tight junctions[a]
non-electrolytes,	Evans blue, Lanthanum	vesicular transport
	microperoxidase	endothelial cell permeability?
E. Irradiations		
X-ray irradiation,	radiotracers,	vesicular transport
a-, b- particle irradiation,	Evans blue,	widening of tight junctions[a]
microwave irradiation,	microperoxidase	endothelial cell permeability
ultrasonic irradiation,		
F. Drugs and venoms		
alcohol and other lipid solvents,	radiotracers,	vesicular transport
bile salts, saponin, lysolecithin,	Evans blue,	widening of tight junctions[a]
Cobra venoms, E. coli endotoxin,	microperoxidase	endothelial cell permeability
G. Toxicity to chemicals		
diodrast, iodopyracet, mercuric chloride,	HRP, Evans blue,	vesicular transport,
nickel chloride, lead, manganese	radiotracers	endothelial cell permeability
H. Lesion/Stimulation		
Locus coeruleus	water	not known

Table 9.2 (continued)

Disease/conditions	BBB breakdown	Possible mechanisms
I. Vascular diseases		
hypertension[a] (mechanical,	radiotracers,	widening of tight junctions[a]
chemical or metabolic)	Evans blue, HRP,	endothelial cell permeability
hypotension, carotid artery occlusion, air embolism, gas embolism, atherosclerosis, periarteritis nodosa, thromboangitis obliterans, diabetic vasculitis	Lanthanum	vesicular transport
J. Loss of autoregulation		
Acute hypertension,	HRP, Evans blue,	widening of tight junctions[a].
hypertensive encephalopathy, intracranial hypertension,	radiotracers	vesicular transport, endothelial cell permeability?
hypovolaemic shock, hypervolaemia		
K. Autoimmune diseases		
Viral encephalitis, experimental allergic	HRP, Evans blue,	widening of tight junctions?
encephalomyelitis, polyneuritis, multiple sclerosis	radiotracers	vesicular transport,
L. Stressful situations		
immobilization[b], forced swimming[b],	HRP, Evans blue	endothelial cell permeability
heat exposure[b], seizures, training in water maze, adrenalectomy,	radiotracers Lanthanum	vesicular transport widening of tight junctions?
electroconvulsive shock, morphine withdrawal/dependence?		
M. Electromagnetic radiation		
Mobile telephony	Evans blue, HRP	vesicular transport
Microwave radiation	Evans blue, HRP, Sucrose	endothelial cell permeability
N. Nanoparticle exposure		
Cu Ag Al SiO_2 TiO_2	Evans blue, Radioiodine endothelial cell membrane permeability	vesicular transport

Compiled from various sources, [a]known to occur, [b]authors own investigations, ? not shown yet
Modified after [11–14, 21, 28, 49, 105–107].

Pathological studies and case reports from such diseases suggest that in wide variety of neurological and psychiatric diseases, marked pathological alteration in the nerve cells, glial cells and axons are found [14, 15, 29]. Probably an increased BBB permeability to several tracers ranging from 9 to 80 Å wide may results in contamination of the CNS structures with peripheral agents [43–45]. These phenomena seem to be one of the major factors in inducing abnormal cell reaction in the CNS leading to brain pathology. It is likely that the day to day stressful situations affecting mental function for long time may also induce BBB disturbances and could be instrumental in precipitating brain pathology leading to neurodegenerative diseases [2, 6–9, 11–15]. In addition, peripheral alterations in neurochemical metabolites or their receptor activation or down regulation could also influence the BBB function [21, 29]. However, further studies regarding role of cellular and molecular stress on the BBB function are needed to clarify these points.

Since, the areas of abnormal brain function in relation to BBB damage are still not well investigated in details, new insight and novel impetus are needed to expand our knowledge in this field of research.

9.7 Neurobiology of Stress

The term "Stress" denotes any external or internal stimuli that could perturb the physiological and/or psychological homeostasis of the organisms [11–13, 24–27, 46–49]. Thus, stress-related disorders include anxiety to post-traumatic experiences [50, 51] all could impair the cognitive functions [52, 53]. However, so, far it is unclear whether alterations in the cognitive functions in stress are related to the stress-induced BBB dysfunction [11–13, 20, 21, 25–27].

Stress could activate or inhibit a select group of neurons or influence the functioning of selective organs, e.g., hippocampus within the brain [5–7, 11–13, 54]. Since stress is able to release several hormones and neurotransmitters that could impair the neuronal activity [4–13, 55, 56] there are reasons to believe that long-term exposure to stress will induce CNS disorders [50].

Thus, prolonged excitation/inhibition of nerve cells leads to brain dysfunction and results in brain pathology and/or neurodegeneration [7–12, 54, 55]. This idea is supported by the fact that abnormal regulation of stress response culminates in chronic systemic diseases including hypertension and several affective disorders, e.g., depression, post-traumatic stress symptoms and Alzheimer's disease [57–61].

Fig. 9.4 (continued) and results in death of the organism. (**c**) Stages of stress response in clinical situations. Mild stress induces short-term alterations in the arousal and emotional response that can affect learning and memory processes. When the magnitude, severity and/or duration of stress increases, several transient and/or permanent changes (synaptic plasticity, changes in neuronal structure and circuitry as well as neurotoxicity) will occur in the CNS. BBB dysfunction can be seen after moderate level of stress overload and may be crucial for short-term or permanent structural changes in the CNS. Modified after [11–14]. For details see [12]

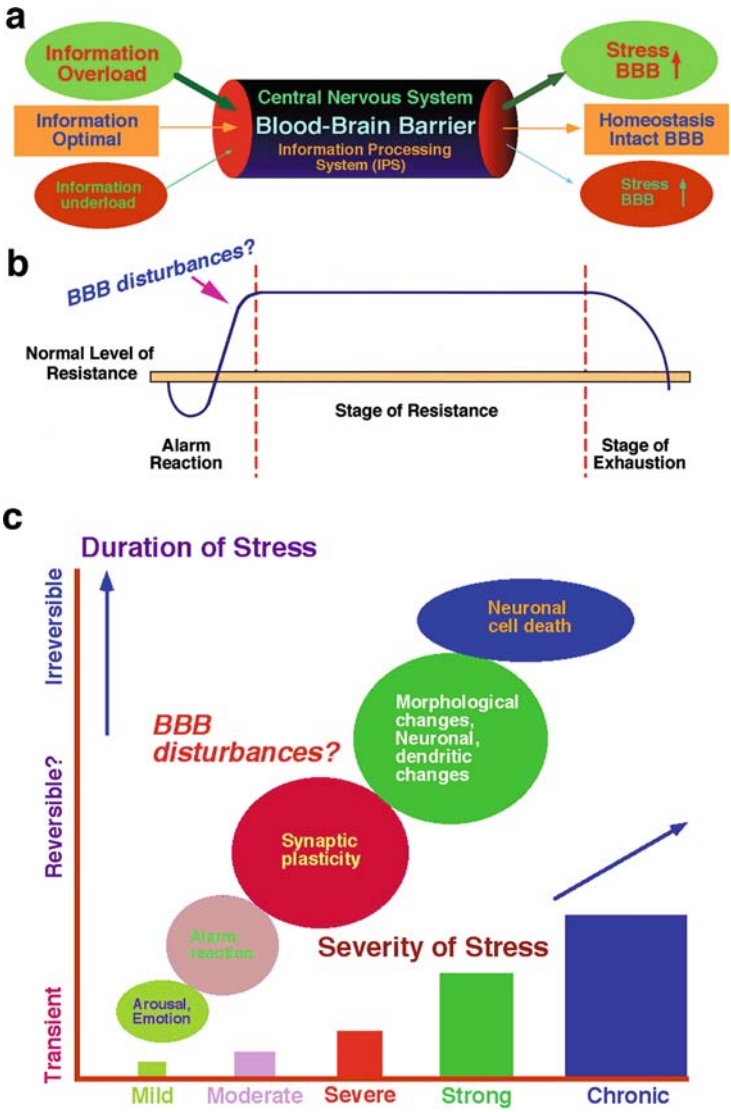

Fig. 9.4 Influence of stress on BBB dysfunction. (**a**) Stress depending on its magnitude and severity can induce CNS dysfunction. The information processing system (IPS) of the CNS can handle certain level of stress without showing symptoms (optimal information). Inadequate handling of stress due to information overload (psychological, environmental, physical exercise or hyperactivity, etc.) may impair IPS and the BBB permeability. Likewise, stressors causing an information underload (hypoactivity, peripheral nerve transection, suturing of one eye-lid, etc.) will also perturb IPS and the BBB function. (**b**) Three stages of stress response. Effects of stress on the organisms can be divided into 3 stages. The initial reaction of an information overload induces alarm reaction showing profound symptoms. When the stress is further continued with same intensity, the symptoms disappear after some time leading to stage of adaptation. Further continuation of stress may finally lead to stage of exhaustion in which the symptoms of alarm reaction may re-appear

9.7.1 Information-Processing System and Brain Dysfunction in Stress

Nerve cells are equipped with a stimulus-response information processing system (IPS) [2–13, 20–27] (Fig. 9.4). Depending on the magnitude and severity of the stress response, the nerve cells may induce short- or long-lasting changes in its phenotype [11–13]. The early stress responses lasting from milliseconds to minutes are normally induced by various neurotransmitters and growth factors (the first messengers) acting on the cell surface receptors to activate the second messenger systems [212, 25–27]. The second messenger systems then by acting through specific protein kinases leads to phosphorylation of specific neuronal proteins resulting in alteration of the structure and function of the nerve cells [62–64].

It appears that disruption of the BBB is one of the most important factors in stress-induced alterations in the IPS leading to subsequent brain dysfunctions [4–13] (Fig. 9.4). This idea is well supported by the fact that an overload on the IPS caused by foot-electroshock, electrical or chemical induced seizures or training in water maze results in BBB leakage in selective parts of the brain within a very short exposure periods [4, 11–13, 14, 15]. Likewise, an underload on the IPS caused by restriction of movement, e.g., restraint or immobilization, peripheral nerve lesion or denucleation of one eye could also results in BBB disruption after considerably long periods of time [4, 11–13, 15, 29]. Similarly, degeneration of noradrenergic neurons (information underload), or stimulation of locus coeruleus (information overload) also results with an increased permeability of water into the brain [12, 13, 21, 27]. Taken together, these observations suggest that stress-induced alterations in the IPS may results in brain dysfunction that is probably mediated through the BBB disruption.

9.7.2 Three Stages of Stress Response in the CNS

The effect of “acute” stress on the organism is often entirely different and sometimes results in opposite symptoms following “chronic” exposure of the same stressor [50]. This led Selye to categorize the whole stress response into three stages (Fig. 9.4) [12, 25, 26, 50]. Some effects like adrenal enlargement, gastrointestinal ulcers and thymicolymphatic involution invariably occur in response to any stressor and classified as “general adaptation syndrome (GAS)” (see [2, 4, 50]).

Thus, the initial stress response that results in immediate reaction within the organisms is characterized as (i) Alarm reaction. When stress is continued further to such an extent that these alarm reactions are disappeared and it seems that organism is not showing any apparent reactions to the continued stress, the stage of (ii) Adaptation ensues. However, continuation of stress further may result in precipitation of alarm reaction type symptoms again after certain period of time and denotes the (iii) Exhaustion stage of the organism precipitated by causing death [46, 50] (Fig. 9.4). The duration and appearance of these stages of stress-responses in the

organism mainly depend on the magnitude and intensity of the primary stimulus and environmental (external) and genetic or metabolic (internal) factors of the organisms [11–15].

Experiments carried out in our laboratory suggest that these three stages of stress-responses are also applicable on stress-induced brain dysfunction [2–13, 20–28]. This is evident from the fact that the intensity and duration of stress closely corresponds to the magnitude and severity of the BBB leakage and brain pathology [2–13, 11–15, 20–28, 65] (Fig. 9.4). Thus, it appears that major brain diseases, e.g., Alzheimer Diseases, depression, psychosis, and other neuropsychiatric illnesses may represent life time stress exposure resulting in sever brain dysfunction over time [46, 50, 56, 65].

9.7.3 "Specific" Vs. "Non-specific" Effects of Stress on the CNS

Although, stress is part of our daily life that includes numerous dissimilar events like fear, frustration, sorrow, joy, fatigue, pain, mental or physical efforts and related events [46, 50] the body responds to all these diverse stressors in almost identical manner [2, 4, 11–13, 25–27]. Thus, the effects of pleasant (*eustress*) or unpleasant (*distress*) stressors on the body function are mainly identical in nature [50]. According to Selye [46, 50] there cannot be different types of stress. Thus, the terms "emotional stress", "heat stress"; "cold stress", "swimming stress", "immobilization stress", "surgical stress", "sleep deprivation stress" and other kind of stimuli denote the stress produced by these stressors [21, 27, 46, 50, 65].

The effect of stress on the organism largely depends on the (i) *non-specific* effects, as well as (ii) the *specific effects* of these stressors [11–13, 25–27, 46, 50, 65]. In addition, the stress-induced effects are finally influenced by several *conditioning factors* such as age, sex, genetic predisposition (endogenous factors) or diet, drugs, environment or hormones (exogenous factors) [13, 27, 46, 50]. Under the influence of these external or internal factors, any stressor or adverse conditions can induce pathogenesis and produce disease of adaptation that affects the specific parts of the body according to sensitivity of the specific stressors or to the above conditioning factors [2–13, 25–27, 50]. Molecular mapping of genes and proteins that are activated by stress in different regions of the CNS are in good agreement with this hypothesis [12]. Our investigation suggests that a selective and specific opening of the BBB under different kinds of stress stimuli could also denotes the specificity of each stressors at the level of CNS response, a feature not described before.

9.7.4 Improper Handling of Stress Leads to "CNS Diseases"

It is widely believed that improper handling of stress by the organisms could lead to CSN diseases [11–13, 25–27, 46, 50]. Thus, any stressor on the organism will

first induce a non-specific response (the first mediator): either a nervous stimuli from the cerebral cortex, reticular formation or limbic system particularly the hippocampus and amygdala; or a chemical substance, the exact nature of which has not established yet [46, 50]. The incoming nervous stimuli act on neuroendocrine cells in the median eminence (transducer) where these signals are transformed into a humoral messenger "corticotropin releasing factor (CRF)" that causes discharge of adrenocorticotropic hormone (ACTH) from the adenohypophysis into general circulation [46, 50]. The ACTH then acts on the adrenal cortex to release glucocorticoids that provide energy to cope with the increased necessary demands of the organism resulted in response to the stressor [4–13, 22–27, 29, 46, 50]. This release of ACTH from the pituitary is controlled by the level of excess ACTH in the blood (ACTH short-loop feed back mechanism), as well as the high level of the corticoid levels (corticoid long-loop feedback mechanism) [4, 11–13, 23–27].

In addition, the stress response is mediated by the catecholamines released from the autonomic nerve endings (noradrenaline) under the influence of acetylcholine, and from the adrenal medulla (mainly adrenaline) [4, 50]. Infusion of noradrenaline and adrenaline in the similar amount released in stress induces a short-term disruption of the BBB function [4, 11, 13, 66]. This indicates that stress-induced release of neurochemicals is capable to influence CNS microenvironment.

Taken together, it is obvious that insufficient, excessive or faulty response of the organism to the stressors either in terms of inappropriate nervous or hormonal responses could leads to "stress diseases" including the CNS disorders [11–13, 22–27, 46, 50].

9.7.5 Stress Pathways in the CNS

Hypothalamus, prefrontal cortex, cingulate cortex, hippocampus, amygdala and the bed nucleus of the stria terminalis are the most sensitive structures that are affected by stress [11–13, 61]. In addition, brain stem could also influences stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and the limbic system through the ascending serotonergic and noradrenergic nerve fibers emanating from the raphé nucleus and locus coeruleus, respectively (Fig. 9.5) [61, 66–69].

Stress caused by several neuropsychiatric diseases, psychostimulants, substance abuse and other neurovascular anomalies results in very selective and specific hippocampal damage resulting in profound memory impairment [23–28]. Stress induced specific cell damages in the hippocampus are seen in the CA 3 and CA4 subfields along with atrophy of dendrites particularly in the CA3 area [11–13, 23–27, 54, 70–74]. Animal experiments show that repeated stress induces reversible synaptogenesis in the CA1 region [75] besides the atrophy of dendrites in the CA3 subfield [76] (for details see [12]). These observations suggest that stress could activate selective and specific pathways in the brain leading to selective brain damage.

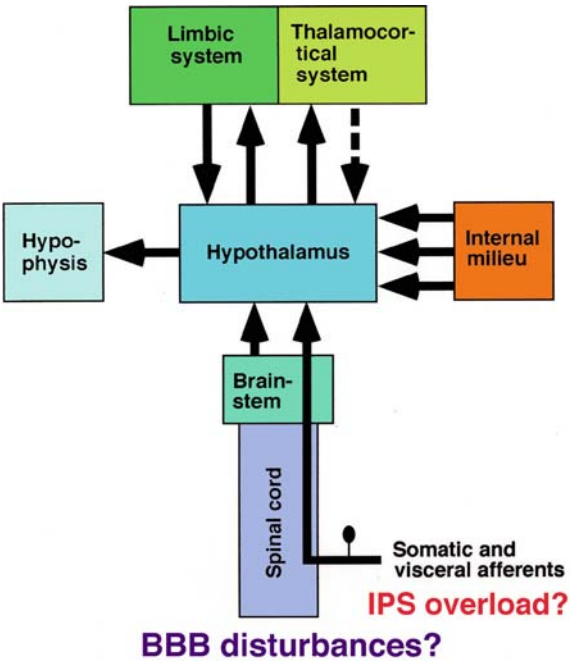

Fig. 9.5 Stress can influence information processing system (IPS) of the CNS. Sensory information is relayed to the hypothalamus through spinal cord. Hypothalamic information is further transmitted to the limbic and thalamocortical systems and hypophysis. Alterations in internal milieu and feedback from the thalamocortical and limbic systems to hypothalamus are the integral part of the CNS to maintain homeostasis. However, an overload or dysfunction of this feedback may lead to alterations in the brain fluid microenvironment and BBB permeability. Modified after [12]

9.7.6 Beneficial Vs. Harmful Effects of Stress

Whether stress is beneficial or harmful to the organisms with special references to the CNS structure and function is still unclear. Available evidences suggest that mild to moderate stress could results in enhancement of adaptive response whereas, excessive stress for sufficient duration leads to brain damage [11–13, 54]. Administration of corticosterone in doses simulating the stress conditions induces loss of nerve cells in the pyramidal cell layer in the CA3 subfield of the rat hippocampus [11, 54]. Glucocorticoid administration normally does not affect nerve cells in the dentate gyrus and in CA1 sector of the hippocampus [77, 78]. On the other hand, stress downregulates the expression or function of neurotrophic factors that may induce slowly developing neurodegeneration.

The neurotrophic factors are intracellular messengers capable to induce gene expression in the target neurons [79]. An increase in the BDNF mRNA in the hippocampus and in cortex is seen following seizures, ischemia and hypoglycemia [80]. This indicates that mild stress could be beneficial for the CNS function [11–13, 81].

On the other hand, 2 h immobilization decreased the BDNF mRNA in the hippocampus of adult rats [15, 82] that is most pronounced in the dentate gyrus followed by CA3 and CA1 hippocampal pyramidal neurons [11–13, 22–26, 82]. A reduction in BDNF expression in hippocampus is also seen 24 h after maternal separation in the rats on the postnatal day 12–20 (see [81]). This stress-induced reduction in the BDNF expression leads to atrophy but is not sufficient to induce

cell death [83]. Thus, stress-induced downregulation of BDNF expression will have profound consequences on neuronal communication and the signal transduction mechanisms in the brain [11–13, 20–28].

Long-term treatment with antidepressants or electroconvulsive seizures prevents the stress-induced decrease in the BDNF expression [84]. Interestingly, an increased expression of BDNF mRNA following stress is also seen in the pituitary and in the hypothalamus [85]. The BDNF is often co-localized with corticotropin-releasing factor (CRF) in the hypothalamus and thyrotropin-releasing hormones (TRH) in the paraventricular nucleus (PVN) neurons [81–84]. An increase in BDNF expression could be seen in the PVN following adrenalectomy or thyroidectomy [11–13]. This suggests that neurotrophic factors could exert trophic effects on the pituitary to regulate the local peptide or hormone secretion into the general circulation [81]. This indicates that stress effects on the brain functions are still unclear and requires further investigations.

9.7.6.1 Stress Releases Neurosteroids: An Antistress Hormone

Although, elevated levels of stress and corticosterone impair memory function in several animal models [11–13, 86–88], highly arousing but sub-threshold levels of stress could enhance memory functions as well [89]. This effect of stress is mediated through a new category of steroids termed, as "neurosteroids" [13, 87]. The neurosteroids are produced endogenously in the periphery as well as in the brain. One type of neurosteroids, dehydroepiandrosterone sulfate (DHEAS) is the most abundant adrenal neurosteroids in humans [90]. The DHEAS antagonizes the functions of corticosterone and thus is often known as an antiglucocorticoid hormone [90]. The DHEAS enhances hippocampal-dependent learning in rats [91] and enhance electrophysiological and cognitive measures of hippocampal function [92]. The complex effects of stress on brain function or behavioural alterations are mainly due to a competitive interaction between corticosterone and DHEAS [87, 88, 91, 92]. These observations suggest that endogenous neurosteroids could act as an antistress hormone and may possibly protect brain dysfunction in stress up to certain extent.

9.7.6.2 Stress Enhances Virus Penetration into the Brain

A variety of stressors exacerbate the effects of several viruses, e.g., herpes simplex [93], influenza [94, 95]; and encephalitic viruses [96, 97] leading to significantly higher increase in the morbidity and mortality [98–102].

Thus, inoculation with attenuated variant of West-Nile virus (WN-25) or neuroadapted noninvasive Sindbis strain (SVN) to mice subjected to stressors like cold, isolation or administration of corticosterone enhanced their mortality by 50–80% as compared to the non-stressed animals [9]). This effect was most pronounced during isolation stress (mortality 80%) followed by cold (about 60%) and corticosterone administration (50%). Moreover, these stressors resulted in fatal encephalitis when avirulent strain of Semliki Forest virus (SFV-A7) was administered as compared to no death seen in normal mice. Laboratory investigations showed that the brain titters of viruses in the stressed mice are 3- to 4-fold higher in comparison to the normal

unstressed group [11–13, 96, 97]. This suggests that stress is able to enhance brain penetration of viruses causing lethality.

It is believed that stress-induced immunosupression could enhance the proliferation of the viruses into the CNS leading to exacerbation of the infection and pathogenesis [11–13, 20–27, 96, 97]. However, an increased permeability of the BBB following stress could be another factor in exacerbation of viruses-induced mortality.

9.8 BBB in Stressful Situation

Although, stress is known to influence brain function, alterations in the BBB permeability following stressful situation is still not well characterized. About 41 years ago, Angel [103] demonstrated an increased uptake of ^{14}C-cocaine in the brain following starvation, training in the water maze or adrenalectomy. This was the first report suggesting that mental abnormalities will result following long-term stressful situation probably due to an increase in BBB permeability. However, the molecular mechanisms of BBB opening following stressful situations were not examined in this investigation.

Since adrenalectomy will induce disturbances in endocrine system, it was speculated that neuroendocrine imbalance seems to be the endogenous factor responsible for BBB dysfunction.

Taking clue from these observations, our laboratory has initiated a series of experiments to study the effects of different stressors on the BBB function [2–9]. Thus, we developed different models of stressors that are known to represent emotional disturbances akin to depression and helplessness [2–13, 20–28, 104]. In addition, we also evaluated effects of unpleasant environmental situations, e.g., heat stress [2–13] and/or effects of microfine particles in the ambient environments, i.e., nanoparticles exposure [13, 27, 105, 106] on the BBB function in relation to brain pathology [107–110]. Results from our laboratory revealed that stress of immobilization, swimming, environmental heat stress or exposure to nanoparticles resulted in an increase in the permeability of the BBB to several protein tracers and induced brain pathology [2–13, 20–27, 107, 111]. Our observations further show that these stressors also alter cognitive and sensory motor functions at the time of the BBB disruption [13, 105–111]. A brief summary of our investigations in the past together with recent observations on stress and brain pathology and their pharmacological manipulations for neuroprotection is summarized below.

9.9 Our Observations on BBB Dysfunction and Brain Pathology in Stress

Studies carried out in our laboratory since last 30 years revealed that several stressful situations are able to disrupt the BBB function [3–13, 112], a feature that was confirmed now by several independent studies [2–13, 20–27, 104–112].

This BBB dysfunction in stress is dependent on the magnitude and intensity of stressors and is primarily responsible for brain pathology. Thus, breakdown of the BBB in stress may be regarded as "a gateway" to neurological diseases and neurodegeneration. This aspect is critically evaluated below based on our own investigations.

9.9.1 Animal Models of Depression and the BBB Dysfunction

Animal models of depression are induced by hypoactivity that is also used as models for post-traumatic disorders [113–115]. Since immobilization and swim stressors are well known animal models for immobility [116, 117], we employed these animal models of stress to understand the state of BBB function of depression and post-traumatic stress disorders [118, 119].

9.9.1.1 BBB in Immobilization Stress

Different animal models of immobilization stress show disruption of the BBB function to various tracers at different time points [4, 6, 7, 112, 120–123]. Thus, mild changes in the BBB function in few brain regions were observed after 5 to 15 min of immobilization [124, 125]. Whereas, 2 and 6 h immobilization results in an increase tracer transport into several brain regions [120, 121]. Our observations show that long-term immobilization stress (7–9 h) is needed to induce leakage of protein tracers across the BBB in many brain regions [4, 6, 7]. These observations suggest that the magnitude and intensity of the BBB breakdown depend on the severity of immobilization stress.

Visual inspection of Evans blue dye in the brain of immobilized rats revealed Evans blue dye extravasation in the cingulate, occipital, parietal and frontal cortex. The cerebellum was also stained (Fig. 9.6). The choroid plexus and other non-barrier regions took deep blue stain. A mild to moderate blue staining is seen in the walls of lateral and in the 4th cerebral ventricles indicating disruption of the blood-CSF barrier by immobilization. The dorsal surface of the hippocampus and caudate nucleus stained mildly. The massa intermedia, hypothalamus and regions surrounding third ventricle showed mild to moderate blue staining (Fig. 9.6). Coronal section of the brain passing through basal ganglia, hippocampus, brain stem and cerebellum showed mild to moderate staining of the deeper tissues across the dorsal, lateral and ventral surfaces of the brain (Figs. 9.6 and 9.8). These observations suggest that immobilization induces selective disruption of the BBB.

Quantitative studies using measurement of Evans blue leakage and radioiodine ([131] Iodine) extravasation showed profound BBB disruption (EBA, +670%; radioiodine tracers +1061%) in the whole brain [2, 4–6]. Whereas, only a slight increase in BBB permeability to Evans blue (135%) and radioiodine (100%) is seen at 4 h immobilization [6, 7]. The permeability changes at 1 h stress are negligible (Fig. 9.7). Since every brain regions are different with regard to stress pathways, we

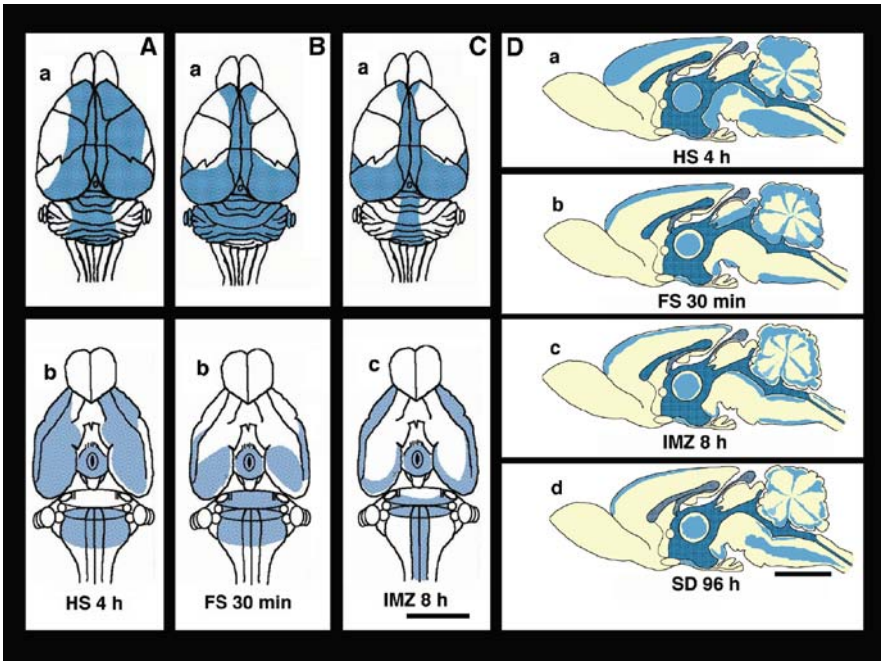

Fig. 9.6 Stress induced leakage of Evans blue albumin indicates disruption of the BBB. Diagrammatic representation of Evans blue albumin (EBA) extravasation in the brain following 4 h heat stress (HS, **A**), 30 min forced swimming (FS, **B**) and 8 h immobilization (IMZ, **C**) stress. The mapping of EBA extravasation in the brain is based on 6 to 8 individual experiments in each category. Heat stress appears to induce most extensive EBA extravasation in the dorsal (**A.a**) and ventral (**A.b**) surfaces of the brain. In forced swimming (FS) the most prominent extravasation of EBA is seen in the cerebellum (FS, **B.a**). Immobilization stress (IMZ) induces most frequent EBA staining in the anterior and posterior cingulate cortices (**C.a**). EBA staining on the ventral surfaces of the brain also varied according to the stressors used. However, the piriform cortex and pons regions always exhibited EBA staining in stress. (**D**) Diagrammatic representation of mid-sagittal section of the rat brain showing extravasation of Evans blue albumin (EBA) following heat stress (HS, **a**), forced swimming (FS, **b**), immobilization (IMZ, **c**) and sleep deprivation (SD, **d**). Differences in the pattern of EBA extravasation are prominent in the cingulate cortex, cerebellum, thalamus and in the brain stem. Staining of cerebroventricular walls of the lateral ventricles, 4th ventricle and median eminence is a frequent finding in all stress experiments. Bar = 5 mm. Modified after [12, 13]

examined the regional BBB dysfunction in 12 brain areas after 8 h immobilization stress using radioiodine tracer [4, 6, 7, 12]. The most marked increase in radioiodine was seen in the occipital cortex (1018%) followed by parietal cortex (622%), hippocampus (563%), inferior colliculi (268%), cerebellum (255%), superior colliculus (225%), hypothalamus (102%), thalamus (94%), caudate nucleus (85%), cingulate cortex (61%), temporal cortex (55.5%) and frontal cortex (25%). The pons and medulla did not show extravasation of radioiodine [3, 6]. The regional extravasation of radiotracer at 4 h immobilization is not significant in any region. At 1 h

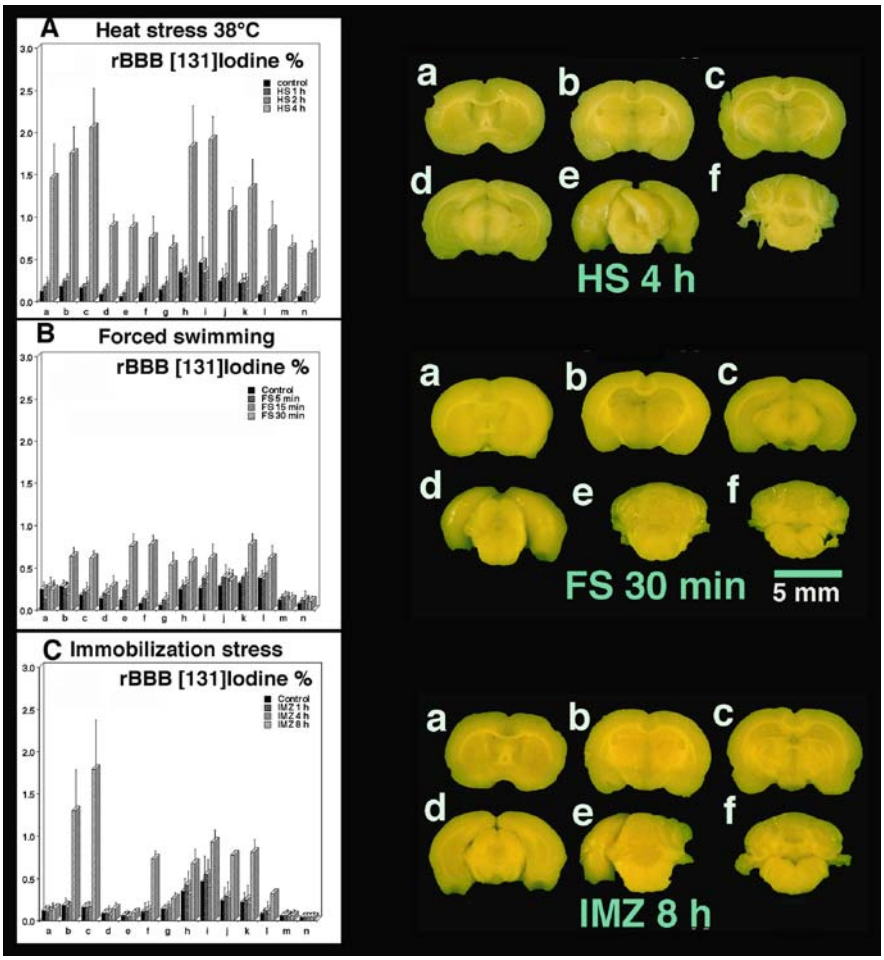

Fig. 9.7 Regional BBB permeability changes in Stress. *Left Panel*: Changes in regional blood-brain barrier (rBBB) permeability following heat stress (**A**), forced swimming (**B**) and immobilization stress (**C**). The rBBB permeability showed significant increase to radiotracer in the all 14 brain regions after 4 h heat stress (**A**), only in 9 brain regions (**b, c, e–i, k, l**) following 30 min forced swimming (**B**), and in 12 brain regions (**a–j**) at 8 h immobilization stress (**C**). Brain regions (**A, C**) : a = frontal cortex, b = parietal cortex, c = occipital cortex, d = temporal cortex, e = cingulate cortex, f = hippocampus, g = caudate nucleus, h = thalamus, i = hypothalamus, j = sup. colliculus, k = inf. colliculus, l = cerebellum, m = pons, n = medulla. Brain regions (**B**): a = frontal cortex, b = parietal cortex, c = occipital cortex, d = ant. cingulate cortex, e = post. cingulate cortex, f = cerebellum vermis, g = cerebellar cortex, h = caudate nucleus, i = hippocampus, j = colliculi, k = thalamus, l = hypothalamus, m = medulla, n = brain stem. Values are mean±SD of 10 to 12 rats. Data modified after [12, 13]. *Right Panel*: Representative examples of coronal sections of rat brain from 6 different levels (**a–f**) showing Evans blue albumin (EBA) extravasation following immobilization heat stress (HS, **A**), forced swimming (FS, **B**) and immobilization stress (IMZ, **C**). The pattern and intensity of EBA showed selective variations in different levels according to the stressors used. Most extensive staining of various brain regions are observed after heat stress (**B**) followed by forced swimming (**C**) and immobilization (**A**). Staining of cingulate (**a–c**),

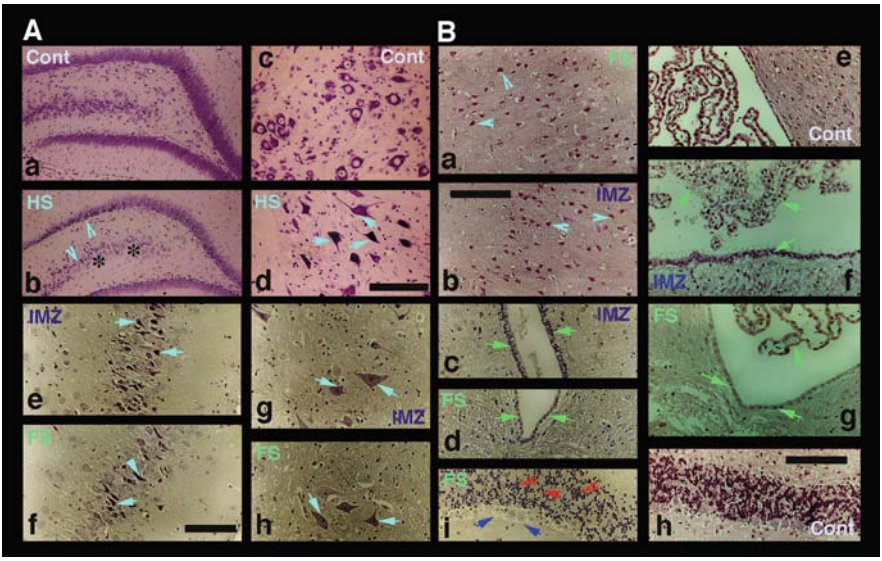

Fig. 9.8 Structural changes in the brain at the time of BBB disruption in stress. Representative examples of light microscopic changes in different brain regions following 4 h heat stress (HS), 8 h immobilization (IMZ) or 30 min forced swimming (FS). Marked degeneration (arrow heads) in dentate gyrus and CA-4 region (*) of the hippocampus is seen following HS (**A.b**) compared to control (**A.a**). Many damaged and distorted nerve cells in the brain stem regions following HS is apparent (**A.d**, *arrows*) compared to control (**A.c**). Mild to moderate cell damage in the hippocampus CA-4 region is seen following IMZ (**A.e**, *arrows*) or FS (**A.f**, *arrow heads*). IMZ (**B.a**) and FS (**B.b**) induces specific and selective cell damage (*arrow heads*) in the cerebral cortex. Cell damage in ependymal cells (*arrows*) in the median eminence following IMZ (**B.c**) and FS (**B.d**) is clearly evident. Degeneration (*arrows*) of choroid plexus epithelial cells and ependymal cells in the lateral cerebral ventricle by IMZ (**B.f**) and FS (**B.g**) is apparent compared to control (**B.e**). In the cerebellar Purkinje cells and granule cells show marked degenerative changes following FS (**B.i**, *arrows*) compared to control (**B.h**). A. a-d Nissl stain; others H & E stain on 3 μm thick paraffin sections. Bars: A. a–b 50 μm; c–d, g–h 25 μm, e–f 30 μm; B: a–i 40 μm. Reproduced with permission after [12]

immobilization stress, a mild extravasation of radiotracer (14–33%) is seen in the mid-brain regions including cerebellum [12, 25, 26]. These observations suggest that immobilization stress increases the permeability of the BBB in selective and specific brain areas.

Fig. 9.7 (continued) frontal (**a**), parietal (**b, c**), temporal (**c**), occipital (**d**), and piriform (**b–c**) cortices are clearly evident (**A.a–c; B.a–d; C.a–c**). Different pattern and intensity of EBA staining in immobilization (**C**), heat stress (**A**) and forced swimming (**A**) are apparent in deep brain structures, e.g., caudate putamen (**a**), hippocampus (**b–c**), thalamus (**b–d**), hypothalamus (**b–d**), amygdala (**b–c**), brain stem (**d–e**) and cerebellum (**f**). (Co-ordinates for coronal sections: a = + 0.10 to +0.45; b –3.25 to –3.90; c = –4.20 to –4.60; d = .5.25 to –6.65; e = –7.10–7.60; f = –10. 60 to –11.90 from Bregma). Bar = 5 mm. Modified after [12]

9.9.1.2 Immobilization Stress and Structural Changes in the Brain

Profound neuronal damages are seen in the brains of immobilized rats at the time of the BBB leakage. Most of the nerve cell damage is seen in the areas showing leakage of Evans blue or radioiodine extravasation [12]. Thus, specific nerve cell damage following 8 h immobilization is seen in the cerebral cortex, hippocampus, cerebellum and in brain stem. Many dark and distorted nerve cells are present in the superficial layers of the cerebral cortex especially in the cingulate, parietal, temporal and in occipital cortex (Fig. 9.8). The hippocampal dentate gyrus, CA4 and CA3 sectors as well as CA1 subfield contain several damaged nerve cells (Fig. 9.8). Dark neurons are frequent in the brain stem reticular formation. Degenerative changes in cerebellar Purkinje cells and granule cells are also quite common (Fig. 9.8).

The choroid plexuses and the ependymal cells around the lateral and third ventricles show degenerative changes. In these regions, sponginess and edema is frequent (Fig. 9.8). These observations indicate disruption of the blood-CSF barrier in immobilization stress [4, 6, 7, 12, 43].

At the ultratstructural level, areas showing BBB disruption reveals membrane damage, vacuolation and distortion of nerve cells (Fig. 9.13). This indicates that stress induced BBB disruption is associated with structural changes in the neuropil.

9.9.1.3 BBB in Forced Swimming

Forced swimming is frequently used as an animal model of depression. When rats are forced to swim in a restricted pool, they quickly acquire an immobility response [127]. The hypoactivity caused by swim stress is associated with selective neurochemical metabolism in the brain. Our laboratory was the first to show that rats subjected to forced swimming exhibit selective disruption of the BBB [104, 126, 128] that is reversible in nature [12, 104].

Thus, extravasation of Evans blue albumin was noted in 5 brain regions following 30 min forced swimming. These regions are cingulate cortex, parietal cortex, occipital cortex, cerebellum and the dorsal surface of the hippocampus the brain is evident (Figs. 9.6 and 9.7). In most cases, the cerebellar vermis took moderate blue staining compared to the lateral cerebellar cortex (Fig. 9.7). The deep cerebellar nuclei were mainly unstained (Figs. 9.6 and 9.7). The walls of lateral ventricle took mild stain, whereas the 4th ventricle exhibited deep blue staining (Fig. 9.6). The areas around the third ventricle were also stained mildly indicating a disruption of the blood-CSF barrier by forced swimming (Fig. 9.7). On the other hand, extravasation of radioiodine after 30 min swimming was observed in 8 brain regions. Thus, besides 5 blue stained regions, the radiotracer is present in another 3 brain areas viz., caudate nucleus, thalamus and hypothalamus (Figs. 9.6 and 9.7) [12, 104].

Subjection of animals to short duration of swimming, e.g., 5 or 15 min did not show extravasation of protein tracers in the brain (Fig. 9.7) [12, 104]. This BBB disruption following forced swimming in young rats is reversible in nature. Thus, the BBB permeability is no longer observed in rats subjected to 2 h rest after 30 min swimming (Fig. 9.7) [12, 104, 128].

9.9.1.4 Swimming Induced Structural Changes in the Brain

Structural changes in the brain were seen in the rats after 30 min swimming in the areas exhibiting BBB disruption [12]. Thus, light microscopy revealed specific changes in the neuropil, and damage of selective nerve cells in the cerebral cortex, hippocampus and in cerebellum in rats subjected to 30 min forced swimming (Fig. 9.8). This selective nerve cell damage is seen largely in the layer III to V in the cerebral cortex that is most pronounced in the cingulate, occipital and piriform cortices (Fig. 9.8). This indicates that BBB disruption contributes to cellular injury.

Degenerative changes in few nerve cells are visible in the brain stem and granule cells of the cerebellum after 30 min swimming (Fig. 9.8). The presence of damaged nerve cells is common in the dentate gyrus, CA 3 and CA 4 sectors of hippocampus.

Mild to moderate degree of ependymal cell damage around the lateral and third ventricle and degenerative changes in the choroid plexuses are also seen at this time (Fig. 9.8) confirming the BCSFB disruption by forced swimming.

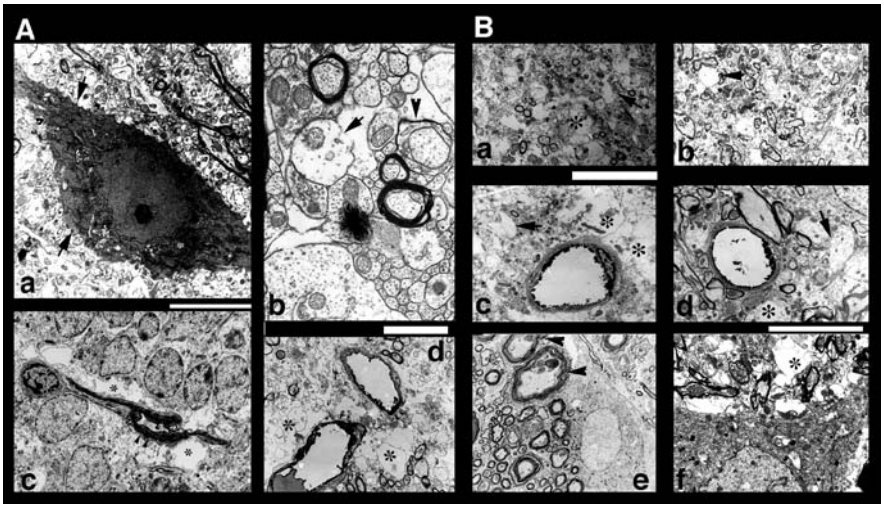

Fig. 9.9 Ultratstructural changes in the brain at the time of BBB opening in stress. Representative examples of ultrastructural changes in the rats brain following 4 h heat stress (HS), 30 min forced swimming (FS) or 8 h immobilization (IMZ) stress. In the cerebral cortex, one nerve cell with dark and electron dense cytoplasm (arrows) is seen following HS (**A.a**) Many degenerative changes can be seen in the neuropil (**A.a**). High power electron micrograph from the parietal cerebral cortex cellular layer III showing damaged synapses (*arrows*), vesiculation of myelin and membrane damage following HS (**A.b**). A completely collapsed microvessel (*arrow heads*) and distorted granule cells in the cerebellum are seen following HS (**A–c**). Perivascular edema (*), membrane damage and leakage of lanthanum across the microvessels (**A–d**) are quite frequent in HS. Vacuolation, membrane damage (*arrows*) and perivascular edema (*) are frequent following FS (**B.a,c**) or IMZ (**B.b,d**). Myelin vesiculation (**B.e**) and nerve cell damage (**B.f**) is very common in hippocampus and in thalamus following HS. Bars: A.a = 0.6 μm; A.c,d = 1 μm; A.b = 0.4 μm nm; B.a–e = 1 μm; B.f = 0. 5 μm. Reproduced with permission after [12]

At the ultrastructural level, vacuolation and damage to neuropil is present in the cortex and in brain stem in rats after forced swimming (Fig. 9.9). These observations are in line of the idea that BBB disruption in swim stress is associated with selective nerve cell damage. Whether these cell changes in such a short duration of 30 min reflect acute nerve cell death or permanent neurodegenerative changes are unclear. To further confirm this point, morphological investigations in rats following several days or weeks after 30 min swimming are needed [2, 12, 13].

9.9.2 Learned Helplessness and the BBB Dysfunction

Learned helplessness induced by sleep deprivation is an experimental model of depression and/or anxiety [129–132]. Sleep structure is a sensitive marker of human affective disorder in depression [133, 134] as well as in anxiety [135]. Thus, sleep deprivation for certain periods could mimic learned helplessness and induce signs of depression (see [12, 13, 136]).

9.9.2.1 Sleep Deprivation and the BBB Disruption

Sleep deprivation for 4 days induces profound cellular and molecular changes in the brain reticular activating system [136, 137] along with expression of c-fos and Fos proteins. In addition, alterations in GABergic and serotonergic neurons could also be seen in the brain stem reticular formation in these sleep-deprived rats [136]. However, alterations in the BBB function following sleep deprivation are still not investigated in details [12, 13].

We examined BBB function in rats following 1–4 days of sleep deprivation using an inverted flower pot model [136–138] that induces a selective deprivation of paradoxical sleep (PS) [138]. Each rat is placed on an inverted flower pot (6.5 cm in diameter) surrounded by water filled in a Plexiglas box up to 1 cm of the surface with free access to food and water [136–138]. The water temperature is maintained at 30±1°C [104, 128].

The animals in this model can undergo to slow wave sleep (SWS) but not the PS [136]. Thus, the loss of muscle tonus with the onset of PS causes animals to fall into the water and awaken them [136–138]. The PS is significantly attenuated 48 h after the sleep deprivation in this model. For BBB experiments, the animals were kept maximum for 96 h under these conditions [12, 13, 136].

Our observations show that sleep deprivation of 48 h induces mild blue staining of the frontal, temporal and cingulate cortices [12, 13]. The cerebellar cortex took faint staining. This increase in Evans blue extravasation was further intensified at 96 h after sleep deprivation. Thus, moderate Evans blue staining in the cingulate, frontal, parietal and temporal cortices is observed (Sharma HS unpublished observations). The walls of lateral cerebral ventricles, dorsal surface of the hippocampus and massa intermedia showed mild blue staining (Fig. 9.5). Some areas in the brain stem reticular system took mild to moderate staining (Fig. 9.5).

Extravasation of HRP and endogenous albumin immunohistochemistry showed a good relationship with the exogenous Evans blue extravasation in the brain (results not shown). Thus, the albumin immunoreactivity was mainly seen around the microvessels in the cerebral cortex, hippocampus, brain stem and thalamus. In 96 h sleep-deprived rats, the albumin immunoreactivity was also seen around few nerve cells in the cortex, hippocampus, brainstem, cerebellum and thalamus (Sharma HS unpublished observation). These observations are the first to show that the sleep deprivation stress depending on its duration is able to induce BBB disruption in specific regions.

9.9.2.2 Structural Changes in the Brain Following Sleep Deprivation

Light and electron microscopy showed profound neuronal, glial and endothelial cell changes in the areas associated with Evans blue leakage in rats following 48 h and 96 h after sleep deprivation (results not shown). Several neurons in the hippocampus CA-3 regions, dentate gyrus and subiculum were dark in appearance and perineuronal edema was most prominent (Sharma HS unpublished observations). Sporadic changes in cerebellar granule cells and purkinje cells were also seen in the vermis region. Most profound alterations in neuronal structures were seen in the brainstem reticular formation. These observations suggest that BBB disruption in sleep deprivation is associated with marked neuronal damages.

9.9.3 Environmental Heat as Stressor and the BBB Dysfunction

Around 60% of the World populations live in the temperate climate where high environmental temperatures during summer seasons could induce serious health problems. Thus, about 10,000–12,000 deaths are recorded during heat waves when the ambient air temperature reaches between 32 and 34°C [139–142]. High incidence of heat-related mortality occurs in cities with high levels of urbanization [12, 27, 105, 110, 142–144]. However, the exact causes of heat deaths are still not well known.

The first scientific report on heat-related death was published in 1743 describing death of 11,000 persons in China during a hot weather in July. Another incidence of heat death was seen in Liverpool in 1841, in which 33 British soldiers died in one hot day in a ship while coming from Muscat to Bushier [105, 110, 143–145]. Similarly, during 1873 in the "Black Hole of Calcutta", 123 out of 186 British prisoners collapsed in one night [21, 27, 49, 105, 145]. More than 700 persons died in 1995 due to hot weather conditions in Chicago during summer [21, 27, 49, 146]. About 1000 deaths in 1996 occurred in nursing homes of Rotterdam, the Netherlands that were related to hot weather conditions [147, 148]. These reports suggest that high environmental heat is a serious life-threatening event probably due to heat-induced severe brain damage [21, 27, 28, 49, 105, 106].

Rise in core body temperatures above 40°C is associated with heat-stroke [105, 106, 149]. More than 50% of heat stroke victims die within short period despite

lowering of the body temperature and/or therapeutic interventions. Those who survive show permanent neurological deficits [21, 27, 28, 49, 105, 106, 150]. This indicates that the brain is a highly vulnerable organ in heat-related illnesses [4, 9, 11–13, 21, 28, 105, 150].

Our laboratory was the first to show that experimental or environmental heat stress induces BBB disruption to protein tracers [2–13, 22–27, 150]. This suggests that environmental heat exposure during summer months could lead to brain damage due to BBB dysfunction [12, 13, 150].

9.9.3.1 Heat Stress Induced BBB Dysfunction

Using an animal model of heat stress we exposed rats at 38° C in an environmental chamber (wind velocity 2.6 cm/sec; Relative humidity 45–47%) for 1–4 h and examined the BBB function using Evans blue and radioiodine [2–13]. Marked increase in the BBB to Evans blue albumin and radioiodine tracer are apparent in animals after 4 h heat exposure (Figs. 9.6 and 9.7). The blue staining is seen in 8 brain regions, viz., cingulate cortex, occipital cortex, parietal cortex, cerebellum, temporal cortex, frontal cortex, hypothalamus and thalamus (Fig. 9.7). Mild to moderate staining of the ventricular walls were observed. The fourth ventricle showed deep blue staining and the structures around the third ventricles were moderately stained (Fig. 9.7). Occasionally the dorsal surface of the hippocampus also took mild stain (Figs. 9.6 and 9.8). indicating disruption of the blood-CSF barrier function as well [43].

On the other hand, extravasation of radioiodine occurred in 14 brain regions examined. Thus, besides the 8 blue stained regions, another 6 region viz., hippocampus, caudate nucleus, superior and inferior colliculi, pons and medulla also showed an increase in radioactivity (Fig. 9.6) [2, 6, 12, 13].

Subjection of rats to shorter periods of heat stress, i.e., 1 or 2 h did not induce BBB disruption [6]. Furthermore, animals subjected to 2 h rest at room temperature after 4 h heat exposure still show a mild leakage of Evans blue and radiotracers extravasation in brain (Fig. 9.7). These animals are still lethargic, however their body temperature returned to normal level (Sharma HS unpublished observations). This suggests that the BBB changes in heat stress are long lasting and can be seen even when the body temperature returned to normal [12, 13, 150].

9.9.3.2 Structural Changes in the Brain Following Heat Stress

Disruption of BBB in heat stress is associated with brain damage [12–14, 24, 151]. Thus, profound nerve cell changes are seen in those brain areas exhibiting leakage of Evans blue and radioiodine after 4 h heat exposure. Neuronal cell injury, edematous expansion and sponginess of the neuropil are common in several brain areas e.g., such as cerebral cortex, brain stem, cerebellum, thalamus and hypothalamus (Figs. 9.8 and 9.9). A selective nerve cell damage in the hippocampus is most pronounced within the CA-4 subfield compared to other regions (Fig. 9.8), although edematous swelling and general sponginess are present throughout this region. This indicates that BBB leakage is associated with brain damage in heat stress.

Damage to ependymal cells around the lateral and third ventricle is quite prominent in heat stress (Fig. 9.12). The choroid plexus from the lateral ventricle, third ventricle and fourth ventricles exhibited degenerative changes (Fig. 9.8) indicating heat stress induced disruption of the blood-CSF barrier [43, 44, 105, 106].

At the ultrastructural level, damaged nerve cells, degenerated nuclei accompanied with eccentric nucleolus are frequent in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus, and brain stem (Fig. 9.9). The nerve cells are dark in appearance and contain vacuolated cytoplasm. The nuclear membrane contains many irregular foldings and the nucleolus often showed signs of degeneration (Fig. 9.9). Interestingly, damage of the one nerve cell is often seen in a region where the adjacent neuron is almost normal in appearance suggesting a selective vulnerability of nerve cells in heat exposure [2–14, 22–28, 49, 105, 106, 150, 151].

Swollen synapses with damage to both pre-and post-synaptic membranes are common in thalamus, brain stem, hypothalamus, cerebellum, hippocampus and in cerebral cortex (Fig. 9.9). In some of these regions damage to post-synaptic dendrites and disruption of synaptic membrane is also common.

Widespread axonal damage, demyelination and vesiculation are most pronounced in the brain stem reticular formation, pons, medulla and the spinal cord (Fig. 9.9). Many unmyelinated axons are also swollen. These observations are in line with the idea that breakdown of the BBB is an important factor in heat induced brain damage.

9.9.3.3 Ultratstructural Changes in the Cerebral Endothelium

Disruption of the BBB at ultrastructural level is clearly seen in heat stress. Using lanthanum as electron dense tracer, we observed several microvessels that exhibit leakage of lanthanum across the cerebral endothelium in a very selective manner (Fig. 9.10). The leakage of lanthanum is often evident in one endothelial cell, whereas the rest of the vessel or the adjacent endothelial cells are completely normal (see Fig. 9.10). This indicates a highly selective nature of the endothelial cell membrane permeability in heat stress [11–13]. Activation of specific endothelial cell transporters, permeability factors, neurochemical receptors or ions channels located on the selected area of the endothelial cell membrane many be responsible for such a selective increase in the lanthanum permeability [13].

In many other vascular profiles, lanthanum is stopped at the luminal side of the tight junctions (Fig. 9.10). Whereas, several microvessels showed infiltration of lanthanum across the endothelial cells membranes including the tight junctions without widening them (Fig. 9.10). These observations support the idea of specific receptor mediated increase in microvascular permeability [12, 13, 23, 24, 105]. Since receptors can also be present on the membranes apposing tight junctions, increased microvascular permeability around the junctions is possible via activation of such receptors [12, 13, 150].

Taken together our morphological studies clearly points out an intimate role of BBB breakdown in brain pathology following heat stress.

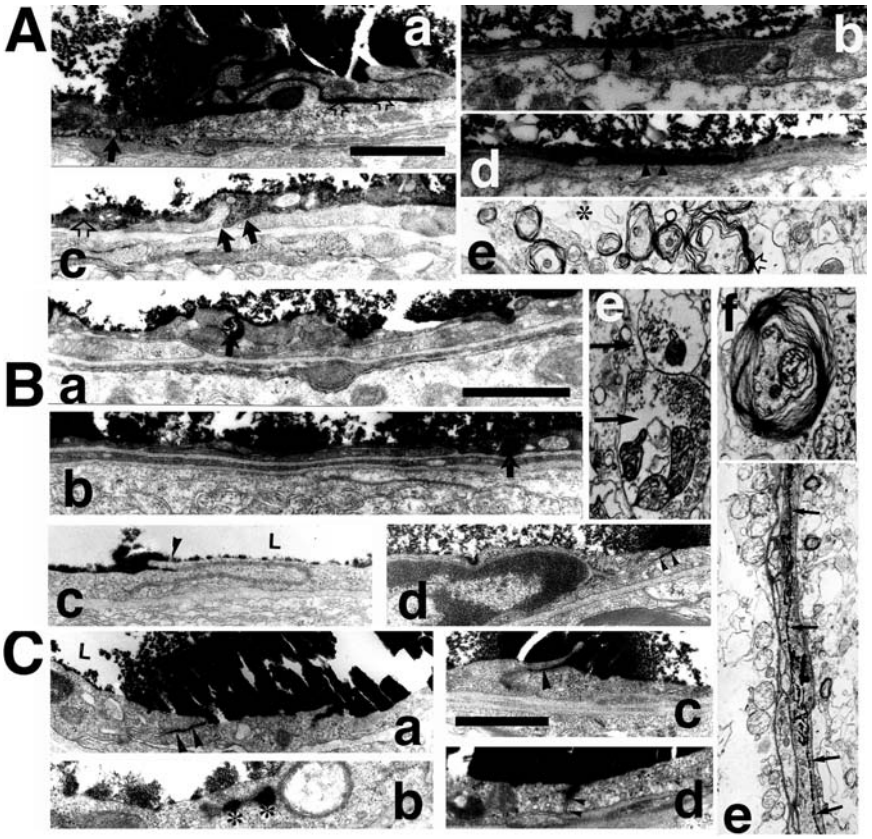

Fig. 9.10 Ultrastructural changes in the cerebral endothelial cells and the adjacent neuropil at the time of the BBB disruption in stress. Ultratstructural studies on the cerebral endothelial cells from various brain regions in heat stress showing lanthanum extravasation and damage to adjacent neuropil. (**A**) Lanthanum, an electron dense tracer (seen as *dark black particles*) is seen across the endothelial cell membrane containing tight junctions (**A.a** *blank arrows*). Infiltration of lanthanum across the endothelial cell membrane is clearly seen (*solid arrows*, **A.b,c**). In some cases lanthanum is seen diffusely infiltrated within the cell membranes of tight junction complex and endothelial cell cytoplasm covering the apposed plasma membranes connected with the tight junctions (**A.a**). However in these cases the tight junctions are not found opened because lanthanum within the intercellular cleft is stopped at the tight junction (*blank arrows*, **A.a**). In some cases only one endothelial cell membrane covering tight junction is found diffusely infiltrated with lanthanum (*filled and blank arrows*) leaving its counterpart completely intact (**A.c**) One endothelial cell showed infiltration of lanthanum in a certain segment of the cerebral endothelium (**A.d**, *arrow heads*). Damage to neuropil (*) and myelin vesiculation are prominent (**A.e**) in the adjacent area. (**B**) Lanthanum is present in endothelial cell and in the basement membrane (**B.a, b**) without widening of the tight junction (**B.a**). In several microvascular profiles, lanthanum is stopped at the tight junctions (**B.c,d**, *arrow heads*). Damage to synaptic membrane (*arrows*, **B.e**), vacuolation, edema and myelin vesiculation (**B.f**) is frequent around microvessels showing BBB disruption to lanthanum (**B.e, f**). (**C**) Many cerebral endothelium show presence of lanthanum in the microvesicular (*) profiles within the cell cytoplasm (**C.b, d**). Normally, the tight junction in these microvessels appears to be closed (**C.a, c, d**). Complete collapse of microvessels with perivascular edema and damage to neuropil (**C.e**) is common in many brain regions during heat stress. Bars: A = 0.3 μm; B = 0.2 μm; C = 0.2 μm. Reproduced with permission after [12]

9.9.3.4 Cognitive and Sensory-Motor Dysfunction in Heat Stress

Whether BBB disturbances in heat stress contribute to cognitive and sensory motor dysfunction is still a matter of speculation [12–14, 23, 24, 28, 105, 106, 143, 144, 150]. Available evidences show that alterations in fluid microenvironment of the brain in specific regions, e.g., hippocampus, cerebellum, amygdala, visual, sensory-motor cortex, hypothalamus, caudate nucleus, colliculi and cerebellum could be responsible for cognitive and sensory motor dysfunctions. Thus, it is quite likely that in heat stress cognitive and sensory-motor functions are affected at the time of the increased BBB permeability [11–14, 105, 106, 150].

Keeping these views in mind, we examined cognitive and sensory-motor functions in animals subjected to heat stress using standard procedures [105, 106, 143, 144, 150].

We used Rota-Rod treadmill (at 16 rpm) to assess motor co-ordination and fatigue in rats. Rats not falling off the Rota-Rod for 2 min were considered normal during a 3 min session [144]. Subjection of rats to 3 h heat stress showed a significant decline in Rota-Rod performance that was progressive in nature up to 4 h period (Fig. 9.11). In these rats, changes in locomotor behaviour, gait and overall walking skill was examined using a grid-walking test in which an elevated level (30°) of stainless steel grid was used with a mesh size of 30 mm [144]. The animals were placed on the grid for 1 min and the total number of paired steps, i.e., placement of both forelimbs was counted. During this period, the number of misplaced limbs error, viz., the forelimbs fell through the grid was recorded. The total number of errors for each forelimb was also counted manually [12, 13, 144, 150].

Rats subjected to 4 h heat exposure were unable to walk normally during a grid walking session. The number of steps taken during a 60 sec grid-walking session was significantly reduced in heat stressed rats at 4 h (Fig. 9.11). These animals exhibited greater placement errors of hind legs on the grid following 3 and 4 h after heat exposure (Fig. 9.11). These observations suggest that BBB disruption following heat stress leads to significant deficits in cognitive and sensory function.

The motor disturbances in the rat after heat stress was determined using the inclined plane test. The angle of inclined plane was set in such a way (60°) that the normal animal could stay on the plane for 5 sec without falling. In this test, a significant motor deficit in heat stressed animals is seen that was most pronounced after 3–4 h exposure (Fig. 9.11). Another good measure of gait and motor disturbances can be judged using footprint analysis [12, 13, 144]. For this purpose, the hind paws were wetted and the animals were allowed to walk on a paper coated with bromophenol blue dissolved in acetone. The imprints of hind paws were used to determine motor function behaviour by measuring the distance (mm) between hind paws from the base of the central pads [12, 13, 21, 23, 24, 28, 105, 106, 150]. In addition, the stride length (mm) was also determined by measuring the distance between hind paws in two consecutive steps.

Animals subjected to heat stress exhibited pronounced motor disturbances at the time of the BBB disruption. Thus, the transverse distance between the hind feet, a measure of disturbed gait was increased significantly at 4 h (Fig. 9.11). On the other

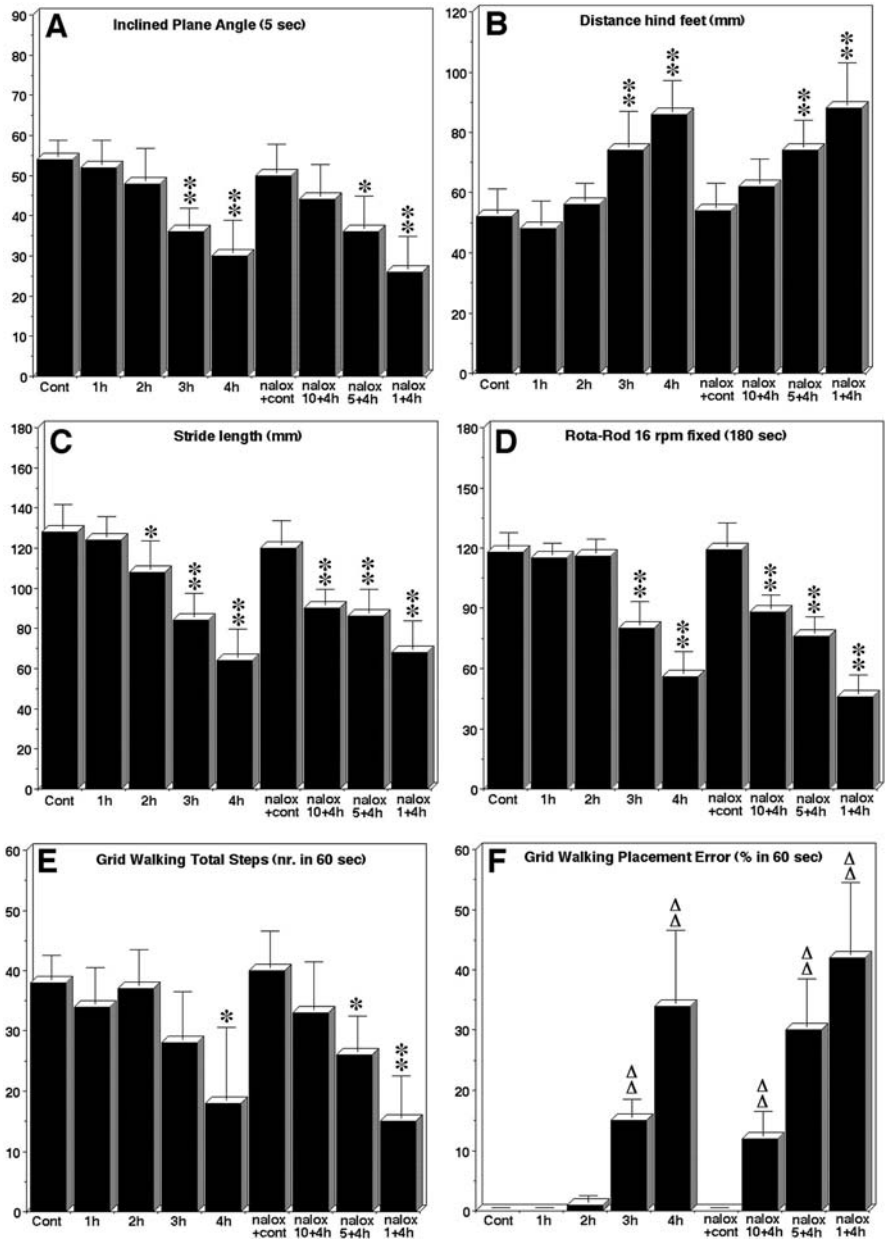

Fig. 9.11 Sensory-motor and cognitive dysfunction at the of the BBB disruption in heat stress and their prevention with Naloxone treatment. A significant decrease in the angle of inclined plane test reflecting alterations in motor functions was noted in rats after 3 h after heat exposure that was progressive up to 4 h (**A**). Marked decline in cognitive function was also seen in heat stressed animals when they are subjected to Rota-Rod performance (**B**). At 3 h heat stress, rats could not stand on Rota-Rod for more than 75 sec that was subsequently reduced to 60 sec at 4 h (**B**).

hand, the stride length calculated as longitudinal distance between hind feet during stepping showed a significant decrease from 2 h heat exposure and was progressive in nature (Fig. 9.11). Taken together, these observations clearly indicate that cognitive sensory-motor functions are definitely altered at the time of the BBB dysfunction in heat stress [12–14, 144]. This suggests that persons exposed to long time period in hot environments, e.g., solders in the Middle East may be highly prone to cognitive and sensory motor disturbances over time even without any external injuries to their CNS.

9.9.4 Nanoparticles as Stressors and the BBB Dysfunction

Several nanoparticles from various sources are present in the environment that could influence human health functions [107, 108, 152]. However, the potential effects on environmental nanoparticles on human brain functions with regard to BBB dysfunction are still not well known [107, 108, 152–155]. Persons exposed to environments around the industrial waste, battlefield, or silica dust in deserts could inhale lots of nanoparticles e.g., SiO_2, Cu, S, Al, C, Ag and Mn etc. that could reach to their CNS easily through translocation [107, 108, 152–156]. Once these nanoparticles reach into circulation that may be deposited in different areas of the body in various organ system leading to alterations in respiratory, cardiac, renal, hepatic or even brain and spinal cord functions [13, 107, 108, 152, 153].

There are reasons to believe that nanoparticles once entered into the body fluid system will induce oxidative stress and production of free radicals and could be thus responsible for cell membrane damage [107]. Thus, nanoparticles, from Cobalt, carbon tubes, quantum dots, and ultrafine particles (20–80 nm) induce production of reactive oxygen species (ROS), especially following concomitant exposure to light, ultraviolet, or transition metals [157–165]. Since oxidative stress alone is capable to induce brain pathology [166–168], it is likely that nanoparticles induce ROS activation may play crucial roles in inducing BBB disruption and neurotoxicity. However, the effects of nanoparticles exposure per se on the BBB function are still not well investigated in details [152–156].

Fig. 9.11 (continued) Measurement of gait and walking pattern revealed deficit in the placement of legs (**c**) and the stride length (**D**) from 3 h and onwards. Heat stressed rats also showed significant deficit in grid walking and placement errors compared to controls (**E, F**). Decrease in the number of steps taken in a grid walking test for 60 sec was evident in rats subjected to 4 h heat stress (**E**). However, placement errors of hind feet can be seen as early as 3 h after heat exposure (**F**). Pretreatment with naloxone in high doses improved the motor and cognitive functions in rats after 4 h heat stress (**A–F**). However, low doses of naloxone (1 and 5 mg) did not alter these deficits significantly. Data at each column represent mean±SD of 5 to 6 rats. $^{*}P < 0.05$; $^{**}P < 0.01$, ANOVA followed by Dunnet's test for multiple group comparison from one control group. DD = $P<0.01$, chi-square test from the control group. Reproduced after permission from [144]

9.9.4.1 Nanoparticles Administration Induces BBB Disruption

Using engineered nanoparticles from metals, i.e., Ag, Al and Cu (50–60 nm) we examined BBB permeability to proteins in rodents after their administration through systemic or cerebral routes. Our observations are the first to show that nanoparticles when administered systemically or in to the brain ventricles, significantly increased the BBB permeability to Evans blue and radioiodine in rats and mice [154]. This effect was most pronounced with Ag and Cu nanoparticles as compared to Al in mice compared to rats (Table 9.3). Intraperitoneal administration of nanoparticles (50 mg/kg) was the least affective in inducing BBB disruption caused by any nanoparticles used these studies (Table 9.3).

On the other hand, intravenous administration of nanaoparticles (30 mg/kg) resulted in pronounced disruption of the BBB 24 h after administration as compared to 4 h period (Table 9.3) and was most marked in mice (Table 9.3). Ag and Cu nanoparticles exerted most powerful disruption of the BBB as compared to Al treatment (Table 9.3).

When nanaoparticles (2.5 mg/kg) were administered into the cerebral circulation through right internal carotid artery (ica) Evans blue or radioiodine leakage was seen in the ipsilateral side of the brains only (Table 9.3). This effect was most prominent with Ag and Cu nanoparticles after 24 h injection (Table 9.3) [154].

Direct injection of nanoparticles (20 μg in 10 μl) into right lateral ventricle resulted in extravasation of Evans blue and radioiodine into the infused side of cerebral hemisphere significantly that was most prominent at 24 h after nanoparticles administration (Table 9.3). Ag and Cu nanoparticles exerted most powerful BBB disruption in rats and mice as compared to Al infusion (Table 9.3) [154].

In most cases, cerebellum, hypothalamus, piriform cortex, brain stem and ventral surface of the brain served by major cerebral arteries showed quite pronounced leakage of Evans blue in nanoparticle treated groups (Fig. 9.12). Blue staining was also seen on the dorsal and ventral surfaces of the brain and spinal cord in nanoparticles treated rats and mice (Fig. 9.12). The leakage of Evans blue was also seen in deeper structures of the brains as coronal sections showing profound blue staining of the deeper areas of the cortex, hippocampus, thalamus and hypothalamus (Fig. 9.12 arrows).

These observations clearly suggest that nanoparticles depending on their type, dosage and route of administration induces BBB disruption. Thus, Ag and Cu are mot effective in opening of the BBB as compared to Al in identical doses. This effect of nanaoparticles appears to be species specific as mice are more sensitive to rats in BBB disruption [154].

9.9.4.2 Structural Changes in the Brain After Nanoparticles Administration

Light and electron microscopy revealed profound brain pathology in nanoparticles treated animals. These structural changes were present in those areas showing BBB leakage [108, 154]. Thus, profound alterations in nerve cells were seen in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem in

Table 9.3 Effect of nanoparticles on Blood-Brain Barrier (BBB) permeability, brain edema formation and cell changes in rats and mice at 24 h after administration

Type of Expt.		n	BBB permeability		Brain edema			Cell changes (LM)		EM		
			Evans blue mg %	[131] Iodine %	Water content %	%f	Na+ mM/Kg dry wt	K+ mM/kg dry wt	Neuron Nissl	glia GFAP	Myelin Luxol Fast Blue	La+++ TEM
1. Control Group (saline treatment equimolar concentration)												
Rats	i.v.	6	0.23±0.08	0.34±0.03	76.34±0.28		316±8	218±10	nil	nil	++++	nil
	i.p.	6	0.24±0.08	0.30±0.08	76.12±0.32		nd	nd	nd	nd	nd	nd
Mice	i.v.	8	0.21±0.10	0.28±0.14	74.56±0.32		280±14	186±17	nil	nil	++++	nil
	c.s.	6	0.24±0.10	0.30±0.08	74.65±0.41		267±18	190±21	nil	nil	++++	nd
	i.p.	5	0.28±0.08	0.34±0.12	74.72±0.32		269±12	195±28	nd	nd	nd	nd
2. Nanoparticles intraperitoneal (50 mg/kg)												
Rats	Ag	5	0.23±0.05	0.32±0.07	76.23±0.12		nd	nd	+/–	+/–	+++/?	nd
	Cu	5	0.24±0.12	0.37±0.17	76.45±0.43		nd	nd	+	+/–	+++/?	nd
	Al	6	0.28±0.23	0.38±0.21	76.78±0.44		nd	nd	+/–	+/–	+++/+	nd
Mice	Ag	5	0.23±0.03	0.28±0.12	74.54±0.21		nd	nd	+/–	+/–	+++/+?	nd
	Cu	5	0.22±0.08	0.29±0.06	74.56±0.32		nd	nd	+/–	+/–	+++/+	nd
	Al											
3. Nanoparticles intravenous (30 mg/kg)												
Rats	Ag	8	0.67±0.12**	0.89±0.11**	77.34±0.21**	+4	350±18*	160±21**	+++	++	++	++
	Cu	6	0.60±0.19**	0.76±0.08**	77.18±0.08**	+4	328±12**	174±10**	++	++	++	++
	Al	6	0.54±0.12*	0.60±0.12*	76.89±0.12*	+2	330±16*	195±12*	+	+/–?	+++	+?
Mice	Ag	8	0.68±0.08**	0.79±0.12**	76.89±0.23**	+8	308±12**	178±12**	+++	+++	++	++
	Cu	6	0.60±0.12*	0.68±0.14*	76.10±0.23*	+7	299±12*	185±8*	++	++	+++	+
	Al	6	0.54±0.08*	0.62±0.06*	75.23±0.12*	+2	280±4*	170±8	+	+	+++/+	+/–?

Table 9.3 (continued)

			BBB permeability		Brain edema			Cell changes (LM)		EM		
Type of Expt.		n	Evans blue mg %	[131] Iodine %	Water content % %f	Na+ mM/Kg dry wt	K+ mM/kg dry wt	Neuron Nissl	glia GFAP	Myelin Luxol Fast Blue		La+++ TEM
4. Nanoparticles Cortical superfusion (20 μg/10 μl)												
Mice	Ag	8	0.76±0.21**	0,89±0.22**	77.48±0.21**	+14	324±12**	140±14**	++++	++++	+	+++
	Cu	8	0.68±0.21**	0.76±0.18**	76.78±0.24**	+10	302±8**	160±8**	+++	+++	++	++
	Al	6	0.48±0.12*	0.54±0.14*	75.23±0.43*	+3	280±8*	189±14	+	+	+++	+

Values are Mean±SD of 5 to 8 animals. *$P < 0.05$; **$P<0.01$, ANOVA followed by Dunnet's test for multiple group comparison from one control group. Data in Rats and mice were compared to rats and mice groups respectively. c.s. = cortical superfusion, nil = absent, nd = not done, LM = light microscopy, EM = electron microscopy, Na = sodium, K = potassium, La = lanthanum ion; % = volume swelling calculated from changes in water content (for details see text). + = faint, ++ = mild, +++ = moderate, ++++ = extensive, +/– = unclear, ? = boarder line. Data from Sharma [150–156]

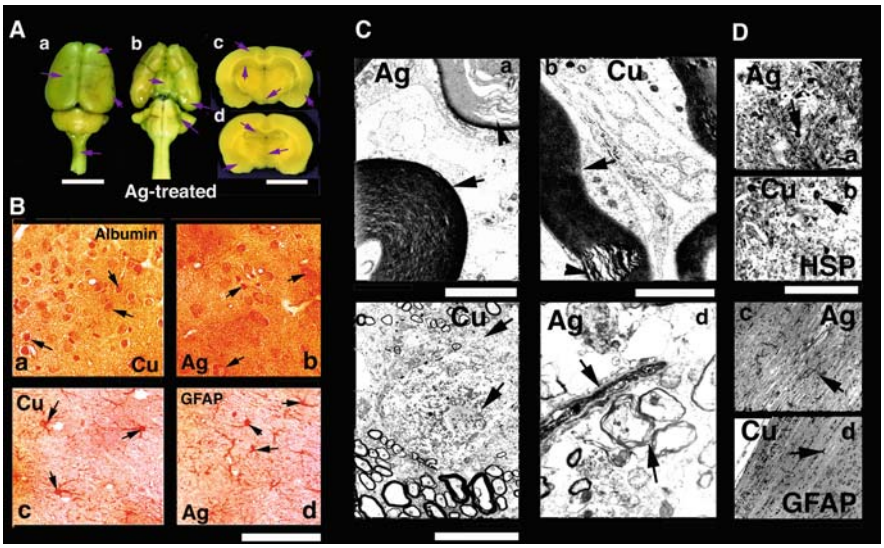

Fig. 9.12 Effects of engineered nanoparticles from metals on the BBB disruption and concomitant structural changes in the brain. (**A**) Shows extravasation of Evans blue on the dorsal (**a**) and ventral (**b**) surfaces of rat brain after Ag nanoparticle treatment. The Ag nanoparticle was administered intravenously (35 mg/kg) and the rat is allowed to survive 24 h after injection. Coronal sections of the brain passing through hippocampus (**c**) and caudate nucleus (**d**) are also shown. Leakage of Evans blue dye can be seen in various brain regions (*arrows*). The deeper parts of the brain, e.g., hippocampus, caudate nucleus, thalamus, hypothalamus, cortical layers including pyriform, cingulate, parietal and temporal cortices showed moderate blue staining. This indicates widespread leakage of Evans blue albumin within the brains after Ag treatment. Bar = 3 mm. (**B**) Shows albumin immunoreactivity (**a, b**) and glial fibrillary acidic protein (GFAP) expression (**c, d**) in the cerebral cortex rats receiving Cu (**a, c**) or Ag (**b, d**) treatment. These nanoparticles were administered separately (35 mg/kg) intravenously in rats and the animals allowed to survived 24 after the administration. Paraffin (3 μm thick) sections showed massive uptake of albumin (**a, b**, *arrows*) by the nerve cells and along with its extravasation in the neuropil. Damage to nerve cell and leakage of albumin in the neuropil is more conspicuous by Ag treatment (**b**) compared to Cu nanoparticles administration (**a**). Overexpression of astrocytes, as seen by GFAP immunoreactivity is present in the neuropil of both Ag (**d**) and Cu (**c**) treated animals. Star shaped astrocytes are more frequent in the neuropil of Ag treated rats compared to Cu treatment. Bar: 50 μm. (**C**) Transmission electronmicrograph of myelin (**a, b**), nerve cell (**c**) and one microvessel (**d**) following Ag or Cu nanoparticles treatment. Low power light micrograph showing immunostaining of heat shock protein (HSP, **e, f**) and glial fibrillary acidic protein (GFAP, **g, h**) in the spinal cord following Ag or Cu nanoparticle treatment. Ag (**a**) or Cu (**b**) nanoparticles selectively induce damage to myelinated fibers (*arrowheads*) in rats after 24 h administration. However, normal myelinated fibers (*arrows*) are also seen in the neuropil. Bar a, b = 600 nm. Degeneration of one nerve cell and disintegration of nucleolus (*arrows*) is clearly seen in the cerebral cortex by Cu treatment (**c**). Damage to myelinated fibers and vacuolation of the neuropil are also seen (**c**). A completely collapsed microvessel (*arrow*) with perivascular edema and myelin damage (*arrow*) is seen in Cu treated rat (**d**). Bars c, d = 1 μm. (**D**) Expression of stress protein seen using heat shock protein expression (HSP 72 kD) is evident in the spinal cord after Ag (**e**) or Cu (**f**) treatment. HSP expression is seen within the neurons (*arrows*) as well as in glial cells. Bars e, f = 40 μm. Expression of GFAP, marker of astrocytes is seen in the longitudinal section of spinal cord (*arrows*) of Ag (**g**) or Cu (**h**) treated rats. Bars g, h = 50 μm. Modified after [13, 154]

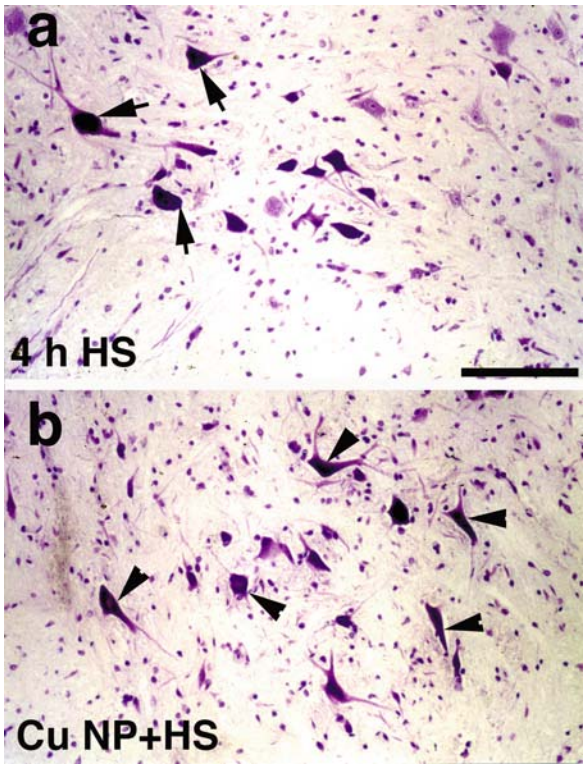

Fig. 9.13 Exacerbation of neuronal damage in heat stress by prior exposure to nanoparticles. Neuronal damage in heat stress (**a**) and its exacerbation with Cu nanoparticles (**b**). Many nerve cells (*arrows*) are dark and distorted in heat stressed rat (**a**) and sign of sponginess and edema are clearly seen. These cell changes appears to be more prominent in rat that received Cu nanoparticles treatment (for 1 week) before heat stress (**b**). The number of dark and distorted nerve cells (*arrow heads*) is much more pronounced in Cu nanoparticle treated rats after heat exposure (**b**) and saline treat rat (**a**). Bar = 100 μm. Reproduced with permission after [107]

nanoparticles treated rats or mice at light microscopy (Table 9.3, Fig. 9.13). These neuronal changes were most marked when Ag or Cu nanoparticles were administered through intravenous, intracarotid or intracerebroventricular routes (Fig. 9.13) [154]. In terms of loss of neurons or nerve cell damage by nanaoparticles, hippocampus (Fig. 9.13) was the most adversely affected organ followed by cerebellum, cerebral cortex, brain stem, thalamus and hypothalamus [154]. These observations demonstrate that nanoparticles are capable to induce brain pathology probably though BBB disruption [107, 108, 154].

9.9.4.3 Cognitive and Sensory-Motor Dysfunctions by Nanoparticles Exposure

At the time of BBB dysfunction, nanoparticles treated rats showed mild to moderate deficits in the cognitive and sensory-motor functions. This was evident by their poor performances seen on Rota Rod, grid walking, inclined plane angle and footprint analysis tests. These sensory motor deficits were most pronounced by Ag and Cu treated group as compared to Al treatment. Moreover, administration

of nanoparticles through intravenous, intracarotid or intracerebroventricular routes resulted in most massive deficits in the above behavioral functions as compared to their intraperitoneal administration (Sharma HS unpublished observations). These observations indicate that nanoparticles induced BBB dysfunction and subsequent brain pathology could be responsible for sensory motor dysfunction (Sharma HS unpublished observations).

9.10 A Combination of Nanoparticles and Heat Stress Exacerbate BBB Disruption and Brain Pathology

Recently, magnetic nanoparticles are used to enhance tumor temperatures [169]. This "Thermotherapy" for tumor treatment is based on controlled heating of intratumoral administered magnetic nanoparticles [170]. Thus, local administration of magnetic nanoparticles within the tumor sites resulted in a 3–4°C higher temperature compared to tumors without the nanoparticles administration. This observation suggests that magnetic nanoparticles could enhance the tumor temperature effectively to kill the cancer cells. Interestingly, this thermotherapy with nanoparticles also limits proliferation by inducing localized necrosis around the peritumoral zones [107, 170]. This suggests that heating in presence of magnetic nanoparticles enhances tumor temperature that could kill more cells as compared to heating without these nanoparticles. However, this is still uncertain whether exposure of nanoparticles may alter the sensitivities of the whole body hypertehrmia (WBH) [150]. Accordingly, this is not known whether soldiers in Middle East that are exposed to silica dust or Cu nanoparticles routinely could be more sensitive to environmental heat induced exacerbation of brain pathology as compared to normal populations [156].

To further clarify these points, we initiated animal experiments in our laboratory in which nanaoparticles exposed rats or mice are subjected to heat stress and BBB permeability and brain pathology was examined. Our novel investigations show that rats exposed to nanaoparticles prior to heat stress exhibited an exacerbation of BBB dysfunction and brain pathology [107, 108, 156].

9.10.1 Exacerbation of BBB Disruption Heat Stress by Nanoparticles

We examined the effects of chronic exposure of three different nanoparticles derived from metals e.g., Al, Ag, and Cu (50–60 nm sizes) on brain function subjected to WBH. The results are compared with heat-stressed rats without receiving any nanoparticles. The metal nanoparticles (Cu, Al and Ag, ≈50–60 nm in size) were suspended in Tween 80 and administered separately in 3 different groups of rats through intraperitoneally route in a dose of 50 mg/kg (weight by volume) once daily

for 7 days. On the 8th day, these animals were subjected to 4 h heat stress at 38°C and barrier permeability and brain pathology were examined [107].

Rats treated with nanoparticles exhibited about 3–30% increase the BBB and about 16–75% increase in blood-spinal cord barrier (BSCB) permeability to protein tracers after WBH as compared to saline treated heat stressed animals (Table 9.4). This effect was most pronounced in Cu treated nanoparticles followed by Ag and Al treatment (Table 9.4). The magnitude of BBB disruption after WBH in nanoparticles treated animals was most marked in the spinal cord. This suggests that nanoparticles are likely to produce severe damage to the spinal cord resulting in more profound sensory-motor dysfunction. The mechanisms behind a selective vulnerability of the spinal cord in nanoparticles treated heat stressed rats compared to brain is still unclear.

Using radioiodine, the tracer extravasation following WBH was most pronounced in different brain regions by Ag treatment followed by Cu and Al treatments (Table 9.4). The highest increase in regional BBB to radioiodine was seen in brain stem (≈400%) followed by hippocampus and cerebellum (≈300%), cerebral cortex (200%) and thalamus and hypothalamus (≈150%, See Table 9.4). These observations indicate that nanoparticles are able to influence regional BBB function in a selective manner depending on their chemistry [107, 153].

9.10.2 Exacerbation of Structural Changes in Brain in Heat Stress by Nanoparticles

Nanoparticles treated animals when subjected to 4 h heat stress; they exhibited an exacerbation of neuronal, glial, axonal and endothelial cell damages (see Table 9.5). The most marked effects of WBH-induced aggravation of cellular injuries are seen in Ag nanoparticles followed by Cu and Al treatment [107]. These brain cell damages are mostly seen in the regions exhibiting BBB leakage.

Thus, large number of distorted neurons is seen in the brainstem, hippocampus, cerebellum, cerebral cortex, thalamus, hypothalamus and spinal cord [107, 156]. The neuronal damage includes chromatolysis, degeneration, distortion of cell nucleus and/or nucleolus that are most marked in Ag and Cu treated stressed animals (Fig. 9.14). A greater expansion, sponginess and edema are common finding in these nanoparticles treated rats after WBH as compared to saline treated animals (Fig. 9.13).

At the ultrastructural level, the endothelial cells of cerebral microvessels showed widespread exudation of lanthanum in Ag and Cu nanoparticles treated rats after WBH as compared to saline treated animals (Fig. 9.14). Diffusion of lanthanum in the adjacent neuropil is quite common in nanoparticles treated rats after WBH. Whereas, in saline treated group, the electron dense tracer is largely confined to the basal lamina (Fig. 9.14). These observations suggest that the magnitude and intensity of endothelial cell membrane dysfunction to Lanthanum (Mol. Diam. 12 Å) is significantly increased in nanoparticles treated group after WBH as compared to saline treatment [107].

Table 9.4 Effect of nanoparticles on BBB permeability, cerebral blood flow and brain edema in normal rats and animals subjected to 4 h whole body hyperthermia (WBH) at 38°C. For details see text

Expt. Type	n	BBB permeability	BSCB Permeability		Blood flow		Edema formation		Volume Swelling		
		Evans blue mg %	[131]Iodine %	Evans blue mg %	[131]Iodine %	ml/g/min Brain	Spinal cord	Brain Water %	Spinal cord water %	*%f* Brain	*%f* Spinal cord
A. Normal animals											
Saline	6	0.28±0.08	0.36±0.06	0.22±0.04	0.30±0.05	1.16±0.06	0.94±0.04	74.23±0.86	65.25±0.21	nil	nil
Cu	8	0.56±0.13**	0.68±0.11**	0.67±0.08**	0.76±0.10**	0.98±0.08*	0.84±0.09*	75.14±0.13*	66.16±0.08*	3.53	2.61
Ag	8	0.64±0.12**	0.74±0.11**	0.64±0.08**	0.75±0.05**	0.96+±0.10*	0.88±0.06*	75.04±0.08*	66.08±0.11*	3.14	2.38
Al	8	0.46±0.08*	0.58±0.06*	0.58±0.06*	0.64±0.08*	1.04±0.06*	0.88±0.08*	74.84±0.13*	65.75±0.31*	2.36	1.43
B. Heat Stressed Animals (38°C for 4 h)											
Saline	5	2.06±0.25aa	2.86±0.24aa	1.21±0.21aa	1.46±0.22aa	0.76±0.08aa	0.70±0.06a	81.34±0.62aa	68.23±0.21aa	27.59	8.57
Cu	6	2.68±0.33b	3.17±0.26b	2.10±0.8b	2.83±0.08b	0.70±0.06b	0.65±0.07b*	83.12±0.34b*	68.76±0.43*	34.49	10.01
Ag	8	2.64±0.33b	3.14±0.12bb	1.87±0.6b	2.08±0.07bb*	0.74+±0.08b	0.68±0.06*	82.32±0.26b*	68.56±0.28*	31.39	9.52
Al	8	2.13±0.36*	3.08±0.21b*	1.46±0.32b*	1.89±0.42b*	0.72±0.06*	0.68±0.10b*	82.16±0.24*	68.34±0.21b*	30.77	8.89

The nanoparticles were suspended in Tween 80 and administered separately intraperitoneally in rats daily once in a dose of 50 mg/kg (weight/volume). Values are Mean±SD from 5 to 9 animals at each data point. *P<0.05; **P <0.01 compared from saline in normal animals, a = P <0.05; aa = P <0.01, compared from 4 h saline treatment in normal vs. heat stressed. b = P< 0.05, bb = P <0.01 compared from saline treated heat stressed animals, ANOVA followed by Dunnett's test for multiple group comparison from one control group. Data from Sharma and Sharma (2007) and Sharma (2009a).

Table 9.5 Effect of nanoparticles on morphological changes in the neurons, astrocytes, myelin and endothelial cells (see Chapter 5 in this volume) in normal rats and animals subjected to 4 h whole body hyperthermia (WBH) at 38°C. For details see text. Semiquantitative grading was done by two independent workers in a blinded fashion and was averaged in the final score

	Neuronal Reaction					Glial Reaction		Axonal Reaction		Lanthanum Extravasation	
Expt. Type Lamina	Distortion Cell shape	Chromatolysis+ degeneration	Nuclear damage	Eccentric+ nucleolus	Sponginess	Reactive gliosis	perivascular+ gliosis	Loss of myelin	Vesiculation of myelin	Endothelial cell cytoplasm	Basal Lamina
A. Normal animals (n= 6–8)											
Saline	nil	nil	nil	nil	nil	+/–	+/–	nil	+/–	nil	nil
Cu	++	+/–	+	+/–	+	++	++	++	+	++	+/–
Ag	+++	+	+	+	+	++	++	+++	++	++	+/–
Al	+	++	+	++	+	++	+++	+	++	+	+/–
B. Heat Stressed Animals (38° C for 4 h, n = 6–8)											
Saline	++++	++++	+++	+++	+++	++++	+++	+++	+++	++++	+++
Cu	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Ag	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++
Al	++++	++++	+++	+++	+++	+++	++++	+++	+++	++++	+++

nil = absent, ++++ = severe; +++ = moderate; ++ = mild; + = present; +/– = possible.
The nanoparticles were suspended in Tween 80 and administered separately intraperitoneally in rats daily once in a dose of 50 mg/kg (weight/volume).
Light microscopy and immunohistochemistry was used to asses neuronal, glial and myelin damage. Ultrastructural changes were used to determine eccentric nucleolus, perivascular gliosis, myelin vesiculation and lanthanum extravasation. Lanthanum as dark electron dense particle can easily be seen within endothelial cell cytoplasm or in the basal lamina as dark particulate deposits under transmission electron microscope, for details see Chapter 5 in this volume (Data from [13, 107, 152])

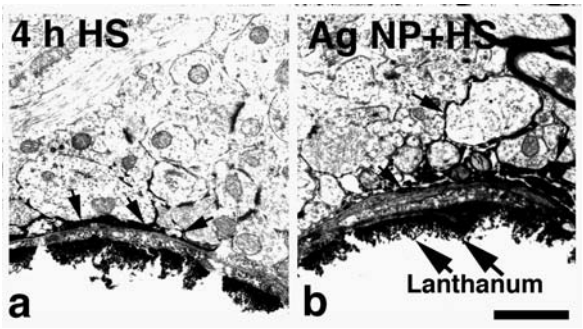

Fig. 9.14 Exacerbation of BBB leakage to lanthanum at the ultrastructural level in heat stress by prior exposure to nanoparticles. Blood-spinal cord barrier (BSCB) to electron dense tracer lanthanum after 4 h heat stress in saline treated (**a**) and in Ag nanoparticle treated (1 week) rat (**b**). High power electron micrograph showing presence of lanthanum in the basal lamina (*arrows*) of one spinal cord microvessel from the ventral horn of the T9 segment (**a**). Deposit of lanthanum within the basal lamina and in extracellular space is clearly seen in the neuropil (**a**). In Ag nanoparticle treated stressed rat, the magnitude and intensity of lanthanum extravasation across the BSCB and in the neuropil is much more pronounced (**b**) Thus, the endothelial cell cytoplasm is heavily infiltrated in nanoparticle treated heat stressed rat (**b**). The extent of penetration of the tracer in the extracellular compartment of the spinal cord neuropil is much deeper within 4 h (**b**) compared to the same period in saline treated stressed rat (**a**). Perivascular edema and myelin degeneration are prominent in the neuropil of heat stressed rats (**a, b**). However, the tight junctions are intact to lanthanum (**b**). Bar = a, b = 600 nm. Reproduced with permission after [107]

9.10.3 Exacerbation of Cognitive and Sensory-Motor Dysfunctions in Heat Stress by Nanoparticles

We observed most pronounced disturbances in cognitive and motor functions in nanoparticles treated heat stressed rats as compared to saline treated group (Table 9.6). Cu treatment resulted in most marked decline in rota-rod performance after WBH followed by Ag and Al treatment. On the other hand, Ag nanoparticles treated rats showed most severe decrease in the number of steps taken during a grid-walking session after WBH than Cu and Al treatment (Table 9.6). However, no significant difference in placement error was seen in saline or nanoparticles treated animals after heat stress (Table 9.6).

A marked reduction in the angle of inclined plane in Ag treated rats was seen after WBH (Table 9.6). The distance between hind feet is also decreased in Ag treated stressed rats. However, no difference in stride length is seen in Ag or saline treated animals after WBH (Table 9.6). Interestingly, inclined plane test or foot print analysis did not show any significant differences from saline or Cu or Al treated rats after WBH (Table 9.6). These observations suggest that disturbances in the cognitive and motor functions caused by WBH in animals is markedly influence by nanoparticles depending on their chemistry [107].

Table 9.6 Effect of nanoparticles on cognitive, motor function and heat-stress symptoms in normal rats and animals subjected to 4 h whole body hyperthermia (WBH) at 38°C. For details see text

		Cognitive function			Motor function			Salivation Prostration		
Expt. Type	n	Rota Rod (180 sec)	Grid-walking total steps (60 sec)	Placement errors (60 sec) %	Inclined plane Angle° Placement error (5 sec)	Footprint analysis Hindfeet distance mm mm	Stride length mm	Saliva spread Stride length mm	Grade	Microhemorrhage in stomach (spots, nr)
A. Normal animals										
Saline	6	120±8	40±6	nil	60±4	50±5	130±6	nil	nil	nil
Cu	8	118±10	35±9	2±1	55±8	45±8	120±12	nil	nil	2±4
Ag	8	110±12	32±9	4±3	52±6*	42±11	118±8*	nil	nil	8±4
Al	8	116±9	36±8	3±4	56±8	46±12	124±16	nil	nil	2±3
B. Heat Stressed Animals (38°C for 4 h)										
Saline	8	80±4**	20±5**	35±6**	35±6**	85±6**	75±8**	22±6**	+++	48±12#**
Cu	8	65±12a	18±6	38±8	38±8	78±8a	80±12	18±6	+++	55±8#
Ag	8	68±6a	16±4a	40±6	30±5*	80±8a	72±6	20±6	++++	60±12#a
Al	8	74±6	18±5	36±7	36±8	81±5	72±9	20±8	+++	36±8#

#Many microhaemorrhages seen; ** $P<0.01$, Chi-Square test, compared from saline treated control; a = Student's unpaired t-test compared from saline treated heat stressed group.

The nanoparticles were suspended in Tween 80 and administered separately intraperitoneally in rats daily once in a dose of 50 mg/kg (weight/volume). Data are Mean±SD from 5 to 8 animals at each data point. Nil = absent. Data from [107, 153]

9.10.4 Possible Mechanisms of Nanoparticles Induced Exacerbation of Brain Damage

The possible mechanisms behind exacerbation of hyperthermia-induced brain damage in nanoparticles treated rats are still unclear. However, it appears that profound oxidative stress induced by nanoparticles will further enhance hyperthermia induced free radical formation and release of oxygen radicals leading to exacerbation of brain damage [107, 153]. This is supported by previous studies showing carbon nanoparticles depending on their sizes could aggravate lung inflammation induced by bacterial endotoxins [171]. This exacerbation of inflammatory changes by nanoparticles is mediated through enhanced local expression of proinflammatory cytokines and induction of oxidative stress [13, 107, 171].

In our studies, nanoparticles in normal animals induced mild brain damage that could also probably by induction of oxidative stress by them. Since heat exposure alone induces profound oxidative stress [12, 13, 166–168, 172], additional exposure of nanoparticles treated rats to WBH could reflect the synergistic effects of two inflammatory agents (heat stress and nanoparticles) resulting in potentiation of oxidative tress and the pathological outcome [107, 153].

Alternatively, BBB damage in WBH will result in transport of nanoparticles into the brain fluid microenvironment that could further generate local oxidative stress [173] resulting in higher brain inflammation and cellular damage.

Moreover, nanoparticles within the body or brain fluid microenvironment could induce a higher increase in local cell and tissue temperatures leading to exacerbation of brain damage [107, 153].

Taken together, our novel study demonstrates that nanoparticles could exacerbate BBB disruption and brain pathology following WBH. These findings have immense strategic significance with regard to defense planning and military exercise, particularly in hot environments at various places in the World. It is likely that both military and non-military personals when exposed to nanoparticles from the ambient air at home or abroad are more vulnerable to additional environmental heat load. However, to understand the basic mechanisms of nanoparticles induced exacerbation of brain pathology in stressful situations, additional studies are needed.

9.11 Pharmacological Manipulation of BBB and Brain Pathology in Stress

Based on the above observations, it is clear that disruption of the BBB in diverse stressful conditions or nanoparticles treatment is associated with brain pathology, cognitive and sensory-motor dysfunction and cell or tissue injuries (see Table 9.6). To further confirm this idea, we examined the effects of several pharmacological agents, neurotrophic factors and antibodies directed against neurochemicals and enzymes on the BBB dysfunction in relation to brain pathology in the above animal models of stress [2–14, 22–28, 49, 105–108, 111, 152–156, 166–168].

9.11.1 Antibodies Against Injury Factors Attenuate BBB Dysfunction and Brain Pathology

Use of antibodies therapy to neutralize the endogenous effects of their antigens in various disease conditions has been emphasized recently [174–176]. The antibodies are considered much more effective in neutralizing the effects of the neurochemicals in vivo than pharmacological blockade of their receptors [12, 13, 25, 26, 174–176]. Our laboratory took one of the earliest initiatives to study the in vivo effects of antibodies directed against several potential injury factors e.g., neuropeptide dynorphin A 1-17 (Dyn A) [174]; neuronal nitric oxide synthase (nNOS) [175]; serotonin (5-HT) [177, 178] and the tumor necrosis factor-alpha [179] to induce neuroprotective effects or in CNS injuries or in stress conditions [12–14, 24–26, 111].

The antibodies directed against Dyn A, nNOS or 5-HT (1:20 dilution) when administered intracerebroventricularly 30 min before or 30 min after immobilization, forced swimming or heat exposure resulted in marked reductions in the BBB disruption and brain damage (results not shown). On the other hand, when these antibodies were administered either 60 or 90 min after onset of stressful conditions, no reductions in BBB permeability or cell injury were noted (Sharma HS unpublished observations). These observations suggest that antibodies to Dyn A, nNOS and 5-HT if effectively neutralize the harmful effects of their endogenous antigens, i.e., dynorphin, nitric oxide and serotonin within minutes after stress results in neuroprotection (see Table 9.6). Whereas, delayed neutralization of these injury factors with antibodies is ineffective. These findings support the idea that the antibodies could be potential neurotherapeutic agents in minimizing BBB dysfunction in stress to achieve neuroprotection [111].

On the other hand TNF-a antibodies when applied 10–30 min after immobilization, forced swimming or heat exposure are quite effective in reducing the BBB disruption and brain pathology [111, 179]. Whereas, TNF-α antibodies if given 10–30 min before stress, they exacerbated the BBB dysfunction and brain pathology (Sharma HS unpublished observations). These observations are the first to suggest that early neutralization of TNF-α is injurious to the brain [111, 179]. This indicates that the TNF-α has a dual role in inducing BBB disruption and brain pathology (results not shown). Taken together it appears that a reduction in the BBB or BSCB disruptions induced by antibodies appears to be largely responsible for neuroprotection following stress induced brain damage.

9.11.2 Neurochemical Synthesis Inhibitors Reduce BBB Permeability and Brain Pathology

The neurochemical mediators such as serotonin or prostaglandins are known to induce BBB disruption and brain damage [10, 12, 13, 23, 25, 26, 73, 74, 151, 180–182]. Thus, it is likely that inhibition of prostaglandin or serotonin synthesis prior to CNS insults in stress is neuroprotective (Fig. 9.15). We

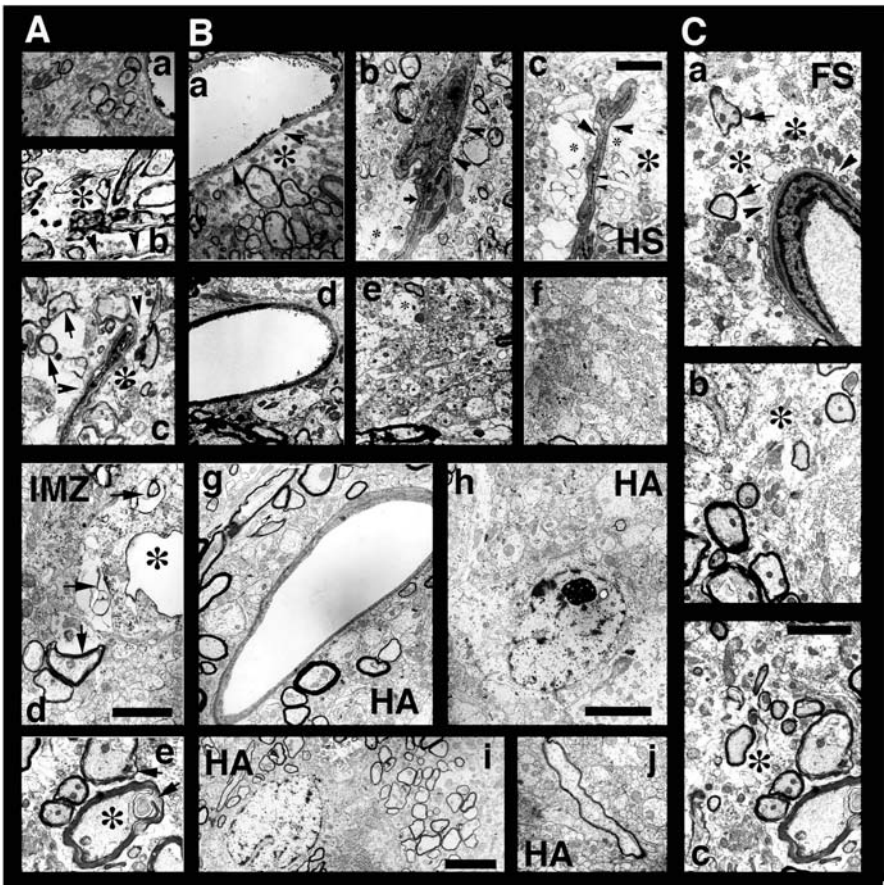

Fig. 9.15 Ultrastructural changes in the brain at the time of the BBB permeability in stress and neuroprotection by various agents. Ultratstructural changes in stress and their modification with drugs. (**A**) Pretreatment with p-CPA markedly attenuated cell damage following 8 h immobilization (IMZ) stress (**A.a**). However, treatment with 5,7-DHT (i.c.v., **A.b**) or 6-OHDA (i.c.v.; **A.c**) did not prevent IMZ induced cell changes. Perivascular edema (*arrowheads*), membrane damage (*arrows*) and vacuolation (*) are quite common in these drug treated rats. Administration of aminophylline (**A.d**) or 5-HT (**A.e**) following 4 h IMZ also induced profound membrane damage and edema. (**B**) Pretreatment with indomethacin (**B.a**), p-CPA (**B.d**), naltrexone (**B.e**) or nimodipine (**B.f**) significantly attenuated 4 h heat stress (HS) induced cell damage at the ultrastructural levels compared to the untreated group (**B.c**). Pretreatment with 6-OHDA (i.c.v., **B.b**) did not reduce cell damage following HS. On the other hand, heat adapted (HA) rats either reared at high environmental temperature (**B. g, h**) or chronically exposed to heat stress for 7 days (**B. i, j**) when subjected to HS did not show any cell damage. (**C**) Pretreatment with indomethacin (**C.b**) or p-CPA (**C.c**) markedly attenuated 30 min forced swimming (FS) induced cell damage (**C.a**) at the ultrastructural level. Bars: A.a–c = 1 μm; d,e = 0.6 μm; B. a–f = 1 μm; g–h = 0.6 μm; i, j = 0.8 μm; C. a–c = 0. 6 μm. Reproduced with permission after [12]

used p-chlorophenylalanine (p-CPA) and indomethacin to inhibit serotonin and prostaglandin synthesis, respectively in separate groups of rats [2–14, 22–28, 150, 151, 181–190] and then subjected them to different stressful situations (see Table 9.6). In these animals, the BBB permeability in relation to neural injuries was determined [12, 13, 25, 26, 185].

Pretreatment with p-CPA or indomethacin significantly attenuated BBB disruption in rats subjected to 8 h immobilization, 4 h heat stress or 30 min forced swimming [2–14, 22–28]. Brain edema formation and cell injury were also considerably reduced in these drug treated stressed animals (Table 9.6). These observations suggest that serotonin and prostaglandin participate in BBB disturbances and brain edema formation in stress. Furthermore, a reduction in BBB function with p-CPA or indomethacin is largely responsible for reduction in brain pathology caused by stress.

9.11.3 Neurochemical Receptor Modulation Influence BBB and Brain Pathology

Several neurochemicals released in stress could influence the BBB function and contribute to brain injury through specific receptor mediated mechanisms [2–14, 22–28, 43–45, 56, 65, 73, 74, 104–112, 150–156, 174–179, 181–192]. Thus, using pharmacological approaches we examined the effects of serotonin, histamine, and opioids receptor blockers on the BBB function in relation to brain damage in stress [2–14, 22–28, 43–45, 56, 65, 73, 74, 150].

Blockade of serotonin 2 (5-HT2) receptors with ketanserin or ritanserin [12, 13, 24–26]; histamine 2 (H2) receptors with cimetidine or ranitidine [12, 13, 24–26, 191, 192]; or multiple opioid receptor blockers with naloxone [12, 13, 144] significantly attenuated heat stress induced BBB disturbances and CNS pathology. However, blockade of histamine 1 (H1) receptor with mepyramine [12, 13, 181–192] or a combination of H1 and 5-HT2 receptor blockers cyproheptadine [2–14, 22–28] did not reduce BBB or cell injury in stressful situations (See Table 9.6; Fig. 9.15). Interestingly, cyproheptadine and mepyramine treatment exacerbated BBB dysfunction and brain injury in stress [12, 24–26]. These observations indicate that blockade of 5-HT2, H2, and multiple opioid receptors before CNS insults are neuroprotective in nature. Whereas, antagonism of H1 or a combination of H1 and 5-HT2 receptor before stress may have neurodestructive effects. Taken together our results show that the BBB disruption is instrumental in inducing in cell and tissue injuries in the brain [13].

9.11.4 Antioxidants Attenuate BBB Breakdown and Brain Pathology

Stress caused by various environmental or mental factors, nanoparticles, trauma and psychostimulant abuse induce oxidative stress and formation of free radicals

and lipid peroxidation in the CNS [13, 166–168]. Generation of free radicals and lipid peroxidation causes neuronal, glial and endothelial cell membrane damages [12, 13, 24, 25, 166–168]. Disruption of endothelial cell membrane will induce BBB breakdown leading to brain edema formation and cell injury. Thus, antioxidants either capable to inhibit lipid peroxidation or are scavengers of free radicals would induce neuroprotection caused by wide variety of CNS insults [12, 13, 166–168, 185, 193]. However the role of antioxidants in BBB disruption in CNS injuries is still not well known. Our laboratory was the first to examine the effects of antioxidants on BBB dysfunction in a wide variety of stressful situation as well as against nanoparticles exposure [2–14, 22–28, 43–45, 56, 65, 73, 74, 104–112, 150–156, 174–179, 181–192].

Using potent inhibitor of lipid peroxidation (H-290/51) [12, 13]; or scavengers of free radicals (EGB-761 and BN 22023) [24–26, 194, 195] we found that antioxidants are capable to attenuate BBB disruption and brain pathology in various stress models (see Table 9.6) [105–107, 153]. Thus, pretreatment with H-290/51 significantly attenuated BBB permeability, edema formation and cell damage in heat stress when administered 30 min before heat exposure [12, 13, 25, 26, 185]. Interestingly, the compound was also effective in reducing BBB disruption and brain pathology in nanoparticles (Ag, Cu or Al) treated rats (Table 9.6) [107, 153]. These observations suggest that inhibition of lipid peroxidation in stress or nanoparticle administration prevents BBB disruption and cell injury.

Similarly, daily treatment with EGB-761 or BN22123 separately in rats for 5 days reduced the BBB disruption and brain pathology in heat stress [12, 13]. However, the magnitude and intensity of BBB disruption and brain pathology were much less pronounced than the H-290/51 treatment [166–168]. This indicates that the compounds able to inhibit lipid peroxidation have superior neuroprotective ability than those drugs that are capable to scavenge the free radicals after their generation [153]. These observations strongly indicate a close correlation between BBB disruption and pathological outcome in various kinds of CNS insults.

9.11.5 Biogenic Amine Neurotoxins Exacerbate BCNSB Breakdown and CNS Pathology

The microvessels of the brain and spinal cord are innervated with serotoninergic, histaminergic, and catecholaminergic nerve fibers [14, 21, 35]. These nerve fibers control the vascular reactivity of neurochemicals and thus may influence the BBB function [35]. We examined the influence of serotoninergic and catecholaminergic nerve terminal degeneration on the BBB disruption in stress in relation to brain pathology.

Our observations show that destruction of central 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) significantly enhanced the BBB disruption to Evans blue albumin and radioiodine in the rat brains following immobilization,

forced swimming or heat stress [2–24, 22–28]. These observations suggest that destruction of central serotoninergic nerve terminals enhances the BBB leakage and thus exacerbate brain pathology [2–14, 22–28].

Interestingly, exacerbation of BBB permeability, brain edema and brain pathology was also observed in rats subjected heat stress after destruction of either central or peripheral catecholaminergic nerve terminals with 6-hydroxydopamine (6-OHDA) [2–14] (Sharma HS, unpublished observation).

Taken together it appears that serotoninergic and catecholaminergic nerve terminals regulate BBB function in stress. Thus, chemical degeneration of serotoninergic or catecholaminergic nerve terminals aggravate BBB dysfunction causing enhanced brain pathology and support the hypothesis that the BBB disruption is instrumental in cell and tissue injury following diverse kinds of CSN insults.

9.12 Possible Mechanisms of Stress Induced BBB Dysfunction and Brain Pathology

Based on our neuropharmacological investigations, it appears that alterations in neurochemicals following stress or nanoparticle administration are responsible for the BBB breakdown. Depending on the magnitude and intensity of stressors, several neurochemicals, i.e., serotonin, prostaglandin, histamine, opioids, amino acid neurotransmitters and other injury factors are released in the CNS as well as in the periphery [2–14, 22–28, 166–168]. These neurochemicals then will act on the luminal and abluminal sides of the cerebrovascular endothelium [14, 21, 35] inducing a cascade of effects leading to alteration in the cell membrane permeability [21, 25, 26]. These neurochemicals could also induce oxidative stress, lipid peroxidation, and generation of free radicals in the CNS that could directly or indirectly affects BBB dysfunction leading to cell damage. Reduction in BBB disruption by blockade of serotonin, prostaglandin, histamine and opioid effects in various kinds of stressors are in line with this hypothesis [12, 21, 24–26, 28, 105, 107, 111, 150, 152–156, 185].

These neurochemicals after binding to their receptors located on microvessels in both the luminal and the abluminal surfaces could induce intracellular signaling, e.g., stimulation of prostaglandins, nitric oxide synthase (NOS), cAMP or cGMP synthesis and/or release [12, 13, 22, 25, 26, 196]. The CNS capillaries contain all necessary enzymes for synthesis and catabolism of prostaglandins, NOS as well as cAMP [13, 22]. Local accumulation of prostaglandins, cGMP or cAMP in cerebral capillaries induces marked vasodilatation and an increases vesicular transport [14, 21, 22, 150]. Obviously, various pharmacological agents could influence this mode of tracer transport. Alteration in the cell membrane transport caused by cAMP and cGMP could also account for increased tracer transfer across the BBB [13, 21, 197–200]. Taken together, our observations suggest that the BBB function strictly regulates the cell and tissue injury in various CNS insults or diseases.

9.13 General Conclusion

In conclusion, our studies demonstrate that the BBB is an important regulator of the brain function in physiological and pathological conditions. Thus, a normal BBB is always responsible for physiological regulation of fluid environment of the brain and spinal in order to maintain their good health. However, leaky BBB caused by a variety of endogenous (neurochemical, alterations, diseases) or exogenous (heat, nanoparticles, forced stressful situations) agents could lead to brain pathology. Damage to specific brain or spinal cord areas in such disease or stressful situations are primarily responsible for deteriorating cognitive, sensory and motor functions. Thus, maintenance of a healthy BBB is the key to maintain good health of the CNS. Neuropharmacological agents including neurochemical synthesis inhibitors, receptor modulators or antibodies directed against neurodestructive elements are able to attenuate BBB dysfunction in a variety of stress situations. A reduction in BBB disruption clearly leads to brain protection in such situations and improve cognitive and sensory motor functions. Taken together, these observations clearly support the idea that the BBB is the gateway to neurodegeneration and neuroprotection.

9.14 Future Perspectives

With advent of nanotechnologies and development of nanomedicine, it remains to be seen how nano-drug delivery could enhance neuroprotection in stress induced brain diseases. Since nanoparticles by itself induce neurotoxicity and exacerbation of brain pathology, further research is needed to see whether nanoparticles could also influence the neuroprotective efficacy of drugs in brain diseases. It is utmost importance for pharmacotherapeutic agents to reduce nanoparticles induced enhanced brain damage in stressful situations. Thus, exploration of suitable therapeutic strategies using nano-drug delivery of various antibodies, receptors blockers or synthesis inhibitors are needed to reduce nanoparticles induces exacerbation of brain pathology and the BBB dysfunction. Our laboratory is currently engaged to identify novel therapeutic agents that can attenuate nanoparticles induced enhanced brain damage in a variety of traumatic and stressful situation in different animals models.

Acknowledgments Author's research described here is supported by Grants from Swedish Medical Research Council (2710), Stockholm Sweden, Göran Gustafsson Foundation, Stockholm, Sweden; Alexander von Humboldt Foundation, Bonn, Germany, European Office of Aerospace Research and Development (EOARD), London Office, UK. Technical assistant of Mari-Anne Carlsson, Kerstin Flink, Ingmarie Olsson and Kerstin Rystedt are highly appreciated.

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Chapter 10
Oxidative Stress and Neurodegeneration: An Inevitable Consequence of Aging? Implications for Therapy

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Abstract Brain aging is one of the most complex issues confronting researchers in neuroscience today. Nevertheless, research on the molecular biology of neurodegenerative disorders, particularly Alzheimer disease, has provided enormous progress in understanding the mechanisms that ultimately lead to the neuronal and glial malfunctions that ultimately damage neurons resulting in death. In this regard, one of the most compelling theories providing a basis for understanding aging and neurodegeneration posits oxidative stress, which results from an accumulation of "free radicals" in the cell that originates from the intense oxidative metabolism in the central nervous system and the diminished antioxidant defenses, as a major contributor. Here we review evidence demonstrating a robust relationship—epidemiological-clinical, molecular-neurobiological, and pathogenetic—between brain senility, mild cognitive impairment, and Alzheimer disease (as well as other neurodegenerative conditions) that places oxidative stress at a pivotal point in these three neurophysiologic and neuropathologic processes. These observations suggest that the three conditions are steps in the progressive decline in cognitive function. First, we focus on classical, clinical, and psychiatric observations of the cognitive ability of elderly people, from normal functioning to declines associated with aging, and then move to mild and severe pathological impairment, with continually worsening clinical and neuropsychiatric status. We show that the term "senile dementia", today removed from the nosological categories, is in fact representative of the clinical observations of progressive age-related brain deterioration. Second, we address oxidative stress and describe the new neurochemical and neuropathological theories of disease pathogenesis, that implicate oxidative stress as the earliest process in brain aging and neurodegeneration in Alzheimer disease. Moreover, we discuss the evidence that amyloid-β, senile plaques, and neurofibrillary tangles may comprise a compensatory defense mechanism against oxidative stress. In addition, the oxidative stress-amyloid-β "cascade" that develops during Alzheimer disease is also described, in which amyloid formation in the brain further exposes neurons

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_10,

to oxidative stress, eliciting a full neurodegenerative response. Finally, we explore how current treatments of Alzheimer disease, such as acetylcholinesterase inhibitors and non-specific glutamate receptor inhibitors/antagonists, may benefit from the inclusion of antioxidants or metabolic agents that target brain aging, mild cognitive impairment, Alzheimer disease, and other neurodegenerative diseases.

Abbreviations

AD	Alzheimer disease
AβPP	amyloid-β protein precursor
CNS	central nervous system
FAD	familial Alzheimer disease
8OHG	8-hydroxyguanosine
•OH	hydroxyl radical
LOAD	late onset AD
MCI	mild cognitive impairment
mtDNA	mitochondrial DNA
PUFA	polyunsaturated fatty acids
PET	positron emission tomography
RNS	reactive nitrogen species
ROS	reactive oxygen species

10.1 Introduction

The "free radical theory of aging" is an accepted hypothesis in current research [1, 2]. Oxidative damage in the brain and body, which originates from reactive oxygen species (ROS) and reactive nitrogen species (RNS), is an inevitable result of aerobic respiration and exposure to ultraviolet rays [3, 4]. Notably, while the central nervous system (CNS) uses 25% of the body's oxygen, it does not have a proportionally increased concentration of antioxidants to counter this load. As such, the brain is subject to high levels of oxidative stress. Additionally, neurons also utilize endogenous neurochemical redox reactions, such as dopamine oxidation [4] and glutamate excitotoxicity. Moreover, the brain, and neurons in particular, contain large amounts of polyunsaturated fatty acids (PUFA) in membranes that are a particularly vulnerable substrate of oxidation [5]. Given these findings, it is not surprising that aging and age-related neurodegenerative disorders are associated with free-radical-mediated damage and are closely related to diminished energy metabolism, trace element toxicity, and excitotoxicity [5, 6]. Oxidative stress is defined as the imbalance between oxidants, such as free radicals, and antioxidants, substances that provide a defense against oxidative damage in cells. Although oxidative stress is well established in aging, as well as in Alzheimer disease (AD), the sources of ROS and RNS, which trigger macromolecular and cellular damage, have not been clearly identified, with some exceptions. One example is redox active metals, such as Fe^{++}, that damage DNA and RNA in vulnerable neurons in the hippocampus (via the Fenton reaction)

and produce the highly reactive hydroxyl radical (•OH) from hydrogen peroxide [7, 8]. Similarly, the amyloid-β peptide found in senile plaques induces formation of hydrogen peroxide. Various other non-specific sources of hydrogen peroxide and the hydroxyl radical, as well as peroxynitrites, oxygen peroxide and many others, are being investigated [6–8]. A current goal is to determine whether endogenous oxidation damages mitochondrial DNA and causes mutagenesis [4] as in the case of dopamine [8].

Three important factors that pertain to the role of metabolic deficiencies in aging and cognitive impairment in mild cognitive impairment (MCI) and AD must also be addressed. First, carbohydrate metabolism is deficient in these disorders; some enzymes, such as pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, both involved in the breakdown of glucose, are lacking and glucose turnover is inhibited [6]. Second, the deficiency in carbohydrate metabolism occurs very early in AD patients with genetic predisposition (20 years before AD was clinically apparent [6]). Third, the cerebral vasculature, the major metabolic exchange surface in the brain, appears to atrophy in AD at about the same time [5]. Notably, the last two findings bring up an essential issue discussed below: genetic predisposition to metabolic deficiencies may be a pathophysiological factor during an individual's entire lifespan, not just one that occurs at the onset of AD symptoms [5–7].

The goal of this article is, then, to propose that oxidative stress is an invariant feature of aging, and, since AD is strongly associated with oxidative stress and aging, that AD reflects one manifestation of life and aging, and is not an illness. This implies that the onset of AD progresses from the beginning of life, becoming clinically apparent when environmental, genetic, and personal factors (diseases, stresses of life, etc.) interact within the brain. As the CNS cannot completely counter oxidative and metabolic change indefinitely, neurodegeneration resulting in AD is one potential outcome [8].

10.2 Clinical and Epidemiological Approach

The clinical diagnosis of dementia from AD is of primary interest to neurologists, psychiatrists, and psychogeriatrists, and provides the most potential for benefit when made in the very early phases of the illness. Accordingly, the DSM-IV and CID-10 are the most widely used criteria for the diagnosis of AD to date [9]. Although many patients present classical symptoms of forgetfulness, word finding difficulties, and slowness in retrieving names, other patients may only present "very mild cognitive changes consistent with their normal aging". Some patients exhibit modest declines in cognition, sometimes for more than 20 years, without suffering any impairment in lifestyle. Finally, some patients present severe, rapid neurodegeneration resulting in death; those having familial Alzheimer disease (FAD) being a prime example [9]. Fortunately, many institutions, such as CERAD, NINCDS-ADRDA work groups, and others, have prepared accurate guidelines and algorithms for diagnosing AD that help physicians reach an accurate diagnosis [10, 11].

Geriatric psychiatrists and other professionals who deal with dementia have provided significant insight into the neuropsychiatric and biological basis of the neurodegenerative processes in AD and similar illnesses [12, 13]. Based on their clinical and research experience, they proposed that aging results in an enhanced vulnerability to the neurodegenerative demented state; for a long time, they referred to these states as "senile dementias". Although this term is considered outdated, we suggest its return to the scientific literature as "demented senility of the Alzheimer type". Interestingly, a study on the populations of elderly people, >80+ years old, from 1980 to 2050 (a statistical projection) [14], predicts an enormous increase in the aged population in the US (from 22 to 102 million), suggesting that it will be the largest age group in the country making dementia and AD increasingly significant in the years to come [15].

Here, we present clinical data, as well as some typically associated situations, related to the events that lead to a diagnosis of AD (i.e., those at the very onset of clinical alterations or in pre-clinical phases). Ultimately, what we conclude is that there is confusion in which the clinician has difficulty understanding what is going on with his/her patient.

Oftentimes, the confusion occurs in the family long before the patient contacts the psychiatrist or geriatrist. The elderly patient's spouse or children prefer, in this situation, to consider him/her as normal; they observe occasional forgetting of some names, objects, words, and nothing more. Occasionally, if the aged individual has an emotional crisis they respond with strange words or phrases. For an extended period of time, the family accepts these instances as normal facets of the aging process. However, as the cognitive and emotional incidents increase with time, the spouse may resort to consulting. This is of limited benefit because the doctor's criteria are not able to provide a definitive diagnosis; he/she might choose to defer any diagnosis and send the patient home as normal with "mild traces of aging" that are also considered normal. Unfortunately, this situation is very common, yet such cognitive decline occurs in almost all people, males and females, who enter the 65–75 year age bracket with a subset that get worse and develop symptoms that permit a diagnosis of either MCI or AD. It is obvious that clear distinctions between "normality," MCI, and "AD" have disappeared and, as a consequence, the doctor's diagnosis is arbitrary. Indeed, in this early phase, he/she has to "decide" who is normal and who is sick, a rather daunting task despite the neuropsychological evaluations, magnetic resonance analyses, and other potential markers of AD. Consequently, clinicians are confronted with an inability to provide an accurate diagnosis. As Scinto and Daffner [9] indicate, this necessitates research into neuropsychological assessment and the identification of more stringent disease markers that might prevent false-positive diagnoses. These authors state that at 65 years of age onward, about 6–10% of the population is diagnosed with AD, and beyond 85 years of age the prevalence increases to 30–47%. [16–18]. Moreover, from 60 years old on, the prevalence of AD doubles every 5 years [19]. As Ritchie and Kildea [17] found in their meta-analysis, the "age-related" evidence of "senile dementia" rather than "aging-related" has no clear statistical correlation; and its exponential increase is a possibility, not a certainty.

Consequently, we could say that, if elderly people do not die from other causes, about 40–50% or more of them would have AD. Recalling that the incidence of a disease, or an event, is calculated in percentages, and that the outcomes are always computed in correlation with normal cases, it is reasonable to extrapolate from the observations of AD incidence in people from 65 to 100+ years old. We may suppose that a cohort of 100 normal elderly people about 65+ years old, randomly chosen, would be assessed for AD, and that none of these people would die until age 100 establishing a closed system. Further, we may suppose that late onset AD (LOAD) is a progressive or final stage of aging and MCI (a hypothesis not yet confirmed or contested). Thus, based on the statistical data presented above:

2 – 65+ year old: AD about 5% = (of 100)= 5 AD. (100–5) = 95 normal
3 – 75+ year old: AD About 10% (of 95) = 9.5 AD. (95–9.5) = 85.5 normal
4 – 85+ year old: AD about 25% (of 85.5)= 21.4 AD. (85.5–21.4)= 64.1 normal
5 – 95+ year old: AD about 45% (of 64.1) =28.9 AD. (64.1–28.9) = 35.2 normal
6 – 100+ year old: AD about 50% (of 35.2) = 17.6 AD. (35.2–17.6) = 17.6 normal

From the above, we observe that by following a group for 35+ years after age 65, and using well established statistics that obey a median profile, we reach the impressive and almost unbelievable result indicating that of 100 healthy individuals—supposing that all live until at least 100 years of age and that no other persons enter this closed system—82.4 persons will have AD and only 17.6 will remain AD-free. Furthermore, if these results are extrapolated to a global scale (i.e., containing 6 billion people), and the same closed-system assumptions were maintained, we would predict the following.

Only 17% of the 6,000,000,000 people (1,020,000,000 people) would not be affected by AD in their 100-year lifetime. Moreover, 4,980,000,000 people would develop AD. However, this incidence would never be realized because millions of aged people die and millions of infants are born every month, effectively negating the closed system model. Additionally, the majority of the elderly die in their seventies or early eighties; thus there is no window for the increasing rates of AD in 85, 95 and 100+ year old people to be manifest. Nevertheless, it is undeniable that AD doubles as age increases 5–10 years in the elderly beyond 65 years of age [19], and that individuals quickly progress from a no-symptoms or AD pathology to a harsh and deadly reality [9], and as such, researchers might entertain the hypothesis that AD is not an illness, but a sign/symptom of aging.

10.3 Neurobiological and Molecular Approaches

Human and animal development is not solely based on the expression of complex macromolecules. Rather, it is the “genomic decision” of when and where these

complex protein machines will be expressed and elicit their functions that also matters, as these aspects constitute the interactions that control life and death. Moreover, transcription itself provides a vital window for this developmental affect: the most significant and adverse effects of the aging process occur due to the damage or bias altering the expression of the huge multitude of factors and co-factors that function during information transfer. Furthermore, the transcriptional macromolecular "machine" is extremely complex: it utilizes about 1000 protein factors and co-factors to control activation and/or repression of specific mRNA synthesis and depends on the three-dimensional interaction of a huge variety of DNA sequences and proteins, with most of the genome maintained in a repressed state [20]. Indeed, this degree of complexity is necessary for providing the redundancy and regulation of the processes required for cellular function [21]. As such, we could question whether it is through this ongoing and lifelong transcriptional activity, with its vast number of regulatory points and its inevitable plethora of biases, deletions, and additions, that the aforementioned defects in cellular function characteristic of aging are elicited. The requirement that the transcriptional machine delivers the right protein to the right place at the right time is critically important for life, and thus, any error or damage to the controlling systems could be serious, if not lethal. Furthermore, mutations, particularly in the mitochondria, increase with age [22], and a simultaneous decay in DNA repair and maintenance systems ultimately culminates in age-related genome alterations [20]. Accordingly, decline of the DNA repair system has a significant role in the theory which states that oxidative stress in aging induces accumulating genome alterations, which are suppressed by genome-maintenance systems [23]. These interactions will be briefly mentioned below.

Although DNA is the carrier of genetic information, it has limited chemical stability. Hydrolysis, oxidation, and nonenzymatic methylation of DNA occur at significant rates in vivo, and are counteracted by specific DNA repair processes. The spontaneous modification of DNA is likely to be a major factor in mutagenesis, carcinogenesis and aging, and also limits the recovery of DNA fragments from fossils. Additionally, reactive products that result from metabolism and radiation may cause DNA damage through oxidative stress [4, 24] that may be detected in urine as glycols (thymine and thymidine), products of the excision of bases in damaged DNA from the repair process. Moreover it is possible to associate oxidative stress with mean life span, as shown in the work of Perez-Campo [2]. His study compared various mammalian species to humans, with respect to several organ systems such as brain, liver, kidney, and fibroblasts, and concluded that the rate of free radical production may determine the rate of aging (Fig. 10.1).

Mitochondria, the main target of oxidative stress in susceptible neurons, were found to be significantly decreased in number in AD, suggesting that oxidative stress may be fundamental to the development of this neurodegenerative disease [25–27]. Additionally, the absence of neurofibrillary tangles in neurons exhibiting mitochondrial damage and energy deficiency places mitochondrial abnormalities as the earliest cytopathological changes in AD [28]. Another common change is the reduced numbers of microtubules in AD, which impair mitochondrial transport to the axon. Notably, mitochondrial damage, improper transport, and the resulting

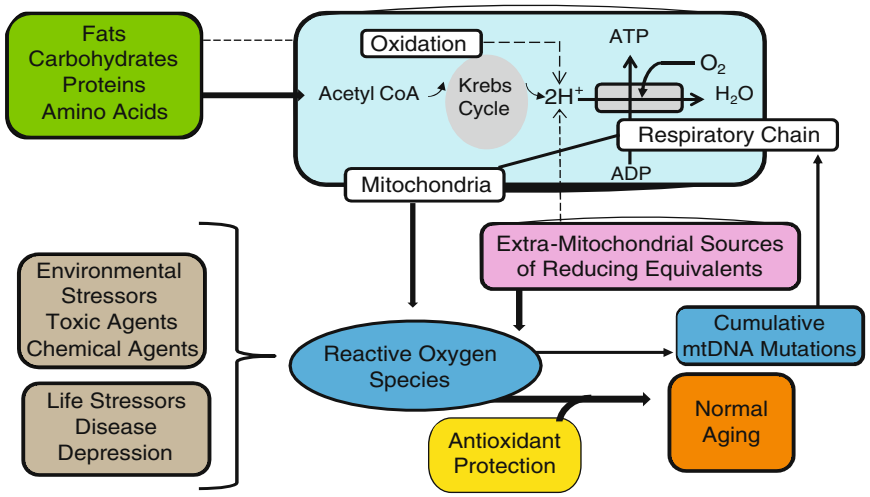

Fig. 10.1 In the metabolic energetic aging cascade, generally the first half of life, stochastic mitochondrial DNA damage mutations, induced by metabolic and energetic oxidation, may find a robust antioxidant resistance to the free radicals generated, and normal aging follows its course (see text)

decrease in energy production, are consistent with early processes that potentially occur before amyloid-β deposition [25, 27, 28]. These structural and functional abnormalities, found in neurons lacking neurofibrillary tangles, confirm that mitochondrial damage is a primary pathology of neurodegeneration in AD. Other changes in AD may also be linked to mitochondria, because reduction of mitochondrial energy production [29] shifts amyloid-β protein precursor (AβPP) metabolism to produce more amyloidgenic forms of amyloid-β [30, 31], induces the production of A68 antigen, and stimulates the mitogen-activated protein-kinase pathway [32], all hallmarks of AD. Furthermore, a continuous, slow, and insidious accumulation (with aging) of mitochondrial DNA (mtDNA) mutations, as a result of ROS and RNS-induced neuronal injury, may lead to a vicious cycle whereby errors in the mtDNA-encoded polypeptide chains are randomly transmitted during mitochondrial and cell division, and where the consequent decrease in electron transfer, decline in oxidative phosphorylation, and reduced protein synthesis, results in higher ROS and RNS production and further neuronal damage [33–35]. Notably, mutations in the mitochondrial genome occur at a much higher rate than in nuclear DNA, leading to heteroplasmy of mtDNA in a random pattern of cytoplasmic segregation—a recently recognized factor in the aging process and neurodegenerative disorders [36].

Mitochondria generate more than 95% of the superoxide anions (O_2^-) produced during normal metabolism through the electron transport chain in the inner mitochondrial membrane. Recent estimates suggest that about 1–2% of the total molecular oxygen utilized by mammalian mitochondria is converted into reactive species such as superoxide [37, 38]. Hence, mitochondria are one of the main cellular targets of ROS induced oxidative damage, and, in fact, relatively

high levels of oxidized nucleic acids, proteins and lipids are detected under normal metabolic conditions [26]. Even so, some authors disagree with this cascade hypothesis and focus instead on mutations in nuclear DNA and the sequelae for causing the rise of ROS and RNS specific aging damage [27, 39]. In either case, however, antioxidants may be protective against neurodegenerative diseases, although they cannot stop, nor control, the rate of aging. Furthermore, the level of mitochondrial oxidants varies in experimental animals; those with long life spans have less ROS derived-oxidants than those with short life spans, and it is known that the rate of pro-oxidant production in different species is inversely correlated with their maximal life expectancy [35]. This corroborates recent findings that anti-oxidants are in higher concentrations in the brain mitochondria of long-lived animals than in short-lived ones. Thus, the rate of oxygen radical attack on mtDNA must be a key factor in animal longevity [25]. That is, DNA damage is repaired at less than 100% efficiency, so DNA damage accumulates with age; consequently, the higher rate of mitochondrial ROS production in short-lived animals must be responsible for their much faster rate of mtDNA mutations. In chimpanzees, this accumulation becomes important after 50 years of age, whereas in mice this occurs after only 2–3 years of age [25, 28]. In humans, this accumulation becomes significant after 70–100 years, a time during which AD, incidentally, first becomes apparent. It now becomes apparent that neurodegenerative diseases, like AD, occur as a consequence of chronic oxidative stress (Fig. 10.2).

Recent studies have shown that oxidative stress is indeed an earlier metabolic process than other pathologies in AD and other neurodegenerative diseases [31], probably due to diminished metabolism during aging and increased ROS generation [40]. These authors report in particular that AD patients exhibit a decline in glucose metabolism and a decline in ATP energy release in the citric acid cycle and in the electron transport chain. Notably, all these age-related changes are manifest in mitochondria, which themselves diminish in number, and create a further increase in free radical production. This dysfunction in mitochondria is perhaps a consequence of DNA vulnerability: its chemical instability subjects it to oxidation, hydrolysis, nonenzymatic methylation, and decay, ultimately eliciting mutagenesis and potentially carcinogenesis or neurodegeneration [23]. Furthermore, the detrimental oxidative cycle is completed as DNA-induced oxidative stress damages mitochondria, which produces additional damage to DNA, and so on. The mechanism of DNA-driven neurodegeneration is still under investigation [41]. We suggest that these stochastic mitochondrial DNA damage mutations continue to accumulate and perpetuate throughout an organism's entire life span, essentially providing the foundation for AD pathology.

These conclusions have been drawn from the investigation of the relationship between neuronal 8-hydroxyguanosine (8OHG) and histological/clinical variables characteristic of AD. 8OHG is a product of DNA damage that is produced from oxidative stress, particularly so within the mitochondria. Concordant with our hypothesis, oxidative stress is present long before deposition of amyloid-β and clinical manifestations of AD [40]. Moreover, it has been shown that oxidative stress decreases with disease progression, particularly when senile plaques and

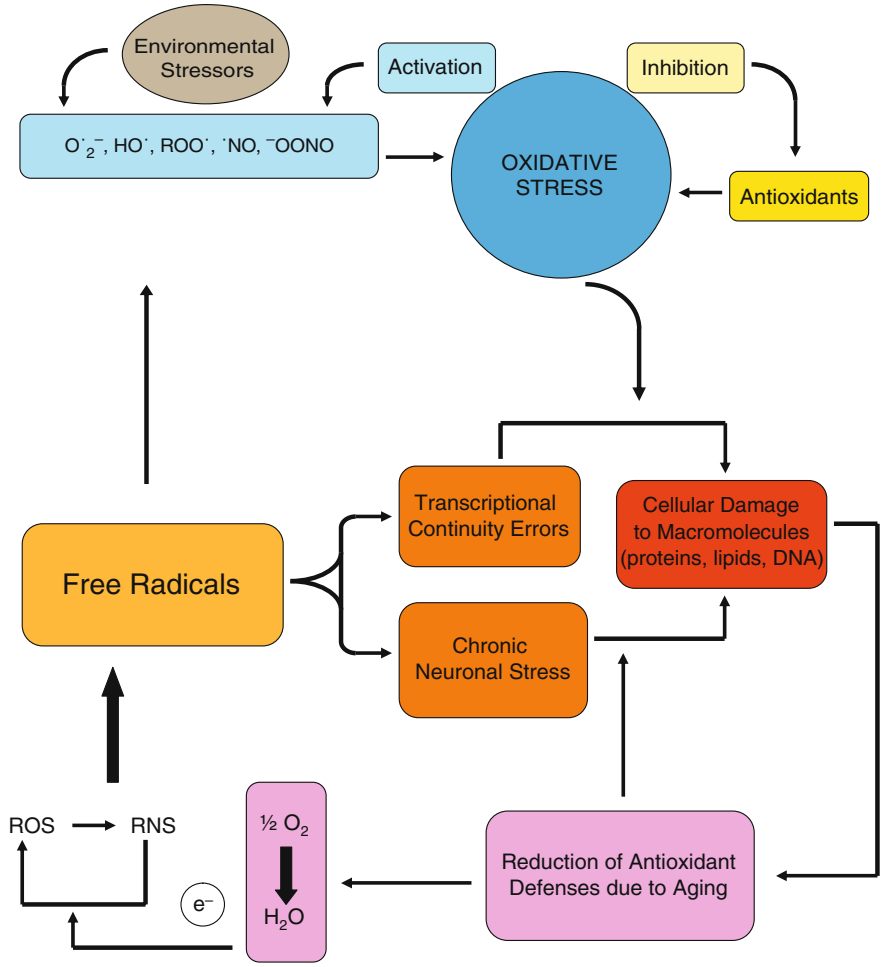

Fig. 10.2 In the neurobiological oxidation aging cascade, continuous aging adds a new factor to DNA lesions. Oxidative metabolism and stress induce stochastic mitochondrial DNA damage mutations, transcriptional and translational continuity mutation biases, and the slow neuronal oxidation stress interacts with the free radicals from long term energetic oxidation. These effects not countered by the declining antioxidant system may increase the risk of neurodegeneration (see text)

neurofibrillary tangles become prevalent, and that AD is associated with compensatory changes that would act to reduce damage from reactive oxygen [40]. These recent observations have important implications in the understanding of AD development. That is, if oxidation reactions, with their generation of free radicals, are present from the beginning of life, and if this is a physiologically natural occurrence, then oxidative stress should always be present in the brain, and should increase progressively throughout life. Moreover, although neurons appear to survive and

function in presence of high oxidative levels (as indicated by the delayed onset of AD pathogenesis), there must still be a surplus generation of hydroxyl radicals, mitochondrial dysfunction [28], redox-active metals [42], and increased intraneuronal amyloid-β deposition and tau hyperphosphorylation [43, 44] that progressively produce neuronal insults and induce degeneration. Further, many studies have suggested that defective glucose metabolism, leading to ATP energy decline, is one of the earliest and most fundamental signs of AD [29, 45]. Positron emission tomography (PET) [46, 47] has also demonstrated deficits in energy metabolism, and other studies have reported that specific areas of the brain, such as the posterior cingulate, temporal, parietal, and prefrontal cortexes of AD and MCI patients, exhibit reduced glucose metabolism [48, 49].

As such, it is increasingly apparent that the neurodegeneration characteristic of AD is actually the natural and inevitable result of cellular alterations such as DNA and RNA damage, enzyme dysfunction, glucose metabolism, and mitochondrial decline. While these changes accumulate throughout an individual's lifetime, however, their associated clinical manifestations are not apparent until well into old age, and this fact severely complicates AD diagnosis and treatment. With this new understanding, we can hopefully institute earlier novel treatment regimens to delay or retard this devastating progressive degenerative condition.

10.4 The Amyloid-β Dilemma

Our proposal challenges the classical idea that amyloid-β and tau protein are the most significant pathological effecters of AD. Rather, because we now know that these markers of AD are preceded by oxidative damage, we must re-examine their role in AD development. Indeed, it has been shown that both peptides possess protective/antioxidant properties [50]. Amyloid-β peptide, in particular, has an antioxidant role, as detected through the levels of 8OHG [51] within neurons. High 8OHG neuronal levels point toward oxidative stress, while an increase in amyloid-β deposition in cortex is associated with decreased 8OHG, and thus a lesser amount of oxidative stress [52]. Furthermore, amyloid-β is a redox-metal chelator and scavenger; senile plaques may therefore be a compensatory response to oxidative stress, reducing its intensity and damage, similar to what is seen in Down's syndrome [53].

However, identifying the exact cellular function of amyloid-β is confounded by its seemingly contradictory effects. That is, despite its aforementioned properties, it also induces oxidative stress, reduces redox metals to their high valence state, and inactivates enzymes through damaging DNA, membrane lipids and lipoproteins [54]. However, it is only when methionine residue 35 on amyloid-β and active redox metals are involved, that oxidative stress develops and initiates the "amyloid-β cascade" [42]. Further, it is undeniable that tau, AβPP, presenilin 1, presenilin 2, and apolipoprotein E are implicated in clearing, transporting, and chaperoning functions [55]; it is only when the aging process is well underway that these proteins exhibit

pathological manifestations characteristic of AD, such as deposition of amyloid-β and hyperphosphorylated tau. Therefore, our model (Fig. 10.3) can be described as follows.

The slow and life-long oxidative stress that occurs in aging, along with the irreversible and cumulative DNA damage processes responsible for stochastic mitochondrial DNA damage mutations, may interact with some critical environmental, social, and psychological stresses that further increase the concentration of oxidative free radicals and produce consequent mutations. During old age, these dynamic interactions, in an already weakened organism, will again act on the long-life transcriptional continuity progression to elicit additional genomic errors and mutations in vital cell signaling systems, such as antioxidant defenses. If genetic vulnerability exposes genomic hereditary pathogenic DNA mutations to essential compensatory proteins against oxidative stress, such as AβPP, presenilin 1, presenilin 2, apolipoprotein E, and tau, there will be a variable aggregation of the amyloid-β peptide and the hyperphosphorylated tau protein in neurons, glia and endothelial vascular cells in the CNS. The aggregates of amyloid-β and hyperphosphorylated

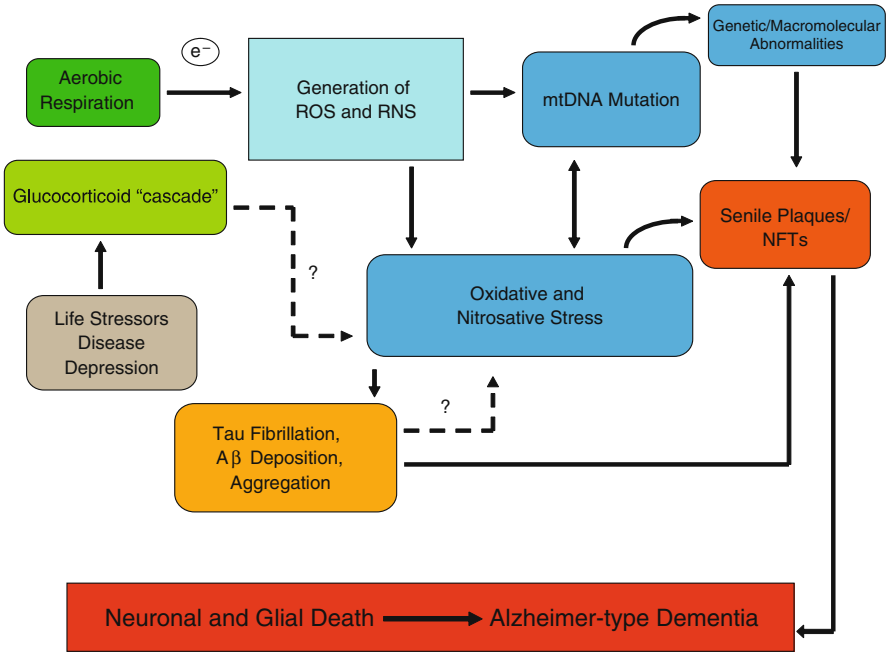

Fig. 10.3 The oxidative stress-amyloid-β aging cascade results from a feedback molecular process between pathological aging (the continuing mitochondrial mutations interacting with an oxidative stress defense system), in addition to the already low antioxidant buffering. Together with the increased deposition of amyloid-β and hyperphosphorylated tau resulting from genomic hereditary pathogenic DNA mutations, aging may lead to neuron and glial death progressing to "demented senility of the Alzheimer type"

tau are a source of additional oxidative stress, closing a life-long and irreparable circle of neurodegeneration: the "oxidative stress- amyloid-β aging cascade", changes the progression from "healthy senility" to "demented senility of the Alzheimer type".

As an illustration of said relationship, we will describe the example of phosphorylated tau isoforms, which have important roles in neurodegenerative disorders as well as in normal brain, as they correspond to oxidative stress. Functionally, tau binds microtubules through microtubule-binding domains, and the assembly and stabilization depends, in part, on their degree of hyper-phosphorylation [56]. Other important functions of tau are in the membrane anchoring of enzymes, and the modulation of the functional organization of the neuron, particularly in axonal morphology, growth and polarity [57]. Interestingly, intraneuronal accumulations of fibrillary materials, neurofibrillary tangles being the most common, may be found in normal aging, since hyperphosphorylated tau exerts protective functions upon the neurons, and some small parcels of the hyperphosphorylated protein may then slowly aggregate. Furthermore, it has been shown that oxidative stress and byproducts of tau modification lead to protein aggregation (neurofibrillary tangles), enabling neurons to survive for decades [58]. Indeed, tau may function as a buffer against neurodegeneration and oxidative stress, and it is not surprising that the precedent long-life oxidation process, with the oxidative stress damage of mtDNA and nDNA genomes, leading to heavy changes in the kinase signaling pathways, may pave the way for, and lead to, hyper-phosphorylation of tau. Like amyloid-β peptide senile plaques, however, hyperphosphorylated tau neurofibrillary tangles are strong pro-oxidant factors as well, both producing ROS and RNS, subsequently increasing oxidative stress, (once they surpass a threshold level of cellular antioxidant defenses). The oxidative stress-amyloid-β aging cascade thus begins with the deposition of significant amounts of senile plaques and neurofibrillary tangles and accelerates aging in healthy elderly people, leading to the significant, but "normal" cognitive decline. Alternatively, in the case of an interaction with the genome hereditary pathogenic DNA mutations carried by predisposed elderly individuals with a frail transcriptional continuity genome mutations, deposition of neurofibrillary tangles becomes severe, leading to senility of the Alzheimer type [32].

Finally, it is important to mention that the understanding of how both genomes, nuclear and mitochondrial, interact in the biogenesis of mitochondria, and in neurodegenerative diseases, remains controversial [59]. A series of studies has indicated mitochondrial DNA mutations as being the origin of some human genetic diseases as well as that of the ailments that occur in senescence [60–62].

That being said, however, it is certain that oxidative damage is increased in AD, and is especially elevated in AD-brain mtDNA. The level of 8-hydroxy-2′-deoxyguanosine in postmortem brain tissue, in particular, was found to be three fold higher in AD patients than in controls. The same measurement of 8OHG in nDNA, although higher in AD than in controls, was present at a significantly lower concentration than in mtDNA. These data confirm that both nuclear and mtDNA are sensitive to oxidative damage which may contribute to the neurodegenerative process [60].

Age dependent damage and deterioration of respiratory enzyme activities may be consistent with neurodegenerative diseases, and there is a possibility that these events constitute the very same processes [63]. One of the consequences of this damage is the age-related loss of hormonal balance that offsets the homeostatic calcium-energy-antioxidant interaction. This interaction, in turn, provokes enhanced amyloid-β protein formation and tau protein hyperphosphorylation during normal aging.

10.5 Depression, Oxidative Stress, and Alzheimer Disease

The most frequent environmental/psychological factors that induce oxidative stress, particularly in the aged, is chronic depression and stress responses. Despite the lack of research on oxidative stress in major depression and/or stress syndromes, it is not difficult to make the following hypothesis in relation to the possible contributing factors of its molecular and neurobiological mechanisms.

Psychoneuroendocrinologists have known for more than two decades that acute and chronic stress disturbs the hypothalamus-pituitary-adrenal axis and increases corticoid concentration in the hippocampus, medial frontal lobe, amygdala and frontal cortex [64], and such consequences persist for long time even after the depression is remitted [65]. The resulting "corticoid cascade" occurs because the corticotrophin releasing factor is unrestrained due to the attenuated atrophic hippocampus's inhibition of the corticotrophin releasing factor hypothalamic neurons. Notably, psychological stress and depression have a clinically demonstrated relation. Nevertheless, in spite of an incomplete knowledge as to which causes which [66, 67], they induce an excessive corticoid-dependent stimulation of glutamate—about four fold higher than normal—and, consequently, an increase in intracellular Ca+ levels which generates neurotoxic and cell death effects [68]. The other important result of increased glucocorticoid secretion during stress is decreased glucose uptake [69] and, accordingly, a declining energy supply [70]. As discussed above, this low level of neuronal energy is insufficient to clear the high level of glutamate and to halt the continued Ca+ influx. High intracellular calcium levels consequently generate ROS and RNS [71], exceeding the antioxidant defenses; once the threshold has been surpassed oxidative stress damage occurs in hippocampal cells. In addition, brain-derived neurotrophic factor is down regulated in chronic stress/depression, in some hippocampal areas, where it influences neuronal survival, differentiation, and synaptic strength [72, 73]. Thus, reduced levels of brain-derived neurotrophic factors may contribute to the atrophy of the hippocampus, and to mitogen-activated protein kinase-signaling malfunction, thus liberating the antiapoptotic factor protein Bcl2 [72, 74]. If chronic depression/stress syndromes indeed induce oxidative stress, it is not difficult to show that depression and oxidative stress may be deeply involved in AD [75] (Fig. 10.4). As such, we can investigate the following: induction of oxidative stress by chronic depression and psychological stress via increased intracellular levels of glutamate and calcium; increased calcium levels

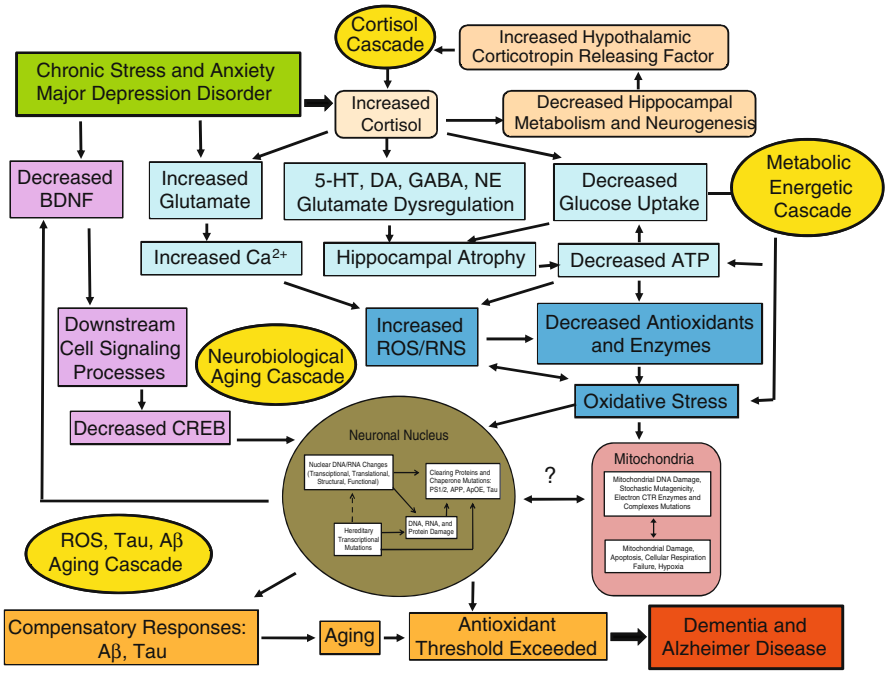

Fig. 10.4 Lifelong stress can induce aging and disease. An example of the "cortisol cascade", the result of chronic stress or major depression disorder in the life of an individual, after interacting with the "oxidation stress aging cascades", may result in demented senility of the Alzheimer type (see text)

are a mechanism of ROS and RNS free radical formation; oxidative stress may increase as antioxidants and the antioxidant defense mechanisms decline with age; stochastic mitochondrial DNA damage mutations are accelerated by depression and stress; and, if some continuity transcriptional DNA stochastic mutations occur, the probability of AD will increase in direct relation to genetic hereditary pathogenic DNA mutations. These hypotheses should be explored in the near future to inform investigation into new therapeutic measures for treating AD.

10.6 Conclusions

We have presented some challenging questions in an attempt to unveil the intimate relationship between aging, oxidative stress and AD. Indeed, we have shown that this relationship is so deep that it is possible to compose a model which places all these processes together as a unique, progressive and changing phenotype that we can refer to as the general aging process. To facilitate understanding of this life-long neurobiological and molecular process, we have split it into generalized phenotypes

or molecular "cascades"; these are by no means final, but rather provide a framework for future investigation. We can state, however, that oxidative metabolism and stress are the earliest processes that lead to aging and cognitive decline, as well as in the demented senility of AD. While the process as a whole is an irreversible cycle of escalating neuronal stress and insult, compensatory mechanisms exist throughout and promote the organism's existence and lengthen its life span. Specifically, the young organism counters oxidative metabolism and stress by increasing antioxidant defenses, and attenuates the stochastic mitochondrial DNA damage mutations by maintaining the metabolic energy state and preserving homeostasis. As such, we named this first molecular phenotype "metabolic-energetic aging cascade". As aging goes on, the concentration of free radicals increases and oxidative stress leads to mutations in mitochondrial DNA; these interact with genomic predispositions (possibly originating in continuity transcriptional errors) and induce DNA mutations and antioxidant concentration decreases. We consequently call this closed circle of events a "neurobiological oxidation aging cascade". Finally, the chronic stresses of life, including psychological ones, may exacerbate oxidative stress-induced DNA damage, and when there are genomic hereditary vulnerabilities, neurodegeneration is inevitable. Eventually, mutations in essential, compensatory proteins result in deposition of amyloid-β and tau that ultimately provokes robust oxidative stress and perpetuates the aforementioned neurodegenerative cascades. In this phase, which we suggest to be in the last quarter of life, "demented senility of the Alzheimer type" is probable. We refer to this process as the "oxidative stress-amyloid-β aging cascade".

Based on the primacy of oxidative stress and mitochondria in neurodegeneration there is a clear need for therapeutics aimed at augmenting or delaying the decline in the body's antioxidant defenses. Additionally, given the temporal sequence of events, effective therapy will likely require early intervention. Thus, the development of sensitive early diagnostics for individuals at risk of developing AD is imperative.

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Chapter 11
Carbon Nanotubes as Electrical Interfaces with Neurons

William Lee and Vladimir Parpura

Abstract Carbon nanotubes (CNTs) are emerging as promising nanomaterials for biomedical applications. Due to their unique structural, mechanical and electronic properties, CNTs can be used as electrical interfaces with the brain in particular with neurons. CNT-based neural interfaces/electrodes have been employed in cell culture and in vivo; they offer advantages over standard metal-based electrodes in terms of monitoring and stimulation of neuronal activity. One of the challenges for interfacing brain and machine is the biocompatibility of the materials used for electrode construction. While CNTs appear biocompatible, the exposure limits have not been set thus far. An appropriate (inter)national standards/rules for the use of CNTs need to be established before CNT-based electrodes/devices can be used in human subjects.

Abbreviations

BBB	blood-brain barrier
CNTs	carbon nanotubes
DRG	dorsal root ganglion
EEG	electroencephalogram
ERP	event-related potentials
MEA	microelectrode array
MWNTs	multi-walled CNTs
PPy	polypyrole
RGCs	retinal ganglion cells
SWNTs	single-walled CNTs
TiN	titanium nitride

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_11,

VACNFs vertically aligned carbon nanofibers
3D three-dimensional

11.1 Introduction

Carbon nanotubes (CNTs) are emerging as one of the most promising nanomaterials for applications in electronics, aerospace and biomedicine. In this chapter we discuss the use of CNTs as electrical interfaces with the brain in particular with its electrically excitable cellular components, neurons. We begin with a primer on CNTs unique structural, mechanical and electronic properties, which have captured the attention of physicists, chemists and material scientists and prompt the use of CNTs in biomedical applications (Section 11.2). This is followed by a discussion of a subset of experimental approaches using CNT-based neural interfaces in cell culture and in vivo to illustrate the advantages that CNTs can offer over standard metal-based electrodes in terms of monitoring and stimulation of neuronal activity (Section 11.3). Finally, we briefly discuss biosafety of CNTs and raise the concern as to the lack of exposure limit guidance to date (Section 11.4).

11.2 Primer on Characteristics of CNTs

Detailed description of the structure, properties and modification/functionalization of CNTs is available elsewhere [1, 2]. Briefly, CNTs are composed of graphene sheets rolled into cylinders, which have a hollow core. The cylindrical ends can be capped with a fullerene dome. Based on the number of concentric graphene cylinders within CNTs, they are classified into single-walled CNTs (SWNTs), double-walled CNTs, or multi-walled CNTs (MWNT). SWNTs commonly have their diameters between 0.7 and 2 nm, although their diameter down to 0.4 nm have been reported [3, 4]. MWNTs have an outer diameter that typically ranges from 2 to 100 nm, while the inner diameter varies between 1 and 3 nm. The length of synthesized CNTs is typically in the μm range, although SWNTs up to 4 cm have been reported [5]. CNTs have an exceptional mechanical strength with a Young's modulus of ~1 TPa. They are chemically relatively inert and non-biodegradable. In addition, CNTs exhibit unique electrical properties. CNT conductivity is endowed by the conformation of their hexagonal graphene lattice, which can be arm-chair, zig-zag, or chiral. They can be metallic or semi-conductive. In metallic CNTs, the graphene hexagonal lattice can be arranged in any of the three configurations, with all arm-chair CNTs being metallic. In semi-conductive CNTs, the lattice can be arranged in a zig-zag or chiral configuration. The combined physical properties make CNTs a durable nanomaterial for bio-engineering, especially in applications where a sustained presence of the material is desirable, such as stimulation/recording electrodes for interface with neural elements as discussed below.

11.3 CNT-Based Neural Interfaces for Stimulation and Monitoring of Neuronal Activity

An in-depth review on neural stimulation/recording electrodes is available elsewhere [6]. One of the challenges in designing electrodes for neural interfaces is to maximize delivery of electrical stimulation to cells with high selectivity, while minimizing tissue damage. In recording mode, electrodes with high sensitivity, as evidenced by high signal to noise ratio, are desirable. Amongst the nanomaterials available to date, CNTs display desirable properties for use in stimulation/recording electrodes. (i) CNT-based electrodes have been successfully miniaturized while they do not seem to inflict tissue damage. (ii) CNTs have the ability to operate as ballistic conductors which aids in lowering impedance and increasing charge transfer. (iii) CNTs display exceptional flexibility and they can be twisted and bent to a large degree, although they are five times mechanically stronger than steel [7]. These traits are advantageous for materials to be used for microelectrodes that would penetrate through the tissue. Such properties of CNTs allowed for their use in stimulating and monitoring of neuronal activity at various levels of spatial domains [see definition of levels in Ref. [8]], which includes (i) stimulation of action potentials/ Ca^{2+} excitability in a small group of neurons in culture using CNT films of multi-electrode arrays, (ii) stimulating and recording from neurons in hippocampal organotypic slice cultures as well and in the whole mount mouse retina, (iii) stimulation of and recording from rat and monkey cortices, and (iv) recording human electroencephalogram (EEG) through a CNT-based attachment to the superficial skin layer. We describe below a subset of experimental approaches demonstrating such usage of CNTs.

Liopo et al. [9] demonstrated the ability to electrically stimulate neural cells directly through a CNT substrate. In this study, the neuroblastoma x glioma NG108 cell line or rat dorsal root ganglion (DRG) cells were cultured on planar and transparent SWNT films deposited onto overhead transparencies, i.e. polyethylene terephthalate sheets (Fig. 11.1a). NG108 cells and DRG neurons were subjected to electrophysiological recordings using a whole-cell patch clamp configuration to monitor the electrical activity of individual cells. These cells were then electrically stimulated either via a patch pipette or through a conductive SWNT film (Fig. 11.1b and c). Recorded currents due to two different stimulation methods appear qualitatively similar, indicating that an SWNT film can be used as a stimulation platform. Subsequent studies have demonstrated that various planar CNT films can be used for cellular growth and direct electrical stimulation of cultured primary neurons [10, 11], NG108 cells [12] and differentiated neural stem cells [13]. The ability of CNTs to deliver electrical stimulation to neurons can be attributed to their conductivity and their intimate contacts with neurons as revealed by electron microscopy [10–12, 14]. It should be noted, however, that while whole-cell patch clamp allows stimulating the same cell that is recorded from, CNT film stimulation excites the entire population of cells that are residing on the film which also serves as a planar growth scaffold/substrate. Since CNTs films/deposition is amenable to miniaturization, one possible solution for achieving the use of CNTs for

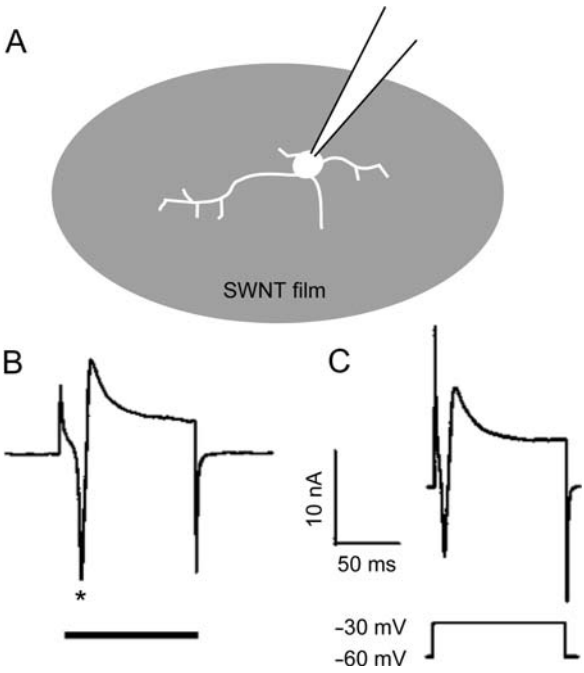

Fig. 11.1 Electrical stimulation of dorsal root ganglion (DRG) neurons via a planar SWNT film. (**a**) Schematic representation of an experimental setup. DGR neurons grown on SWNT films (*grey*) were subjected to electrophysiological recordings using a whole-cell patch clamp configuration. (**b**) Stimulation (direct current, *horizontal bar*) of a DRG neuron through the SWNT film causes an inward current (*asterisk*), similar to that seen when voltage steps were applied via a patch pipette (**c**). (**b–c**) Modified from [9], with permission

recording/stimulation of individual neurons, or a small group of these cells, within the network is to generate a so-called microelectrode array (MEA).

Wang et al. [15] developed a CNT-based MEA comprised of pillars made of vertically aligned conductive MWNTs (Fig. 11.2). The size, the geometry and location of the CNT pillars can be precisely controlled by lithography. CNT pillars electrodes have rectangular (30×30 μm, 50×50 μm, or 100×100 μm) or circular (50 μm in diameter) geometry with a height of 40 μm (Fig. 11.2a–c). Pillars were integrated onto a pre-patterned microcircuit and they were individually addressable. These electrodes have high charge injection capacity and operate without faradic/electrochemical reactions, that otherwise can lead to irreversible damage of the electrodes and surrounding tissue. Thus, they represent a prototype for efficient and biocompatible interfacing for neural prosthesis. Indeed, the authors demonstrated a potential of the MEA approach for neuronal stimulation by using cultured neurons. Dissociated hippocampal neurons were plated onto MEAs. Cells displayed viability and neurite outgrowth consistent with the previously shown biocompatibility of MWNTs [16, 17]. Rather than directly recording electrical activity of neurons, the authors assessed neuronal intracellular Ca^{2+} excitability due to electrical stimulation via CNT pillars. For dynamic Ca^{2+} imaging a fluorescent Ca^{2+} indicator was used. Since an inverted microscope was used while CNTs were nontransparent, the fluorescence emission from cells directly on the CNT pads could not be visualized. However, neurons that were in near proximity of stimulating electrodes

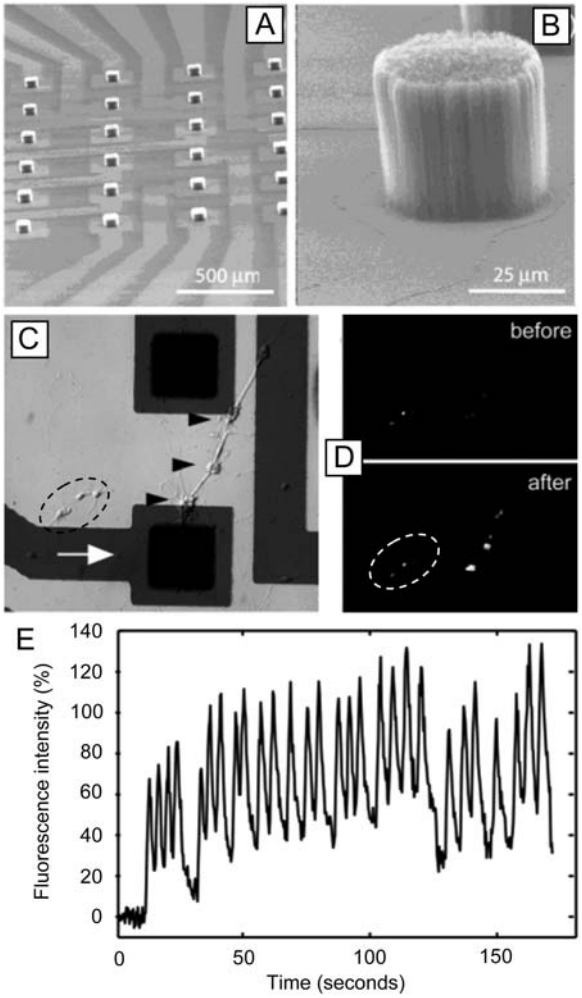

Fig. 11.2 Electrical stimulation of hippocampal neurons grown on an MWNT-based microelectrode array (MEA). (**a**) CNT-based MEA comprised of pillars made of vertically aligned conductive MWNTs and having either rectangular (30 × 30 μm) or (**b**) circular (50 μm in diameter) geometry with a height of 40 μm. (**c**) Pillars are integrated onto a pre-patterned microcircuit and they are individually addressable. Hippocampal neurons grown on MEA (100 × 100 μm pillars) were electrically stimulated (*white arrow*). (**d**) Neurons loaded with a fluorescent Ca^{2+} indicator before and after electrical stimulation, which causes the increase in intracellular Ca^{2+} levels in neurons in contact with each other and a CNT pillar (*black arrowheads* in **c**). Note that neurons that were not in contact with CNT pillars show no changes in intracellular Ca^{2+} excitability (*left, dashed oval*; also see **c**). (**e**) Time course of intracellular Ca^{2+} dynamics due to repetitive cell stimulation. Modified from [15], with permission

showed increases in intracellular Ca^{2+} levels seen as the increase in the fluorescence intensity (Fig. 11.2d). Repeated stimulation paradigm caused transient increases in intracellular Ca^{2+} levels (Fig. 11.2e). In contrast, neurons that were not in contact with CNT pillars show no changes in intracellular Ca^{2+} excitability. Taken together, these experiments show that MEA made out of CNTs have potential for use in tissue and show promise as bio-compatible and efficient electrodes.

Yu et al. [18] used vertically aligned carbon nanofibers (VACNFs), a form of carbon material closely related to MWNTs, to generate MEAs which they utilized to stimulate neurons and record from these cells in cultured organotypic hippocampal slices. In this study, 40 individually addressable VACNF electrodes, 10 μm in height and spaced 15 μm apart, were arranged in a linear array with a total length of 600 μm (Fig. 11.3a). Individual VACNF electrodes assumed a cone-like geometry, which aids their penetration of the tissue leading to improved electrical interface with neurons (Fig. 11.3b). VACNF electrodes had effective radius of up to ~17 μm [see Fig. 2 in Ref. [18]]. Although such size of VACNF electrodes is smaller than traditional metal surface electrodes, the electrical noise level recorded for VACNFs electrodes was comparable to that of various metal-based MEAs reported elsewhere

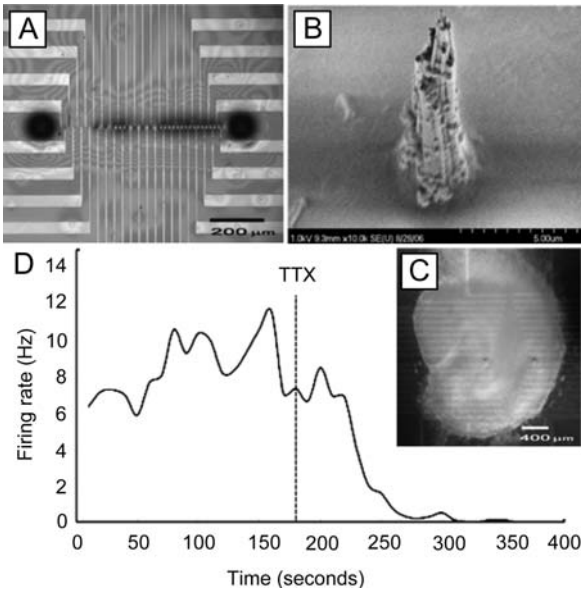

Fig. 11.3 Vertically aligned carbon nanofibers (VACNFs) forming MEA record spontaneous electrical activity within the slice. (**a**) Individually addressable VACNF electrodes were arranged in a linear array (light micrograph). (**b**) An individual VACNF electrode assumes cone-like geometry (electron micrograph). (**c**) A hippocampal slice on a VACNF MEA (light micrograph). (**d**) Spontaneous action potential discharges/firing from a slice recorded by MEA is sensitive to tetrodotoxin (TTX; the time point of application indicated by the *vertical dashed line*), a blocker of voltage-gated Na^+ channels, confirming a neuronal source for spontaneous activity. Modified from [18], with permission

[see detailed comparison on p. 2190 of Ref. [18]]; the average noise level was inversely proportional to the electrode dimensions. The recording noise level of VACNF MEAs assessed in solution was favorable to enable extracellular recordings from cultured organotypic hippocampal slices. Prior to use of VACNF MEA for recordings from slices, the chips were treated with a mixture of cell adhesion permissive substrates poly-l-lysine and laminin to aide the adherence of slices to the chip. Slices cultured separately were then applied onto chips (Fig. 11.3c) and were held in place using a nylon mesh. Spontaneous electrical activity was simultaneously recorded in CA3 pyramidal and dentate gyrus granule cell layers; this activity could be blocked by tetrodotoxin, a blocker of voltage-gated Na^+ channels underlying action potentials producing spikes in the recordings (Fig. 11.3d). Conversely, the removal of the inhibitory inputs by the addition of bicuculline, a blocker of gamma-amino butyric acid receptors type A, resulted in epileptiform activity. In addition to recording of spontaneous neuronal electrical activity, the application of stimuli between two VACNF electrodes resulted in evoked field potentials. Taken together, this study demonstrated that VACNF-based MEAs can deliver stimuli to the tissue and record from it with improved spatial control compared to CNT films. The three-dimensional (3D) cone-like protrusions offer recordings from single units with amplitudes doubled from those seen in metal-based MEAs [19]. A lingering issue with the use of VACNF-based MEA, shared with conventional metal-based electrodes, is the rigidity of their surfaces. Namely, neural cells display sensitivity to the mechanical stiffness of the scaffold [20, 21]. Consequently, to improve the use of VACNF MEAs, Nguyen-Vu et al. [22] implemented VACNF brush-like electrodes that have been additionally coated with the conductive polymer polypyrole (PPy). Such co-deposition approach found applications when using CNTs for recordings of neuronal activity in vivo [see below Ref. [23]].

Shoval et al. [24] implemented the use of MWNT MEAs, which were produced and packaged as previously reported [25], to record from whole-mount retinas. Here, the deposition of CNTs onto titanium nitride (TiN) patterned substrate results in highly conductive and porous/rough CNT islands/electrodes with low impedance [25], which represent a good cell-adhesive surface as neurons entangle into a 3D CNT matrix [26]. Bare TiN electrodes were designed to be porous achieving high surface area and low impedance as well. Freshly isolated whole mount retinas where placed onto MEAs with the retinal ganglion cells (RGCs) layer facing down. It should be noted that RGCs represent the output cells from the retina encoding the information transfer to the cortex by the frequency of their action potential discharges. In many of the retinal dystrophies, these cells may remain intact to transmit information, while photoreceptors degenerate. Consequently, there is an urge to develop retinal implants that would by pass photoreceptors and directly opto-electrically couple to RGC. Nonetheless, whole-mount retinas were restrained onto chips using a polyester membrane filter. For comparison two chips were used: bare TiN MEAs and MEAs containing additional coating with CNTs. In both cases, some of the CNT or TiN islands on the chip were not electrically accessible, thus do not represent any of the 60 active electrodes within the MEA (Fig. 11.4a). These “spare” islands appear to assist in stabilizing the whole tissue. All islands

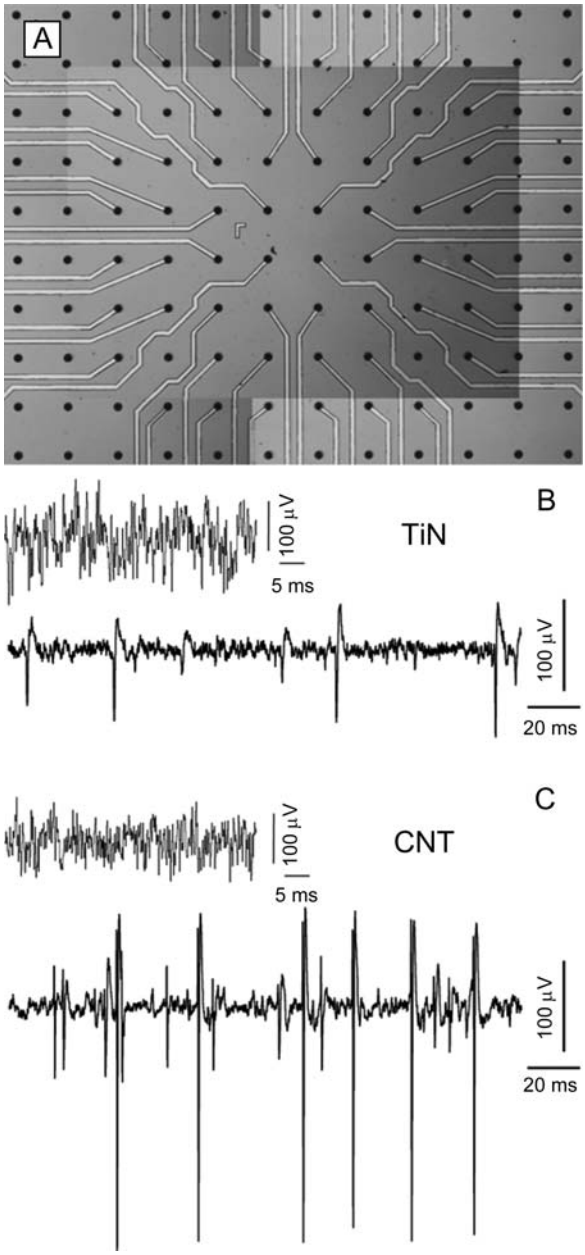

Fig. 11.4 Electrical recordings of neural activity from the whole-mount retina using an MWNT MEA. (**a**) CNTs islands (*black dots*) deposited onto titanium nitride (TiN) patterned substrate. Note that not all CNT island are electrically active, since they are not connected to individual leads. (b**–c**) Recordings obtained from a whole-mount retina using a bare TiN and an MWNT-coated hybrid electrode, respectively. Top traces in b and c represent baseline noise level, while lower traces disclose spontaneous discharges of action potentials from retinal ganglion cells. Modified from [24], with permission

were 30 μm in diameter and spaced 200 μm apart; CNT islands had heights of several μm. Electrical recordings from retinas indicate that both types of electrodes can record spontaneous activity with RGCs discharging burst of action potentials (Fig. 11.4b and c). Individual electrodes of both types of MEAs appeared to record from at least two RGCs simultaneously as two sets of signal amplitudes

were clearly designated. The quality of recordings, however, were much better with CNT-coated electrodes, as evidenced by decreased levels of baseline noise to half of that seen in TiN electrodes and by more than doubled amplitudes of recorded action potential amplitudes, resulting in a exceptionally high signal-to-noise ratio of the CNT-coated electrode and clear single unit recordings (Fig. 11.4c, high amplitude signals). Over time (minutes to hours) the number of electrodes that could record RGCs activity increased on both type of chips. The amplitude of action potentials recorded from TiN electrodes were stable, while recordings from CNT-coated electrodes kept improving over time, showing enhanced amplitudes of recorded action potentials with a 2% per minute increase; recordings lasted up to several hours. This time-dependent improvement in recordings made by CNT-coated electrodes could be attributed to improved coupling between electrodes and the tissue. Moreover, the stimulation proof-of-concept experiments were executed. Stimulation via an individual CNT-coated electrode (80 μm in diameter) can be used to record evoked action potentials generally on a single neighboring electrode (200 μm spacing). Taken together, the results of this study indicate that CNT-based MEAs have promise for use in vivo.

Keefer et al. [23] established a procedure to coat planar and 3D electrodes with MWNTs and compared their performances with the uncoated electrodes using cultured neurons, the motor cortex of anaesthetized rats, and the V4 region of the visual cortex of a conscious trained monkey. The initial experiments were done on planar MEAs in the absence of any brain cells. Deposition of CNTs on indium-tin oxide based MEAs reduced the impedance of electrodes by ~20 fold and increased the charge transfer by ~45 fold. Follow-up experiments used dissociated cultures of frontal cortical neurons plated onto MEAs with bare gold surfaces or MEAs containing an additional coating with CNTs. Both gold and CNT surfaces were permissive substrates for neuronal growth. Spontaneous activity of the established neuronal networks could be recorded for up to 3 months in culture with similar success with either of MEA. However, the stimulation delivered via CNT-coated MEAs was more effective than that of the bare gold-based MEAs, a finding consistent with the CNTs' ability to lower impedance and increase charge transfer. One consequence of such an effect by the CNTs was a decrease in noise levels by ~65%, which led to the increased sensitivity of CNT-coated MEAs, without a change in their selectivity. These proof-of-principle experiments using planar MEAs were followed by 3D electrodes and work in vivo. Commercially available 3D tungsten and stainless steel sharp electrodes were over-coated with CNTs. Once again, when tested in solution, these electrodes outperformed the bare metal electrodes by offering lower impedance and higher charge transfer. This performance could be further enhanced if CNTs were co-deposited with conductive polymers such as PPy which itself has been previously proven successful in experiments in vivo [27]. Two different animal models were used to test CNT-coated sharp electrodes: motor cortex (controlling limb movement) of anesthetized rats and V4 cortex (involved in perception of form-with-color) of an awake trained monkey. In experiments with rats, two parallel electrodes, referred to as stereotrodes, one coated with CNTs and the other bare tungsten, spaced apart by 125 μm, were used. For experiments with the monkey cortex, the animal's task was to look at a flashing color square

(form-with-color) on a screen, while recordings were performed using "stereotrodes" spaced 1 mm apart and containing one uncoated stainless steel (control) and another CNT-coated electrode (Fig. 11.4). In both in vivo experimental models, CNT-coated electrodes outperformed their paired control electrodes in terms of reduced noise (~17 dB) and increased sensitivity of detection (on average 7.4 dB more power) of spontaneous electrical neuronal activity throughout various ranges of acquisition frequencies (1–1000 Hz) relevant to brain (patho)physiology (Fig. 11.5a). As one would predict from their mechanical strength, CNTs endured the advancement of electrodes through the dura mater and remained intact even after recordings were completed, as assessed by electron microscopic investigation of the used electrodes (Fig. 11.5b). Taken together the coating of planar and 3D electrodes with CNTs allowed for their enhanced performance while recording neuronal electrical events in culture and in vivo. Thus, these hybrid electrodes could now be readily tested in exciting ongoing projects in the field of brain-machine interfaces, such as, the restoration of movement in tertraplegia [28]. Of course this should not occur with adequate testing for biosafety of CNT-based devices.

Thus far, we outlined the development of CNT-based electrodes used in direct contact with neural cells/brain tissue. However, it is commonly accepted that the safest way to record neural electrical activity is by using the EEG, since it is non-invasive procedure where electrodes are placed on a scalp. The location of such electrodes in humans is about 2–3 cm away from the surface of the cortex so that the electrical signal has low amplitude. Consequently, EEG recordings are generated by a rather large population of neural cells that are synchronously active. It

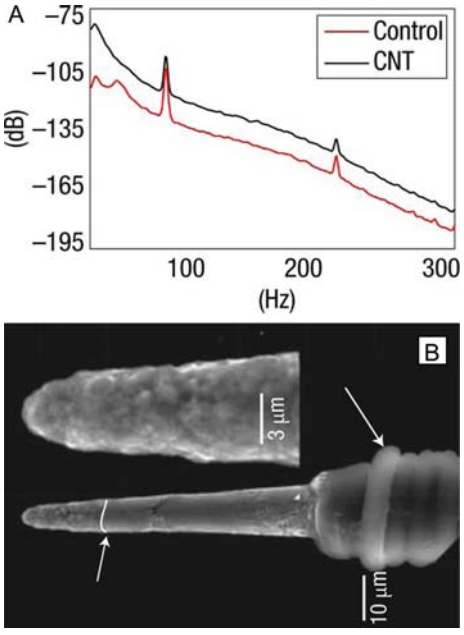

Fig. 11.5 CNT-coated electrodes record electrical activity in the primate visual cortex. (**a**) Power spectra obtained from CNT-coated and bare (control) metal electrodes indicate enhanced performance by CNT-coated electrodes. (**b**) CNT-coated electrode after recording from the monkey cortex. While insulating thin layer is peeled back (*right arrow*) from its original near the tip position (*white line, left arrow*) during penetration through the dura mater, the covalently attached CNTs at the electrode tip remain intact (inset). Modified from [23], with permission

should be noted that in addition to neuronal signals, the current flow created by glial cells contributes to the EEG. For example, glial Müller cell potentials have a time course similar to a component in the electoretinogram, referred to as the b wave [29]. Additional consequence of low amplitude measurements presents itself in the skin preparation for EEG recordings, which requires the use of electrolytic gels and takes several minutes per electrode. Owing to the diffusion of such gels into the skin, there is an additional requirement for stabilization of recordings. To overcome some of these limitations, Ruffini et al. [30] developed a dry electrophysiology sensor based on MWNTs to record the EEG from humans. Arrays of MWNTs were grown on silicon disks to form brush like structures (Fig. 11.6a); individual MWNTs were 50 nm in diameter and 10–15 μm length/height. Disks containing an array of MWNTs were then diced into squares (1 × 1 cm) and mounted onto commercial active electrodes with on-site amplification (Fig. 11.6b). As a standard for comparison, conventional electrodes were used. Both types of electrodes were connected to a commercial research electrophysiology recording system. Prior to the use on human, a series of tests were run on a pig skin to record test signals applied beneath the skin. Here, commercial electrodes were applied using an electrolytic gel, while CNT-based electrodes were applied "dry" without skin preparation. The

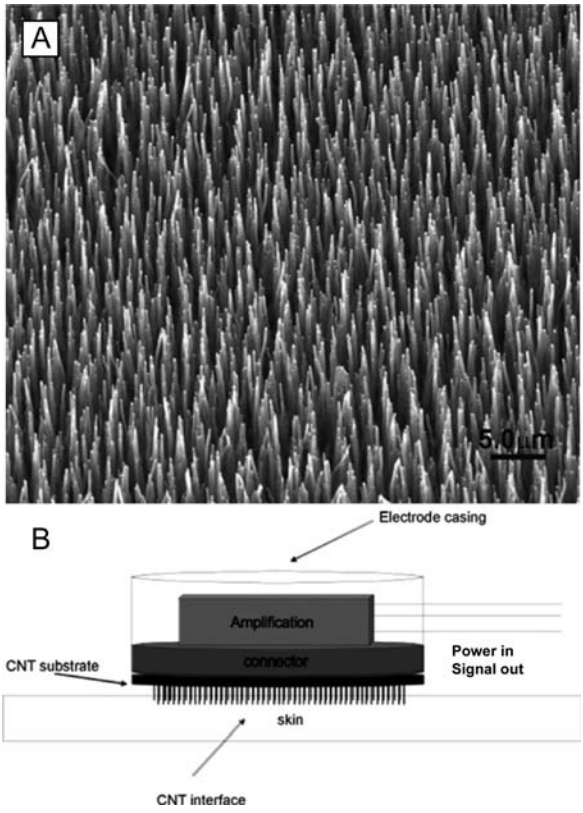

Fig. 11.6 MWNT-based electrode for EEG. (**a**) Scanning electron microscope image of an array of MWNTs grown on a silicon disk, which can be mounted onto a commercial active electrode with on-site amplification (**b**). (**b**) Schematics of CNT-based EEG electrode. MWNT array penetrates a superficial layer of the skin owing to the height/length of CNTs. Modified from [30], with permission

results showed similar performance of two types of electrodes. It should be noted, however, that CNT-based electrodes only penetrated the superficial layer of skin owing to the MWNT length/height. They caused neither pain nor any skin reaction as reported by a human subject who was subjected to EEG recordings when dry CNT and wet commercial electrodes were placed next to each other on the scalp for comparison of their performances. The recordings were done simultaneous using both types of electrodes using the protocol that encompassed spontaneous EEG and event-related potentials (ERP). Spontaneous EEG consisted of two periods, one with eyes open and the other with eyes shut, where the subject did not perform a task, allowing recording of β and α waves, respectively. ERP were tested in response to an auditory stimulus. In all conditions, dry CNT electrodes performed similarly to the commercially available state of the art research-oriented wet electrodes.

11.4 Biosafety of CNTs

The promising and exciting possibilities for the use of CNTs in biomedical applications also raise concerns about their safety and exposure limits [reviewed in Refs. [31, 32]]. Besides the above described use of CNTs in electrodes for neural interfaces, they are also emerging as a tool for targeted drug delivery [reviewed in Ref. [33]] and, in this context, understanding of their biodistribution and biostability is necessary. This knowledge could also be relevant to the applications of CNTs in neural interfaces, since there is a possibility that CNTs could leach out from electrodes and get distributed systemically if they can pass the blood-brain barrier (BBB). Biodistribution of a variety of functionalized SWNTs indicate that, following their delivery to the circulation, their blood retention can vary between 1 hour and 1 day, depending on the animal model used and modifications of SWNTs; the excretion/clearance of SWNTs via biliary and renal pathways is evident [34–37]. Interestingly, the biodistribution of CNTs in the brain 24 hours after their intravenous injection is much lower than that in the spleen and lungs, although these organs are all highly vascularized, suggesting that the intact BBB can effectively shield the entrance of CNTs into the brain [35]. Conversely, whether the BBB prevents CNTs that leach out from implanted electrodes in the brain to exit into the circulation has not yet been determined. However, even if this proves to be a clearance pathway, CNTs available in the circulation would be at very low quantities, so that such a scenario highly likely represents a trivial issue. Consequently, a pressing concern presents itself in the possible direct effects that CNTs may have on neural cells, which they contact as being an integral part of CNT-based electrodes.

The current literature on safety exposure limits and toxcitiy of CNTs on neural cells is limited. Most of the CNT toxicity studies were done using non-neural cells or cell-lines; these in vitro studies reported that the exposure to CNTs increased oxidative stress and cell death in a dose-dependent manner [38–43]. However, some of these studies were performed in such a manner that they do not directly address the toxicity effect solely attributed to CNTs. For instance, the synthesis of CNTs

often involves the use of a metal catalyst, in which the residual content in CNTs could be responsible for some of the observed toxic effects. Hence, non-purified CNTs, containing higher content of a metal catalyst, have been shown to be more potent in inducing oxidative stress in macrophages than the purified CNTs [44, 45]. Nonetheless, the length of CNTs can contribute to differential cellular response. The degree of inflammatory response to subcutaneously applied CNTs in vivo was greater for 825 nm in length CNTs than for shorter 220 nm in length CNTs [46]. It should be noted, however, that no severe inflammatory response, such as necrosis, was observed around both CNTs examined. Therefore, an evaluation of CNTs' effect on cells/tissue must take into consideration the type of CNTs and the presence of impurities.

The development of CNT-based materials for biomedical applications is still at its infancy, and it is timely to investigate effects of CNTs on neural cells/tissue before CNT-based devices become wide-spread in use. A recent in vitro study showed that CNTs are biocompatible with neural cells [47]. However, a more systematic approach, using a variety of CNTs, is needed to address acute and long-term effects that CNTs may have on the brain and the whole living organism in order to establish safety guidance for the use of this promising nanomaterial in biomedical applications in the near future.

11.5 Concluding Remarks and Future Directions

The intent of this chapter was to review the use of CNT-based neural interfaces for stimulation and monitoring of neuronal activity. Owing to unique physical properties of this nanomaterial, CNT-based electrodes have potential to replace bare metal electrodes for many of the medical applications, most notably brain-machine interfaces. Further improvement in CNT-based electrodes may include their chemical modifications, so they can detect various transmitters [discussed in Ref. [48]]. However, the use of CNT-based electrodes in human subjects must not occur without adequate testing. It is encouraging that systemic administration of CNTs [36] indicates that this form of carbon does not have any deleterious effect on mammalian health. This pilot study could be used as a springboard to determine safe exposure limits, both general and brain-specific, in humans. From the stance of implantation in the brain, the chronic response of the tissue to the resident CNT-based electrodes will have to be compared to the "classical" electrodes that have been in use for decades [49]. Although the retention of CNTs on planar electrodes over the period of ~3 months has been demonstrated [23], similar studies will need to be performed with the CNT-coated 3D electrode arrays using even longer implantation times, before we can make a full assessment of CNTs biocompatibility.

Acknowledgements We thank J. Robert Grammer for his comments on previous versions of this manuscript. The authors' work is supported by a grant from the National Institute of Mental Health (MH 069791) and National Science Foundation (CBET 0943343). We dedicate this chapter to the late Glenn I. Hatton, whose work inspired new views of astrocyte-neuronal interactions.

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Part II
Neuroprotective Treatment and Neuromodulation

Chapter 12
Is a Neuroprotective Therapy Suitable for Schizophrenia Patients?

Michael S. Ritsner

Abstract Schizophrenia is a chronic and disabling mental disorder characterized by positive, negative and mood symptoms, disturbed coping abilities with elevated distress and a significant decline in cognition, quality of life and psychosocial functioning. About one-third of all patients with schizophrenia do not respond adequately to drug treatment. Today neuroscience and clinical research have sufficiently advanced to introduce a novel generation of compounds with neuroprotective properties. The use of neuroprotective agents in schizophrenia is not yet significantly established. An in-depth review of new compounds such as neurosteroids, estrogen, omega-3 fatty acids, S-adenosylmethionine, cannabinoids, piracetam, modafinil, L-theanine, bexarotene with neuroprotective properties is discussed. The mechanisms underlying the neuroprotective effects of these compounds vary and differ from classically defined dopamine and serotonin receptors. This review highlights selective evidence supporting a neuroprotective approach in the search for novel compounds, and suggests future directions for this exciting area. Neuroprotection strategy may be a useful paradigm for treatment of prodromal and first-episode schizophrenia patients and might have a significant impact on the subsequent course and outcome of the illness. The clinical effects of neuroprotective agents clearly merit further investigation in schizophrenia spectrum disorders.

Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived neurotrophic factor
CANTAB	Cambridge Automated Neuropsychological Test Battery
CGI-S	Clinical Global Impression – Severity scale
CNS	Central Nervous System
CSF	Cerebrospinal fluid
Delta9-THC	Delta(9)-tetrahydrocannabinol

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_12,

DHA	docosahexaenoic acid
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DHEA(S)	DHEA and DHEAS together
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EPA	eicosapentaenoic acid
EPS	Extrapyramidal symptoms
ESRS	Extrapyramidal Symptom Rating Scale
$GABA_A$	gamma-aminobutyric acid
HPA	hypothalamic-pituitary-adrenal axis
HRQL	the health-related quality of life
ICD-10	International Classification of Mental and Behavioural Disorders
NMDA	N-methyl-D-aspartate
PREG	pregnenolone
PREGS	pregnenolone sulphate
PREG(S)	PREG and PREGS together
PANSS	Positive and Negative Syndrome Scale
RARs	retinoic acid receptors
RXRs	retinoid X receptors
SANS	Scale for the Assessment of Negative Symptoms

12.1 Introduction

Schizophrenia is a clinical syndrome that affects about 1% of the general adult population. Whether schizophrenia represents a single disorder of markedly variable expressions or a family of clinically related brain disorders is unclear. Treatment of schizophrenia patients typically includes a combination of pharmacotherapy with antipsychotic agents and psychosocial interventions. Antipsychotic agents ameliorate symptoms in the early phases of disease but become less effective over time, as the underlying disease progresses [1, 2]. The clinical benefits of second-generation antipsychotic agents are modest; their greatest advantage is fewer extrapyramidal side effects. Furthermore, the earlier hope that these agents would have a primary effect on negative and cognitive symptoms is harder to sustain [3, 4]. Despite the effectiveness of antipsychotic medications in the treatment of schizophrenia, in a large scale multi-center study [5] about 74% of the patients discontinued study medication within the first 18 months: 64% of those assigned to olanzapine, 75% of those assigned to perphenazine, 82% of those assigned to quetiapine, 74% of those assigned to risperidone, and 79% of those assigned to ziprasidone. The majority of patients in each group discontinued their assigned treatment owing to inefficacy or intolerable side effects or for other reasons. Furthemore, about one-third of all patients with schizophrenia do not respond adequately to drug treatment. Thus, although antipsychotic agents heralded a major breakthrough in the treatment of

positive symptoms in schizophrenia, negative symptoms and cognitive dysfunction continue to account for poor quality of life, reduced functioning and inferior employment status of the patients.

The most critical question facing clinical psychiatrists today is how to treat schizophrenia patients. Is the concept of brain protective therapy relevant for schizophrenia? What are the challenges and opportunities?

This chapter begins with a brief overview of the basic models of schizophrenia and a neuroprotective approach to establish a context for subsequent detailed discussions on several potential neuroprotective compounds, addressing a wide range of mechanisms.

12.2 Models of Schizophrenia

12.2.1 Psychopathological Models

Schizophrenia is characterized by psychopathological symptoms, a significant decline in cognition and quality of life, a decrease of psychosocial functioning, elevated emotional distress and disturbed coping abilities that exact considerable human and economic costs. The clinical picture includes a range of positive and negative symptoms such as delusions, hallucinations, agitation, hostility, emotional and social withdrawal, lack of spontaneity, poverty of speech and a wide range of mood symptoms and neurocognitive effects (Fig. 12.1) [6].

Positive symptoms usually emerge in adolescence or early adulthood, but are often preceded by varying degrees of negative symptoms, cognitive and quality of life impairments. Deterioration occurs primarily in the early stages of the illness and is generally confined to the first 5–7 years after onset. Schizophrenia's course over time considerably differs from person to person with varying degrees of functional impairments, reduced quality of life, social disability, frequent comorbid substance abuse, and decreased longevity. Overall, schizophrenia tends to be a chronic and relapsing disorder, with only partial remissions (Fig. 12.2) [6].

Categorical Models. Most major psychiatric classification systems are based on a categorical system of assessment and assume that individuals fall into distinct categories of pathology, each with unique configurations of symptom and behavioral expressions. For exapmle, two diagnostic systems – e DSM-IV [7] and ICD-10 [8] are based on a categorical model of the schizophrenia syndrome and its core symptoms, in which differences between psychotic symptoms and their normal counterparts are considered to be qualitative, an approach not dissimilar to that proposed by Kraepelin over a century ago. However, this categorical approach does not take into account several central concerns [9]:

1. Many mental disorders are in fact on a dimensional spectrum such as an affective spectrum, an obsessional spectrum and in our case a psychotic spectrum.

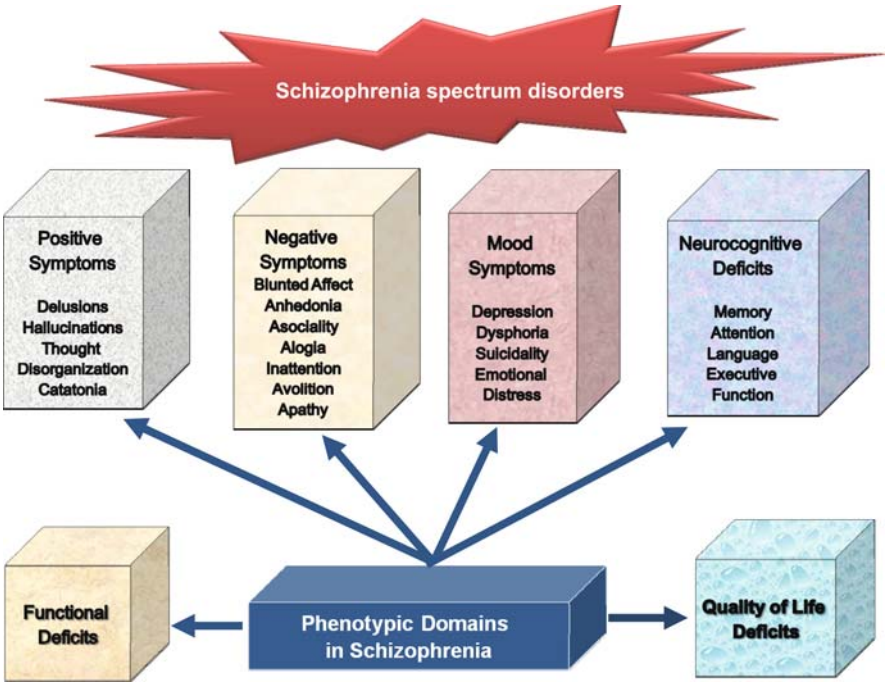

Fig. 12.1 Phenotypic domains in schizophrenia and related disorders [6]. The term *positive symptoms* refers to symptoms that most individuals do not normally experience. They include delusions, auditory hallucinations, and thought disorder, and are typically regarded as manifestations of psychosis. Negative symptoms are so-named because they are considered to be the loss or absence of normal traits or abilities, and include features such as flat or blunted affect and emotion, poverty of speech (alogia), inability to experience pleasure (anhedonia), lack of desire to form relationships (asociality), and lack of motivation (avolition). For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care, are markedly below the level achieved prior to the onset. A third symptom grouping, the *disorganization syndrome*, is commonly described, and includes chaotic speech, thought, and behavior

2. Each of these disorders actually includes several discrete dimensions such as a cognitive dimension, an impulsivity dimension, dimensions of positive, negative, and mood symptoms and so on.
3. As such, by utilizing a dimensional approach we would be treating the particular pathological dimensional symptoms and not an entire categorical disease entity.
4. Moreover, the validity of the categorical approach is further questioned by the vast heterogeneity of the diagnosis – the "x symptoms out of y" approach employed by the DSM-IV or ICD-10 leads to numerous different clinical combinations with little in common apart from the diagnosis.

The problem of non-homogeneous categories, the difficulty in drawing boundaries and individual progression of the severity of psychopathologic phenomena necessitate a change of paradigm from categorical to dimensional diagnostics [10].

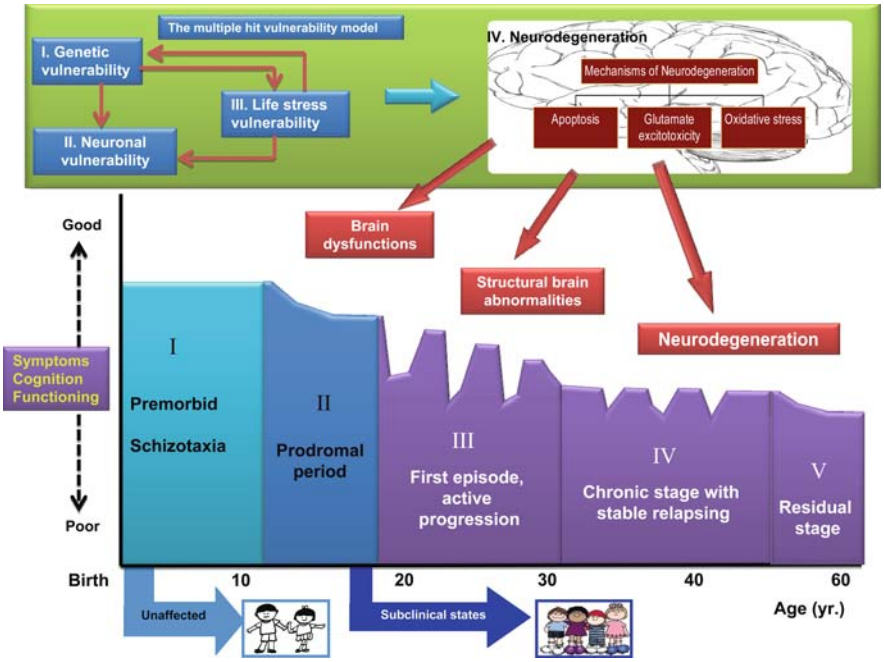

Fig. 12.2 The three-hit vulnerability model and course of schizophrenia and related disorders (Reproduced from [6]). Schizotaxia – term for the predisposition to schizophrenia

Continuous or Dimensional Models. An alternative, dimensional approach assumes that schizophrenia is not a distinct illness entity, but that psychotic symptoms are diversions from normal experiences and behaviors [11, 12]. Clinical psychiatrists have developed numerous psychopathological models based on items from rating scales: the Positive and Negative Syndrome Scale [13–16] (PANSS), the Scale for the Assessment of Positive Symptoms [17] and the Scale for the Assessment of Negative Symptoms [17] (SANS), the Calgary Scale for Depression in schizophrenia [18], the Overt Aggression Scale [19], and others. These dimensional measures are usually used as outcome variables in clinical trials with psychopharmacological and neuroprotective compounds.

Inclusion of dimensional elements in psychiatric diagnostic systems have been advocated for many years, however the concept was resisted due to concerns of clinical utility. Kraemer et al. [20] argue that categorical and dimensional approaches are fundamentally equivalent, but that one or the other approach is more appropriate depending on the clinical circumstances and research questions being addressed. Using an example from the Infant Health and Development Program, the authors illustrate the importance of using dimensional approaches for hypothesis testing, identify the problems with power and with interpretation that arise from employing a categorical approach, and underscore the importance of identifying the appropriate cutpoints when a categorical approach is necessary. A comparison between

categorical and dimensional approaches to the diagnosis of psychosis revealed that the categorical approach is beneficial primarily in terms of reliability, whereas the dimensional approach would enhance validity [21].

Thus, to date, diagnosis of schizophrenia remains problematic for the following reasons:

1. diagnosis is based on a "categorical model" with inclusion and exclusion criteria for symptoms according to the DSM-IV and ICD-10 classification methods;
2. psychopathological symptoms and pathological forms of behavior are evaluated through observation and clinical interview with or without psychiatric rating scales;
3. similar symptoms may be found among schizophrenia patients and individuals with other mental disorders;
4. no laboratory diagnostic tests or biomarkers are currently available to determine a diagnosis of schizophrenia.

12.2.2 Neurocognitive Domains and Tools

Neuropsychological studies of patients with schizophrenia have demonstrated that neurocognitive impairment is a prominent feature of the illness that is present at illness onset and generally remains stable over time [22]. Neurocognitive impairment may be prognostically important, and the enhancement of cognitive functioning has become an important target for both psychosocial and pharmacological interventions. As a result, the assessment of neurocognitive deficits in schizophrenia presents a major challenge to the clinician and researcher, and efforts are being made to develop a reliable and valid cognitive battery especially for use in clinical trials.

Computerized testing has multiple advantages, including increased time-efficiency, a wider range of stimulus options and response forms, and increased psychometric reliability. Computerized cognitive testing has the potential to effectively address the limitations posed by traditional paper-based neuropsychological measures. Technical innovations for accurate measurement of reaction time and frequency of errors enhance overall sensitivity, and on-line adjustment of level of difficulty may minimize ceiling or floor effects. A computerized testing session is usually of shorter duration and is less expensive than traditional paper-based neurocognitive testing. With rapid advances in technology and an emphasis on efficiency of neurocognitive testing, it has become necessary to further investigate the value of computerized cognitive examinations. Although there is no gold standard for assessing cognitive function in schizophrenia patients, a number of computerized cognitive batteries have been developed, such as the Computerized Neuropsychological Test Battery [23], the Computer Administered Neuropsychological Screen for Mild Cognitive Impairment [24], the ECO computerized cognitive battery [25], MicroCog Cognitive Battery [26], the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) [27], the Mindstreams

Battery [28], and the Cambridge Neuropsychological Test Automated Battery [29] (CANTAB). The CANTAB tests run on an IBM-compatible personal computer with a touch-sensitive screen. The nonverbal nature of the CANTAB tests makes them largely language-independent and culture-free. Overall, neuropsychological tests are grouped into five cognitive domains: visual and movement skills, attention and memory, learning, sustained attention, and executive function. The CANTAB battery has been shown to be effective in detecting cognitive deficits in schizophrenia [28, 30, 31].

12.2.3 Health-Related Quality of Life Deficit Model

Despite increasing relevance of health-related quality of life (HRQL) measures in mental health, the theoretical conceptualization of the construct remains poorly developed (reviewed in [32]). Since many valued aspects of life, such as income, freedom and quality of the environment are not usually considered "health related", the term HRQL came to refer to the physical, psychological, and social domains of health. HRQL is multidimensional in the sense that the subjects may simultaneously evaluate several dimensions to arrive at an overall assessment. Two persons with the same mental health status may have different HRQL levels since components such as personality differences and illness related factors influence one's perception of health and satisfaction with life. Perceptions of HRQL are based on a cognitive process that involves identifying the domains relevant to one's life quality, and integrating the various domain evaluations into an overall HRQL assessment. Each health-related domain has many components that need to be measured.

HRQL is a heterogeneous concept, as reflected in the different perceptions of this construct by psychiatrists and their patients. Such differences are reflected in observer rated versus self-report HRQL evaluation instruments. HRQL differs somewhat from subjective well-being, in that the latter concerns itself primarily with affective states, both positive and negative. According to the HRQL impairment concept, the HRQL deficit syndrome refers to the vulnerability to illness, and, consequently, should be viewed as a definitive expression or a particular syndrome of severe mental disorders, such as psychopathology or cognitive impairment [33].

The Distress/Protection Vulnerability Model [33], suggests that:

1. HRQL impairment is a particular syndrome observed in most psychiatric and somatic disorders.
2. This syndrome is an outcome of the interaction of an array of *distressing factors*, on the one hand, and putative *stress process protective factors*, on the other. HRQL impairment increases if distressing factors overweigh protective factors, and vice versa.
3. There are primary and secondary factors that influence HRQL impairment. Primary or vulnerability related factors are those usually considered inborn or personal characteristics, while secondary factors are related to illness and

environment. Primary factors such as harm avoidance, high levels of neuroticism, poor coping skills, elevated emotional distress, emotion-oriented coping, and weak self-constructs might lower the vulnerability threshold, and, consequently, result in severe HRQL impairment. Secondary factors influence HRQL impairment via primary factors.

4. HRQL impairment syndrome is characterized based on underlying neurobiology that may lead to improved understanding of severe mental disorders and more effective treatment decisions.

Factors influencing the HRQL impairment syndrome in schizophrenia according to this model are summarized in Fig. 12.3 [6]. Since the year 2000, this model has been extensively used to compare HRQL impairment among patients with severe mental disorders [34, 35], to examine the role of side effects [36], to test the mediating effects of coping styles [37], to search for longitudinal predictors of general and domain-specific quality of life [38–40], to explore the association of HRQL impairment with suicide behavior [41], temperament factors [42], and sleep quality [43], and to examine the impact of antipsychotic and neuroprotective agents [44–46].

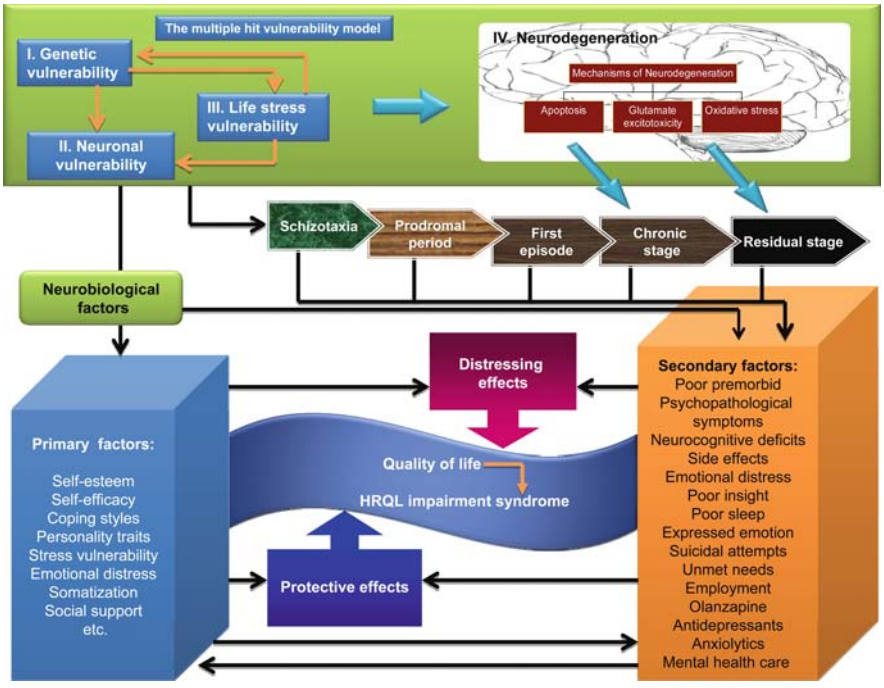

Fig. 12.3 The Distress/Protection Vulnerability model of HRQL impairment and the three-hit vulnerability model of schizophrenia and related disorders [Reproduced from [6]]. HRQL – health-related quality of life

Thus, in addition to symptom dimensions, neurocognitive and HRQL outcome measures have reshaped our understanding of schizophrenia and should be essential tools for designing neuroprotective interventions.

The neurodevelopmental and neurodegenerative models are two alternative, but not mutually exclusive, pathophysiological hypotheses relating to schizophrenia.

12.2.4 Neurodevelopmental Model

Understanding the etiology and pathogenesis of schizophrenia is a major challenge facing psychiatry. In the last two decades schizophrenia has been increasingly viewed as a neurodevelopmental disorder [47–49] due to several advances, principally related to developments in neuroimaging, electrophysiological and neuropathological approaches (reviewed in [50]).

While multiple theories have been put forth regarding the origin of schizophrenia, by far the vast majority of evidence points to the neurodevelopmental model in which developmental insults as early as late first or early second trimester of preganancy cause the activation of pathologic neural circuits during adolescence or young adulthood leading to the emergence of positive or negative symptoms [47–49]. There is evidence from brain pathology (enlargement of the cerebroventricular system, changes in gray and white matters, and abnormal laminar organization), genetics (changes in the normal expression of proteins that are involved in early migration of neurons and glia, cell proliferation, axonal outgrowth, synaptogenesis, and apoptosis), environmental factors (increased frequency of obstetric complications and increased rates of schizophrenic births due to prenatal viral or bacterial infections), minor physical anomalies, and gene-environmental interactions, which support of the neurodevelopmental model [50–53]. Furthermore, premorbid characteristics of schizophrenia patients combined with structural brain changes observed in first-episode neuroleptic-naïve patients are consistent with a neurodevelopmental pathophysiology for schizophrenia [54, 55]. In addition, findings from both cross-sectional studies of first-episode patients and longitudinal studies in childhood-onset and adolescent onset schizophrenia support the concept of early-onset schizophrenia as a progressive neurodevelopmental disorder with both early and late developmental abnormalities [56].

12.2.5 Neurodegenerative Model

Investigation of the long-term course of schizophrenia with progression to different residual syndromes has suggested that schizophrenia may be a neuroregressive illness. In particular, various lines of evidence indicate the presence of progressive pathophysiological processes that occur in the brains of patients with schizophrenia (see review [57]):

(1) Progressive MRI changes in longitudinal studies were revealed in childhood-onset schizophrenia [58], before and after transition to psychosis [59], and in the course of early psychosis [60, 61].
(2) Progressive MRI changes were seen in subgroups of patients with chronic schizophrenia [60–63].
(3) Some, though not all studies revealed more pronounced progressive brain changes in patients that are associated with poor outcome, more negative symptoms, and a decline in neuropsychological performance [64–66];
(4) Neuroimaging studies documented progressive increases in ventricular size, accelerated loss of brain tissue, progressive delays in treatment response, and neurochemical (magnetic resonance spectroscopy) and neurophysiological (P300) indices, all of which are consistent with ongoing cerebral degeneration in a significant subgroup of schizophrenia patients [67].
(5) In addition, the most-affected brain regions were consistently found to be the frontal and temporal cortices, the hippocampus, the amygdala, and the thalamus. Compared to healthy controls, the amygdala appears to be decreased in size among schizophrenia patients [68, 69].
(6) Cerebellar abnormalities have also been noted in schizophrenia [70].

Although brain alterations in schizophrenia patients have contributed to the neurodevelopmental model of pathogenesis, a progressive neurodegenerative process has also been suggested. Genetic and prenatal adversity may underlie the pathophysiological process that led to a shift from a neurodevelopmental to a neurodegenerative model (*a combined model*) of schizophrenia with multiple biochemical abnormalities involving the dopaminergic, serotonergic, glutamate, and gamma-aminobutyric acidergic systems [71], and brain alterations.

12.2.6 Vulnerability Models

Though recent studies have shown that several neurobiological alterations in domains of brain structure, physiology and neurochemistry may reflect diverse pathophysiological pathways from the "genome to the phenome" (reviewed in [50, 72, 73]), a conclusive identification of specific etiological factors or pathogenic processes in the illness has remained elusive. Attention has focused on disease models involving genetic, neurodevelopment, neurodegenerative, and environmental factors and mechanisms. The stress-vulnerability models of schizophrenia and other psychotic disorders have dominated etiology theories for over three decades.

The stress-vulnerability model has become established as a framework for explaining how environmental factors interact with preexisting vulnerability in the etiology and course of the disorder [74]. This model postulates that vulnerability to illness is stable, enduring, and largely attributable to genetic and environmental factors. Greater vulnerability is associated with higher risk for developing schizophrenia, but the actual expression of this predisposition depends on a host

of personal and environmental factors, some of which are noxious, while others are protective. The interaction of vulnerability, stressors and protective factors influences both the onset and the course of the disorder. More recently, with advances in our understanding of the biological processes that mediate the effects of stress, these models have incorporated mechanisms to account for the adverse impact of stress on brain function [75].

The neural diathesis–stress model proposes that the constitutional diathesis for schizophrenia depends on neuroendocrine pathways through which stress exposure, specifically cortisol release mediated by the hypothalamic-pituitary-adrenal (HPA) axis, influences dopamine transmission [74, 76]. The neural diathesis–stress model of schizophrenia can be expanded to account for the heterogeneity of effects of psychological stressors.

A tentative model of schizophrenia is presented, based on the evidence that certain personality characteristics may serve as vulnerability factors and that environmental stressors may precipitate psychotic periods in vulnerable individuals. Certain information-processing deficits, autonomic reactivity anomalies, and social competence and coping limitations are viewed as potential vulnerability factors. Stressors in the form of discrete life events as well as the prevailing level of social environmental stress are considered factors that interact with preexisting vulnerability characteristics to produce vicious cycles that lead, in turn, to psychotic episodes. A distinction among stable vulnerability indicators, mediating vulnerability factors, and episode indicators is suggested to differentiate types of abnormalities that characterize individuals prone to or manifesting schizophrenic disorder [75, 77].

"Multiple hit" models have been formulated including two and three hit models of schizophrenia, which suggest the importance of additive and interactive effects of environmental risk factors against a background of genetic predisposition [78–80]. Genetic factors, most likely multiple genes of modest effect, play a major role in its etiology, but an environmental "second hit" may be necessary for clinical expression. Stress has been postulated as a factor in so called "two hit" models of schizophrenia in which two independent insults (e.g., an aberrant genetic trait and stressful experience) are thought to be necessary for the occurrence of the disorder. In this model, genetic or environmental factors disrupt early central nervous system (CNS) development. The adaptive plasticity of chronic stress involves many mediators, including glucocorticoids, excitatory amino acids, brain neurotrophic factor (BDNF), polysialated neural cell adhesion molecule and tissue plasminogen activator [81]. Inappropriate neurotrophic support during brain development could lead to structural disorganization in which neuronal networks are not optimally established. Inadequate neurotrophic support in adult individuals could ultimately be an underlying mechanism that may lead to decreased capacity of the brain to adapt to changes and increased vulnerability to neurotoxic damage [82, 83]. Keshavan [78] proposed that these factors might interact cumulatively during successive critical "windows of vulnerability" during brain development and during the early course of the illness to lead to the clinical manifestations of the illness. Velakoulis et al. [84] suggest *a three-hit model* in which an early neurodevelopmental lesion renders

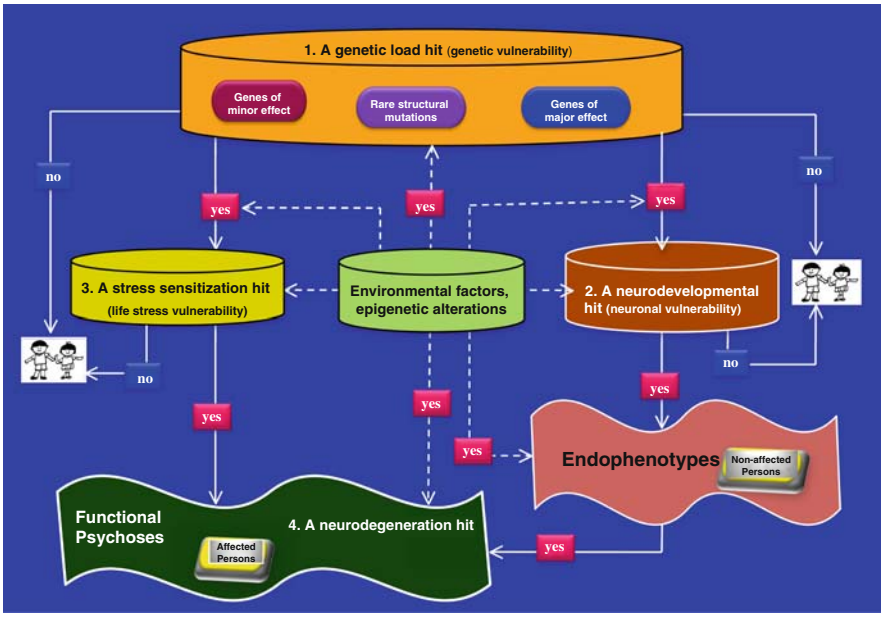

Fig. 12.4 The "multi-hit" vulnerability model of schizophrenia and other functional psychoses. Figure presents the *four-hit model*, which postulates that schizophrenia and other functional psychoses have caused by interaction between: (1) genes with major and minor effects with the possibility of disorder specific and nonspecific effects, respectively, gene-gene interactions and a diversity of genetic causes in different families or populations (a genetic load first hit); (2) a neuronal vulnerability to triggers during early neurodevelopment is a second hit (its underlying causes both genes with major effects and environmental stress); (3) a stress sensitization that could act as a third hit, facilitating (4) mechanisms of neurodegeneration such as apoptosis, excitotoxicity or oxygen radical formation due to environmental factors (a fourth hit)

the hippocampus vulnerable to further insult later in life during the transition phase to active illness. Figure 12.4 presents the integrative conceptualization of the *four-hit model*, which postulates that schizophrenia and other functional psychoses have caused by interaction between:

1. *genes with major and minor effects* with the possibility of disorder specific and nonspecific effects, respectively, gene-gene interactions and a diversity of genetic causes in different families or populations (a genetic load first hit);
2. a *neuronal vulnerability* to triggers during early neurodevelopment is a second hit (its underlying causes include both genes with major effects and environmental stress); and
3. *a stress sensitization* that could act *as a third hit*, facilitating mechanisms of neurodegeneration such as apoptosis, excitotoxicity or oxygen radical formation due to environmental factors (a degeneration *fourth hit*).

Thus, currently schizophrenia is best conceptualized as a "multiple hit" illness or spectrum disorder similar to cancer.

12.3 Neuroprotective Approach

The neuroprotective approach is a treatment paradigm, that is theoretically based on both neurodevelopmental and neurodegenerative models of schizophrenia. This approach aims to protect against gray matter loss and slow functional decline following the onset of psychosis, and to maintain functional integrity of the brain in response to neurobiological stress. Neuroprotective therapy is the administration of an agent (medication, compound etc.) that can reverse some of the damage or prevent further damage. By definition, neuroprotection is an effect that may result in salvage, recovery or regeneration of the brain, its cells, structure and function [46, 85–88].

During the past few years research has focused on developing neuroprotective agents for the therapy of various degenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and glaucoma [89]. Regarding schizophrenia and related disorders some neuroprotective agents (e.g., erythropoietin, glycine, D-serine, neurosteroids, memantine, celecoxib, and others) are currently being evaluated as add-on therapies. Ehrenreich et al. [90] reviewed the neuroprotective approach using erythropoietin that represents a novel frontier. The "Gottingen EPO-stroke trial" represents the first effective use in man of a neuroprotective therapy in an acute brain disease.The experimental erythropoietin therapy to combat cognitive decline in patients with schizophrenia was introduced as a neuroprotective strategy for a chronic brain disease. There is ample evidence that neurotrophins and endogenous cannabinoid systems have numerous neuroprotective effects (see below).

12.4 Targets for Neuroprotective Therapy

Although the molecular mechanisms of neurodegeneration and pathogenesis of schizophrenia remain largely unknown, a significant body of literature indicates that the main mechanisms implicated in the disease process may include apoptosis, excitotoxicity, oxidative stress, stress and others.

12.4.1 Apoptosis

Apoptosis (programmed cell death) is a normal physiologic process that occurs during embryonic development as well as in the maintenance of tissue homeostasis. Studies have shown that various neurochemical events at the synapse can

induce apoptosis [91]. Triggering actions include excessive glutamate stimulation, calcium influx reactive oxygen species all of which may induce caspase activation in dendrites.

Apoptosis is a one of the most recent mechanisms implicated in the pathophysiology of schizophrenia [92, 93]. While schizophrenia is generally considered a neurodevelopmental disorder, evidence for progressive clinical deterioration and subtle neurostructural changes following the onset of psychosis has led to the hypothesis that apoptosis may contribute to the pathophysiology of schizophrenia [92]. A role for apoptosis in schizophrenia has long been hypothesized, but studies investigating this hypothesis have only recently begun. Several postmortem studies have demonstrated that apoptotic vulnerability may be increased in the brains of patients with chronic schizophrenia, even though active cell death does not occur [94]. Apoptosis appears to be downregulated in the cortex of patients with chronic schizophrenia that could reflect either a pathophysiological failure to mount an effective response to an apoptotic insult or an appropriate compensatory response to an earlier insult [95].

12.4.2 Oxidative Stress

Oxidative stress has been defined as "a disturbance in the pro-oxidant–antioxidant balance in favour of the former, leading to potential damage" [96]. Oxidative stress can cause cellular damage and subsequent cell death because the reactive oxygen species oxidize vital cellular components such as lipids, proteins, and DNA. Elaborate antioxidant defense systems exist to protect against oxidative stress (reviewed in [97]). Oxidative stress has been implicated in the pathophysiology of many neurodegenerative diseases, in particular, Parkinson, Huntington, and Alzheimer disorders, amyotrophic lateral sclerosis, and other disorders.

Accumulating evidence points to many interrelated mechanisms that increase production of reactive oxygen or decrease antioxidant protection in schizophrenia patients [53]. For example, there is evidence suggesting that peripheral activities of antioxidant enzymes and lipid peroxidation are abnormal in schizophrenic subjects [98]. Decreased activity of key antioxidant enzymes in schizophrenia was found [99]. Mahadik and Scheffer [100] found increased lipid peroxidation products and altered defence systems in both chronic and drug-naive first episode schizophrenic patients. Pavlović et al. [101] examined the erythrocyte levels of lipid peroxidation products and reduced glutathione and the activities of antioxidative defence enzymes – superoxide dismutase, glutathione peroxidase and catalase – as well as erythrocyte susceptibility to H_2O_2-induced oxidative stress in schizophrenia patients. The obtained results suggest a misbalance in pro/antioxidant status of chronic schizophrenics, which is more expressed in patients with positive symptoms of the disease. The accumulated results indicate that oxidative stress is integral to this disease and not the result of neuroleptic treatment [102]. Wood et al. [53] argue

that a better understanding of the mechanisms and pathways underlying oxidative stress will assist in developing the therapeutic potential of this area.

12.4.3 Glutamate Excitotoxicity

Excitotoxicity is the pathological process by which nerve cells are damaged and killed by glutamate and similar substances. In other words, too much glutamate release can be destructive and literally excite a neuron to death in a process called excitotoxicity. Glutamine synthetase constitutes an endogenous mechanism of protection against glutamate neurotoxicity in neural tissues by catalyzing the amidation of the neurotoxic amino acid glutamate to the non-toxic amino acid glutamine (for review see [103]). Deficits in N-methyl-d-aspartate (NMDA) receptor function play a critical role in the pathophysiology of schizophrenia. Patients who have excitotoxic damage would be expected to have poor outcomes characterized, perhaps, by anatomic evidence of progressive neurodegeneration, pronounced negative symptoms and cognitive deficits, and profound psychosocial deterioration [104]. Blocking the excitotoxicity process may be brain protective for schizophrenia patients.

12.4.4 Stress Sensitization

The brain is the key organ for responding to stress because it determines what is threatening and, therefore, potentially stressful. The brain also influences the physiological and behavioral responses which can be either adaptive or damaging [105]. The stress system orchestrates brain and body responses to the environment. Adverse conditions during early life are a risk factor for stress-related mental disorders. Psychosocial stress, such as life events, childhood trauma, or discriminatory experiences powerfully affect the brain and body and last throughout the entire life span, influencing brain function, behavior, and the risk for a number of systemic and mental disorders [81, 106, 107]. There is evidence that environmental factors, which interact with multiple genes, and epigenetic factors, psychological or physiological alterations, induce *persistent sensitization to stress* [107, 108]. Stress sensitization may be critical in the development or relapse of schizophrenia.

The neurobiological substrate of stress sensitization involves dysregulation of dopaminergic and noradrenergic systems. Glutamatergic regulation activates HPA axis in stress response [74, 109]. The HPA axis is one of the primary neural systems triggered by stress exposure, in the expression of vulnerability for schizophrenia. The results indicate that psychotic disorders are associated with elevated baseline and challenge-induced HPA activity; that antipsychotic medications reduce HPA activation, and that agents that augment stress hormone (cortisol) release exacerbate psychotic symptoms (reviewed in [75]).

Glucocorticoids modulate early life programming of stress reactivity and are a significant factor in brain plasticity underlying adaptation, the aging process and vulnerability to disease [110]. In rodents after a variety of experiences, even minor ones, during postnatal life, permanent changes in emotional and neuroendocrine reactivity have been observed. In particular, the results clearly demonstrate that early experiences trigger immediate changes in the stress system that may permanently alter the brain and behavior [111]. A fundamental question in the neuroendocrinology of stress-related psychopathology is why some individuals flourish and others perish under similar adverse conditions. We focus on the variants of mineralocorticorticoid and glucocorticoid receptors that operate in balance and coordinate behavioral, autonomic, and neuroendocrine response patterns involved in homeostasis and health. The data suggest that mineralocorticorticoid and glucocorticoid receptors contribute to individual differences in resilience and vulnerability to stressors [112]. Recent evidence shows that corticosteroid hormones exert rapid non-genomic effects on neurons in the hypothalamus and the hippocampal CA1 region [113]. Although many of the physiological effects of corticosteroid stress hormones on neuronal function are well recognised, the underlying genomic mechanisms are only beginning to be elucidated [114].

Brain regions such as the hippocampus, amygdala, and prefrontal cortex respond to acute and chronic stress by undergoing structural remodeling, which alters behavioral and physiological responses. Lyons et al. [115] suggest that small hippocampi reflect an inherited characteristic of the brain of monkeys. It has been reported that volume reductions in the amygdala, hippocampus, superior temporal gyrus, and anterior parietal cortex common to both patient groups may represent vulnerability to schizophrenia, while volume loss of the prefrontal cortex, posterior parietal cortex, cingulate, insula, and fusiform cortex preferentially observed in schizophrenia may be critical for overt manifestation of psychosis [108]. Genetically informed clinical studies should assess whether inherited variation in hippocampal morphology contributes to excessive stress levels of cortisol through diminished neuroendocrine regulation. In humans with mood and anxiety disorders, small hippocampal volumes have been taken as evidence that excessive stress levels of cortisol induce hippocampal volume loss. Translational studies in humans with structural and functional imaging reveal smaller hippocampal volume in stress-related conditions [116], and major depressive illness [117]. Laruelle [118] proposed that, in schizophrenia, neurodevelopmental abnormalities of prefrontal dopaminergic systems might result in a state of enhanced vulnerability to sensitization during late adolescence and early adulthood. It is also proposed that dopamine D_2 receptor blockade, if sustained, might allow for an extinction of this sensitization process, with possible re-emergence upon treatment discontinuation.

Changes of protein expressions in the amygdala in the categories of synaptic, cytoskeletal, oxidative stress, apoptosis, and mitochondria related proteins could be associated with mechanisms underlying behavioral sensitization [119].

The burden of chronic stress and accompanying changes in personal behaviors (smoking, over eating, drinking, poor sleep quality; otherwise referred to as “lifestyle”) is called allostatic overload [81]. Behavioral sensitization to daily life

(environmental) stress may therefore be a vulnerability marker for schizophrenia, reflecting dopaminergic hyper-responsivity in response to environmental stimuli [120]. There is evidence that emotional reactivity to daily life stress may be related to a familial liability to develop schizophrenia. In order to test a hypothesis that a persistent higher level of emotional distress in schizophrenia subjects is associated with a positive family history of schizophrenia, Ritsner and associates [121] recorded data for 69 multiplex family and 79 singleton patients at admission and about 16 months thereafter. Authors found that

- patients with negative family history reported improvement in distress severity measured by the Talbieh Brief Distress Inventory and depression severity measured by the Montgomery-Asberg Depression Rating Scale 16 months after admission, while those with positive family history experienced persistent elevated emotional distress, mainly, on obsessiveness, and depression subscales;
- both groups of patients are characterized by elevated emotional distress at follow-up examination compared to healthy subjects, and
- familial schizophrenia is characterized by higher severity of dysphoric mood factors that also may represent impaired emotional reactivity [121].

Thus, it appears that there is a strong association between positive family history and persistent elevated emotional distress.

Stress sensitization is most often *unspecific for schizophrenia and other brain disorders*, since its can trigger high blood pressure, diabetes, ulcers, asthma and digestive and lung ailments among others.

12.4.5 Neurotrophic Factor Expression

Neurotrophic factors (or neurotrophins), known as the nerve growth factor, BDNF, neurotrophin-1, neurotrophin-3, and neurotrophin-4/5, are small proteins that exert survival-promoting, development and function of neuronal cells [82, 122, 123]. They belong to a class of growth factors, secreted proteins, which are capable of signaling particular cells to survive, differentiate, or grow [124].

Neurotrophins have established roles in neuronal development, synaptogenesis, response to stress stimuli, in the regulation of neuronal plasticity and neuron protection.These agents are neuromodulators of monoaminergic, gamma-aminobutyric acid ($GABA_A$), and cholinergic systems [125]. This hypothesis is mainly based on new experimental evidence that psychiatric disorders are associated with neuronal atrophy and cell loss, impairments of structural plasticity and cellular resilience due to neurodevelopmental disturbances and morphological abnormalities of the brain.

The neurotrophin hypothesis proposes that repetitive neuronal activity enhances the expression, secretion and actions of neurotrophins to modify synaptic transmission and connectivity thereby providing a connection between neuronal activity and synaptic plasticity. Moreover, there is ample evidence that neurotrophins have

numerous neuroprotective effects under pathological conditions, which might be important in particular for neurodegenerative diseases with a possible role in most psychiatric diseases including schizophrenia and mood disorders [126]. Indeed, since neurotrophic factors play a crucial role in neurodevelopment, they are plausible candidates for contributing to the pathophysiology of schizophrenia. In line with this hypothesis, accumulating preclinical and clinical data indicate that dysfunctions of neurotrophins may contribute to impaired brain development, neuroplasticity and synaptic "dysconnectivity" that lead to the schizophrenic syndrome, or at least some of its presentations [82, 127].

Moises et al. [128] proposed that functional deficiencies of glial growth factors and growth factors produced by glial cells are among the distal causes in the genotype-to-phenotype chain leading to the development of schizophrenia. The growth factor deficiency and synaptic destabilization hypothesis suggests that a functional deficiency of glial growth factors and of growth factors produced by glial cells such as neurotrophins and glutamate that lead to a weakening of synaptic strength may be implicated as one of the important causes of schizophrenia. This hypothesis suggests that glial cells are the locus of gene-environment interactions in schizophrenia, and that glial asthenia is an important factor for the genetic liability to the disorder. In addition, an increase of prolactin and/or insulin may be a putative working mechanism of traditional and atypical neuroleptic treatments.

There is evidence that the levels of *growth factors* in peripheral blood are disturbed in schizophrenia. The S100 protein family with pro- and antiapoptotic members, are mainly produced by astroglial cells, and essentially involved in the regulation of cell survival and death [129]. The most robust results are reported for S100B protein, which seems to be elevated in acute psychosis and in patients with predominant negative symptoms [130]. Large-scale longitudinal multivariate studies, that simultaneously investigate the levels of several growth factors might provide insight to etiological processes and may identify clinically useful subsets of patients within the heterogeneous schizophrenia sample. Since the mid-1960s, a wide variety of intracellular and extracellular activities of S100B has been elucidated, and it has also been implicated in an increasing number of CNS disorders. S100B is a calcium-binding peptide produced mainly by astrocytes that exert paracrine and autocrine effects on neurons and glia. It regulates the balance between proliferation and differentiation in neurons and glial cells by affecting protective and apoptotic mechanisms. Findings from in vitro and in vivo animal experiments relevant for human neurodegenerative diseases and brain damage are reviewed together with the results of studies on traumatic, ischemic, and inflammatory brain damage as well as neurodegenerative and psychiatric disorders. Information about the functional implication of S100B secretion by astrocytes into the extracellular space is scant but there is substantial evidence that secreted glial S100B exerts trophic or toxic effects depending on its concentration. Recent findings relating S100B to a diversity of CNS pathologies such as traumatic brain injury, Alzheimer's disease, Down's syndrome, schizophrenia, and Tourette's syndrome were discussed [131].

Several studies have reported elevated S100B serum levels in schizophrenia patients. Rothermundt et al. [132] examined S100B serum levels and

psychopathology (PANSS) among 98 chronic schizophrenic patients with negative symptoms upon study admission and after 12 and 24 weeks. They showed significantly increased S100B concentrations upon admission and after 12 and 24 weeks of treatment. High PANSS negative scores were correlated with high S100B levels. Regression analysis comparing psychopathology subscales and S100B identified negative symptomatology as the predicting factor for S100B. S100B is not just elevated during acute stages of disease since it remains elevated for at least 6 months following acute exacerbation. This might indicate that S100B in schizophrenia patients either promotes apoptotic mechanisms by itself or is released from astrocytes as part of an attempt to repair a degenerative or destructive process. In the next study of this group the relationship between astrocyte activation and cognitive performance, S100B serum concentration, memory performance, and psychopathology were assessed in 40 first-episode and 35 chronic schizophrenia patients upon admission and after 4 weeks of treatment [133]. Chronic schizophrenia patients with high S100B were impaired concerning verbal memory performance (Auditory Verbal Learning Test) compared to chronic and first-episode patients with low S100B levels. These findings support the hypothesis that astrocyte activation might contribute to the development of cognitive dysfunction in schizophrenia.

Thus, various aspects of the potential role of neurotrophins in psychiatric disorders have been studied [82, 126, 127, 130].

1. Animal studies indicate the involvement of neurotrophins in psychopharmacological therapies and show that gene expression of cerebral neurotrophins is altered in animal models of several psychiatric disorders.
2. A reduction in BDNF production and availability in the dorsolateral prefrontal cortex of schizophrenics, suggests that intrinsic cortical neurons, afferent neurons, and target neurons may receive less trophic support in this disorder [134].
3. Neurotrophin serum changes have been observed in most psychiatric disorders. Whether or not these alterations represent primary-causal or secondary-reactive changes remains to be determined.

Although many issues related to the role of neurotrophic factors in schizophrenia are still unclear, these factors are attractive candidates for therapeutic agents in many clinical conditions and chronic neurodegenerative diseases including schizophrenia.

12.4.6 Neurosteroids: Modes of Action and Alterations

Pregnenolone (PREG) and its metabolites such as pregnenolone sulfate (PREGS) [together abbreviated PREG(S)], dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) [together abbreviated DHEA(S)] are neurosteroids. Neurosteroids are synthesized in the central and peripheral nervous system, particularly but not exclusively in myelinating glial cells, from cholesterol or

steroidal precursors imported from peripheral sources [135]. DHEA is formed from PREG by the microsomal cytochrome P450c17 enzyme (17a-hydroxylase/17,20-desmolase) in the brain and the adrenals. DHEA(S) concentrations in the human brain were found to be much higher than in peripheral circulation but also exceeded their very low cerebrospinal fluid (CSF) levels, ranging from about 1 to 5% of the corresponding plasma concentrations. DHEA(S) levels decrease markedly with age in humans, and levels in elderly populations are reduced to 20–30% of peak levels in young adulthood (reviewed in [136]).

Biological actions of PREG(S) and DHEA(S) include neuroprotection, neurite growth, and antagonistic effects on oxidants and glucocorticoids, a modulatory effect on neuronal excitability and synaptic plasticity, they have many functions associated with response to stress, mood regulation and cognitive performance [136–138] (reviewed in [136–138]).

Neuroprotective effects. The main effect of PREG and DHEA and their sulfates is neuroprotective [139–142]. Indeed, many lines of investigation demonstrate that neurosteroids have neuroprotective properties.

DHEA(S) inhibits *apoptosis* in human peripheral blood lymphocytes through a mechanism independent of either androgen receptors or estrogen receptors [143]. These neurosteroids also protect sympathoadrenal medulla cells against apoptosis via antiapoptotic Bcl-2 proteins [144]. DHEA(S) exhibit reduction of neurodegeneration [145, 146]. Yapanoglu et al. [147] evaluated the effects of DHEA on apoptosis of testicular germ cells after repair of testicular torsion in rats. The results suggest that DHEA may be a protective agent for preventing apoptosis caused by testicular torsion. Animal and in-vitro studies have shown that DHEA and DHEAS stimulate neuronal outgrowth and development [148, 149] and improve glial survival, learning and memory [141, 148]. Recent studies described a protective effect of DHEA on neuronal survival after oxidative, ischemic or traumatic damage [144, 150]. This specifically extends to a protective effect of DHEA on the hippocampus [151], which supports the proposed anti-glucocorticoid effect of DHEA, as glucocorticoids are known to affect hippocampal structure and function. DHEA(S) protect chromaffin cells and the sympathoadrenal PC12 cells (an established model for the study of neuronal cell apoptosis and survival) against serum deprivation-induced apoptosis [152].

PREG(S) and DHEA(S) modulate HPA axis activity and cerebral BDNF protein levels in adult male rats. In detail, they induced corticotropin-releasing hormone and/or arginine vasopressin synthesis and release at the hypothalamic level, thus enhancing plasma adrenocorticotropin hormone and corticosterone concentrations. This stimulation of the HPA axis occurred concomitantly with BDNF modifications at the hippocampus, amygdala and hypothalamus levels [142]. These results highly suggest that part of the HPA axis and antidepressant effects of neuroactive steroids could be mediated by BDNF, particularly at the amygdala level. They also suggest that neurosteroid effects on central BDNF could partially explain the trophic properties of these molecules.

PREG has neuroprotective effects against both glutamate and amyloid beta protein neuropathology and glutamate neurotoxicity [140]. Likewise, DHEA(S)

demonstrated neuroprotective effects on *NMDA-induced neurotoxicity* in primary cultured rat hippocampal neurons [153]. In addition, DHEA(S) block the neurotoxic effects of cortisol on hippocampal cells [154], protect neurons against glutamate and amyloid ß-protein toxicity [155], and glucocorticoid toxicity [156]. The participation of aromatase in the neuroprotective effect of these neurosteroids was assessed in a study that suggested that estradiol formation by aromatase mediates neuroprotective effects of PREG and DHEA against excitotoxic-induced neuronal death in the hippocampus [157]. Recently, Akan et al. [158] investigated the effects of PREG and PREGS on cell viability and amyloid beta peptide toxicity in a concentration and exposure time-dependent manner in rat PC-12 cells. PREG showed a dose-dependent protective effect against amyloid beta peptide in PC-12 cells. But its sulfate ester did not have the same effect on amyloid beta peptide toxicity. Leskiewicz et al. [159] examined the effect of PREG, DHEA(S), and allopregnanolone on staurosporine-, glutamate-, and NMDA-induced damage in primary cortical neuronal culture. It was shown that glutamate-induced cell damage was attenuated by PREG, DHEA, and DHEAS, but not by allopregnanolone. The results of the present in vitro studies suggest that excitatory neurosteroids PREG and DHEA(S) at physiological concentrations participate in the inhibition of cortical neuronal degeneration elicited by staurosporine and glutamate. DHEA(S) supplement greatly increases neuronal survival and differentiation and reduces astroglial proliferation rates in mouse brain cells in cultures [160]. Treating adult male rats with subcutaneous pellets of DHEA increased the number of newly formed cells in the dentate gyrus of the hippocampus, and also antagonized the suppression of corticosterone. In other words, DHEA regulates neurogenesis in the hippocampus and modulates the inhibitory effect of increased corticoids on both the formation of new neurons and their survival [161].

DHEA has been shown to display *antioxidant properties*. DHEA protects hippocampal cells from oxidative stress-induced damage [162].

DHEA and DHEAS exhibit *anti-stress properties* [163, 164], in particular, as a mediator of the HPA axis adaptation to stress and other psychiatric symptoms [165].

CNS receptors. Neurosteroids directly affect major CNS receptors, especially the gamma-aminobutyric acid ($GABA_A$), NMDA and sigma receptors [145, 166]. More specifically, certain naturally occurring PREG can enhance $GABA_A$ receptor function in a direct manner, and consequently have anxiolytic, analgesic, anticonvulsant, sedative, hypnotic and anaesthetic properties [167]. In particular, they may act as potential signaling molecules for neocortical organization during brain development, regulate the neuronal function by affecting the neuronal excitability through prominent modulatory effects on the $GABA_A$, sigma-1, and NMDA [145, 168, 169], cholinergic [170], and dopaminergic [171] systems. These modulations may lead to important changes for neuronal excitability.

There is evidence supporting a receptor-dependent basis for the direct physiological effects of DHEA(S). The data supporting an intracellular receptor for DHEA(S) are relatively weak and do not allow us to determine whether DHEA(S) directly, or a metabolite of DHEA(S), acts as a direct receptor ligand [172].

Neurosteroids demonstrate cognitive-enhancing effects and have been reported to improve memory [173]. In preclinical studies, memory-enhancing effects of PREGS and DHEAS have been attributed to their NMDA-agonistic properties [174].

While several neurotransmitter systems have been linked to neurocognitive abnormalities in schizophrenia, including prefrontal glutamatergic, cortical dopaminergic and cholinergic neurotransmission function, the association between neurosteroids and neurocognitive function is not yet fully understood and has been sparsely investigated. Early DHEA studies that investigated neurocognitive functions in schizophrenia patients noted that neurocognitive impairment was associated with low DHEAS levels [175, 176], high DHEAS levels [177] or high DHEA levels [175], or no association was found altogether [178].

Alterations. There is accumulating evidence that alterations in PREG(S) and DHEA(S) may be involved in the pathophysiology of schizophrenia, mood and cognitive disorders [73, 136, 178–180]. Previous clinical studies demonstrated low circulating levels of PREG in the elderly, including those with dementia [181], in individuals with schizophrenia [182], in male patients with generalized anxiety disorder [183], and in non-medicated male patients with generalized social phobia [184].

Comparison of the values of blood DHEA and DHEAS levels of schizophrenia patients with healthy controls were found to differ between studies, ranging from normal to low, and to high levels. Overall, serum DHEA and DHEAS concentrations range from 15.7 to 90.9 nmol/L, and from 4,928 to 12,777 nmol/L, respectively, among schizophrenia patients, as well as, from 24.0 to 68.8 nmol/L, and from 5,375 to 13,477 nmol/L among healthy subjects, respectively. Meta-analysis of differences in mean concentrations of serum DHEA(S) between schizophrenia patients and control subjects show significant non-zero effect ($p < 0.001$), and significant heterogeneity of data ($p < 0.001$; see more details and references in [136]).

Contradictory and confusing reports on serum DHEA levels in schizophrenia led us to compare the serum concentration of PREG, and DHEA between 15 medicated schizophrenia patients and 12 healthy subjects at four time points: at the start of the study, after 2, 4 and 8 weeks [182]. Controlling for age, serum concentrations of PREG were lower, while the DHEA level and the molar ratio values of DHEA to PREG were higher in schizophrenia patients compared to healthy controls. PREG and DHEA levels and their molar ratio did not change significantly during the study period either among schizophrenia patients or healthy controls. The blood levels of PREG appear to be associated with trait-anxiety scores in schizophrenia patients, while associations of clinical symptoms with two neurosteroids did not reach a significant level when the confounding effect of emotional distress, and anxiety scores was controlled. Thus, low serum PREG concentrations in schizophrenia appear to be associated with trait-anxiety scores independent of symptoms. PREG and DHEA to PREG molar ratio may represent a trait-like marker of impaired hormonal response to stress in schizophrenia. Although, the role of PREG and DHEA in non-specific response to distress and anxiety or in the pharmacotherapy of schizophrenia is as yet unclear, this report adds evidence to the assumption that, for example PREG and DHEA concentrations, are not related to specific diagnoses, but to more general

psychological states such as anxiety, that can occur in various disorders. A further longitudinal large-scale case-control comparison of PREG and DHEA levels in treated and an untreated, as well as in washed-out schizophrenia patients is warranted.

Thus, some neurosteroids may act as endogenous neuroprotective factors. The decline of neurosteroid levels during aging and schizophrenia may leave the brain unprotected against neurotoxic challenges. Therefore, PREG and DHEA may be suitable candidates for the treatment of schizophrenia and schizoaffective disorder patients.

12.5 Brain Protective Compounds

There are some compounds with neuroprotective properties that may be able to protect brain maturational processes disturbed in schizophrenia, mood and cognitive disorders, such as neurosteroids (PREG, DHEA), estrogen, omega-3 fatty acids, memantine, erythropoietin, S-adenosylmethionine, cannabinoids, piracetam, modafinil, L-theanine, bexarotene. The main targets for neuroprotective therapy may be divided on: (1) neurodegenerative processes in schizophrenia (e.g., apoptosis, excitotoxicity, oxidative stress, stress sensitization, neurotrophic factor expression, and alteration of neurosteroids); and (2) psychopathological symptoms,

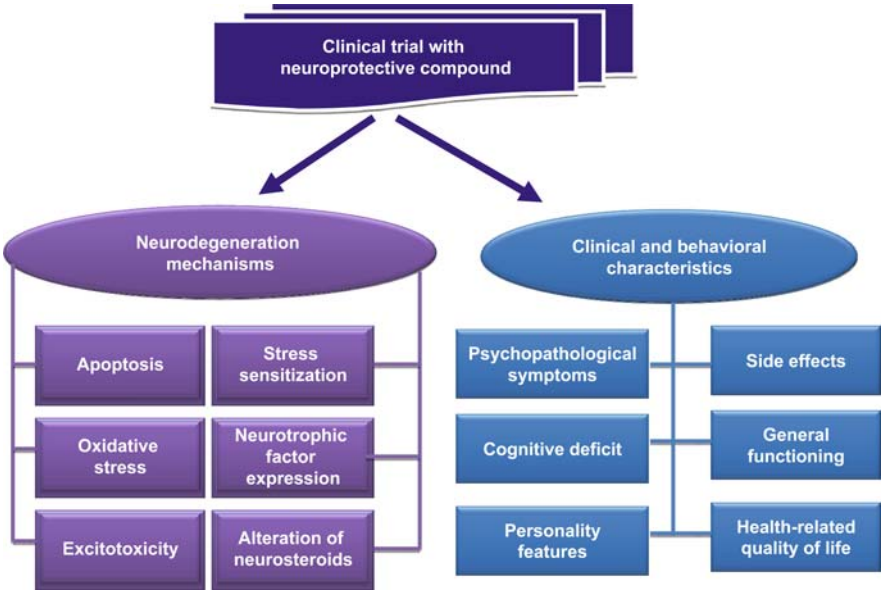

Fig. 12.5 Neurodegenerative, clinical and behavioral targets for neuroprotective therapy in schizophrenia and related disorders (Reproduced from [6])

and behavioral characteristics of schizophrenia patients, which used as "outcome measures" in order to test efficacy and safety of a new candidate for treatment agent (Fig. 12.5).

12.5.1 Pregnenolone and DHEA

Several clinical trials have been conducted with these neurosteroids for treatment of schizophrenia patients. DHEA augmentations (50–200 mg/day) for a period of 1–12 weeks were examined in cross-sectional [185–187] and crossover [188–190] designs. Comparative analyses of the obtained findings are presented in the review [191]. Briefly, the first compared patients receiving DHEA (n=15) and placebo (n=12) and indicated a significant efficacy of DHEA augmentation (100 mg/day) after 6 weeks in the management of negative, depressive, and anxiety symptoms of schizophrenia [185]. Subjects receiving DHEA demonstrated a significant increase in DHEA(S) plasma levels that were correlated with improvement in negative symptoms, but not with improvement in depressive and anxiety symptoms. Limitations of the small sample size and lack of cognitive and quality of life assessments are noted.

A second study investigated the effect of DHEA administration during 7 days on medication-induced extrapyramidal symptoms (EPS) among inpatients with schizophrenia or schizoaffective disorder that were randomized in a double-blind fashion to receive either 100 mg DHEA or placebo in addition to a constant dosage of antipsychotic medication [186]. Parkinsonism showed a favorable effect of DHEA with a significant time effect, as well as a significant group by time interaction and with no change noted on akathisia. Change of DHEA blood levels was negatively associated with change of Parkinsonism ($p < 0.05$) as well as with change of total EPS ratings ($p < 0.05$). Authors concluded that DHEA appears to demonstrate a significant effect on EPS, with improvement observed particularly in Parkinsonian symptoms.

A third study performed by the same research group included 40 patients with chronic schizophrenia stabilized on olanzapine. The subjects were randomized in a double-blind fashion to receive either DHEA (150 mg/day) or placebo augmentation for a period of 12-weeks [187]. 16 patients who received DHEA and 15 patients who received placebo completed the study. DHEA augmentation was not superior to placebo in improving the scores on rating scales (SANS, PANSS), measures of side effects, in cognitive performance, and aggressive behavior. Thus, these cross-sectional DHEA trials [185–187] did not replicate one another in terms of the depressive and anxiety symptoms, and in the medication-induced adverse side effects. They did not show a consistent and unequivocal significant favorable effect of DHEA administration on negative symptoms compared to placebo.

In order to resolve some of the concerns that have risen in the cross-sectional trials, a randomized, double-blind, placebo-controlled crossover study was conducted in two mental health centers [188]. During this trial 55 patients received either DHEA (200 mg/day) or placebo in identical capsules for 6 weeks following which

they were switched to either placebo or DHEA for a further 6 weeks. Patients continued to receive their regular treatment with daily doses of antipsychotic medication kept constant for at least 2 weeks prior to entering the study and throughout the study period. The crossover analysis revealed no statistically significant treatment effect of DHEA on severity of illness symptoms (PANSS), side effects, or on quality of life measures compared with placebo treatment. However, this investigation, while preliminary, supports prior findings of some improvement noted in visual sustained attention, visual and motor skills due to DHEA administration. DHEA treatment was well tolerated without any serious adverse effects.

Recently, Ritsner and Strous [190] explored changes in circulating neurosteroids and neurocognitive deficits in schizophrenia. In order to study the association, they conducted multiple regression analysis for predicting sustained attention, memory, and executive function scores across three examinations from circulating levels of DHEA, DHEAS, androstenedione, and cortisol through DHEA administration in schizophrenia. Data were collected among 55 schizophrenia patients for a double-blind, randomized, placebo-controlled, crossover trial with DHEA at three intervals: upon study entry, after 6 weeks of DHEA administration (200 mg/day), and after 6 weeks of placebo [188, 189]. DHEA augmentation was associated with elevations of both DHEA and DHEAS serum concentrations. Six weeks of DHEA treatment was associated with significant improvement in cognitive functions of visual sustained attention, and motor skills compared to placebo conditions, while DHEA administration did not produce significant improvement in clinical symptoms, side effects and quality of life scores. Obtained findings indicated that circulating DHEAS and androstenedione levels were positive predictors of cognitive functioning, and DHEA levels was a negative predictor. Overall, blood neurosteroid levels and their molar ratios accounted for 16.5% of the total variance in sustained attention, 8–13% in visual memory tasks, and about 12% in executive functions. In addition, effects of symptoms, illness duration, daily doses of antipsychotic agents, side effects, education, and age of onset accounted for variability in cognitive functioning in schizophrenia. Thus, this study suggests that alterations in circulating levels of neurosteroids and their molar ratios may reflect pathophysiological processes, which, at least in part, underlie cognitive dysfunction in schizophrenia.

Early human trials with PREG conducted on healthy volunteers under stressful conditions, demonstrated significant improvements in mood, general well-being, psychomotor performance and learning [192–194]. Low-doses of oral PREG (30 mg/day) were generally well tolerated in 17 healthy volunteers who received pregnenolone for 4 weeks [195].

Given the neuroprotective potential roles of PREG, it was hypothesized that PREG augmentation to ongoing and unchanged antipsychotic therapy may be able to improve psychotic symptoms and cognitive performance in chronic schizophrenia and schizoaffective disorder patients compared to DHEA and placebo administration. An 8-week, controlled, double-blind, randomized, parallel-group trial with "low dose" and "high dose" of PREG (30 and 200 mg/day, respectively), and 400 mg/day DHEA augmentation to on-going antipsychotics in the treatment of chronic schizophrenia and schizoaffective disorders patients was conducted for the

first time in two large state referral institutions: Sha'ar Menashe Mental Health Center and Be'er-Sheva Mental Health Center [196]. Data were collected from February 2005 until June 2007 (70 patients). A total of 58 patients were randomized, 44 patients (12/13 women and 32 /45 men) completed the trial. Ten patients met criteria for schizoaffective disorders; all other subjects met criteria for schizophrenia. After an 8-week period, compared with placebo, PREG-30 administration was associated with significant reduction in positive symptom scores, EPS, and improvement in attention, and working memory performance, whereas subjects treated with PREG-200 did not differ on outcome variable scores for the study period. The condition of patients receiving placebo and PREG-30 improved more than those subjects treated with DHEA- in general psychopathology severity, and general functioning, while DHEA was superior to placebo in improving EPS. No significant main effect of the type of antipsychotics or type of antipsychotics x time interaction on the Clinical Global Impression – Severity scale (CGI-S), PANSS subscale, the Global Assessment of Functioning Scale, Extrapyramidal Symptom Rating Scale (ESRS) and Barnes Akathisia Rating Scale ratings was observed for patients receiving PREG-30, PREG-200, DHEA and placebo (all p values >0.05). Interestingly, a significant efficacy of DHEA augmentation was observed with 50–150 mg/day [185, 187], but not with 200 mg/day [188] or 400 mg/day [196]. Moreover, the augmentation of 400 mg/day of DHEA resulted in significantly less improvement of CGI-S, and PANSS general psychopathology scores compared to placebo. Therefore, we suggest an inverted-U clinical response on a daily dose of PREG and DHEA augmentations (although there could be other reasons for the inconsistent results: methodological issues, different sample characteristics, different baseline severity of illness, and varying durations of combination treatment). Negative symptoms and akathisia did not significantly benefit from any treatment. The administration of PREG and DHEA was well tolerated.

Circulatory pregnenolone was found significantly higher among the patients treated by both neurosteroids compared to the placebo group, however, it was significantly higher among those receiving 200 mg/day PREG compared to PREG-30 and the DHEA groups. This study demonstrates no effects of PREG administration on the other hormones measured in this trial, while treatment with DHEA significantly elevated blood levels of pregnenolone (but to a lesser extent than PREG 200 mg/day), as well as DHEA, DHEAS, androstenedione, 3a-androstane-3a-17b-diol-glucoronide, testosterone, and estradiol compared to PREG-30, PREG-200 and placebo. No between group differences in the levels of progesterone, 17-OH-progesterone, and cortisol were demonstrated. The patients receiving PREG do not have a risk for elevation of androgenic metabolites, like DHEA, which may in turn potentially predispose them to various problems such as prostatic hypertrophy in men and hirsutism in women. PREG's treatment effects cannot be explained by an impact of their neuroactive metabolites like DHEA, DHEAS, androstenedione, 3a-androstane-3a-17b-diol-glucoronide, testosterone, and estradiol. Considering the lack of any significant effect of PREG on the measured hormonal profile in this study, it may be suggested that PREGs therapeutic effects as noted, are mediated by

other mechanisms, including further potential hormonal influences not investigated in this study. In addition, direct neuromodulatory effects on the GABAA, NMDA, sigma-1, dopaminergic, cholinergic or neurotrophic systems may mediate PREG's effect.

Thus, although based on a relatively small sample size, this study suggests that low-dose PREG treatment for 8 weeks, used as an adjunct to antipsychotics, has a valuable ameliorating effect on positive symptoms, attention and memory impairments and antipychotic-induced extrapyramidal side effects, in chronic schizophrenia and schizoaffective patients. Although the results of this study are notable, it is crucial to replicate the trial with a larger sample of chronic and non-chronic schizophrenia or schizoaffective patients, and for a longer duration of treatment. Further double-blind controlled studies are needed in order to investigate the clinically significant benefits of pregnenolone augmentation.

12.5.2 Estrogen

In recent years, we have become increasingly aware that estrogen is a gonadal hormone that exerts diverse non-reproductive actions on multiple organs and in multiple physiological systems. Three major forms of estrogen exist in humans and rodents: the biologically most prevalent and potent estrogen 17b-estradiol and, in order of decreasing potency, estrone and estriol. These estrogens are known to exert their actions through members of the nuclear hormone receptor superfamily, estrogen receptor-a, and the more recently identified estrogen receptor-b [197, 198]. Estrogen is now recognized to have centrally mediated neuromodulatory actions in both in males and females [199–201]. Estrogen receptors are expressed in brain regions that are involved in sex differentiation and maturation. Wise et al. [202, 203] found that estrogen receptors play a pivotal functional role in neuroprotection. However, the cellular mechanisms by which estrogens exert neuroprotective effects are not clearly understood. In vitro models of neuroprotection, 17bestradiol treatment exerts neuroprotective effects on diverse neuronal cell types under b-amyloid–induced toxicity, excitotoxicity, and oxidative stress [204]. Signaling mechanisms underlying estrogen-induced neuroprotection and synaptic plasticity, including the important concepts of genomic versus nongenomic mechanisms, types of estrogen receptor involved and their subcellular targeting, and implicated downstream signaling pathways and mediators were reviewed by Brann et al. [205]. This review demonstrates the remarkable body of work that has been conducted on the neuroprotective and neurotrophic actions of estrogen in the brain with particular emphasis on estrogen actions in the hippocampus, cerebral cortex and striatum.

In addition estradiol exerts rapid membrane effects on neural cells, modulating ion channels, neurotransmitter transporters, levels of intracellular calcium and other second messengers and phosphorylation of different kinases [206].

Several clinical observations support the estrogen protection hypothesis, which proposes:

- fluctuation of psychotic symptoms in women with schizophrenia, during their menstruation cycle;
- indications of a higher efficacy of antipsychotic treatment in women with schizophrenia than in men [207];
- reduction of levels of plasma estrogen in both male [208] and female [209] schizophrenia patients.

Estrogen has now been used as an adjunct to standard antipsychotic medication in quite a few studies of female schizophrenia patients [210]. However, most of these are not double-blind, randomized, controlled trials. Three randomized double-blind placebo-controlled trials and an open-label study showed that adding *estradiol* to women's usual antipsychotic medications was associated with significant abatement of schizophrenia symptoms [211]. Several trials indicate a protective effect of estrogen against onset of schizophrenia and the severity of negative symptoms [212] (reviewed in [213]). Although estrogen appears to be a useful treatment for schizophrenia, further research is required to determine the appropriate dose and duration of use of estradiol augmentation.

12.5.3 S-Adenosylmethionine

S-Adenosyl-Methionine (SAMe) is a naturally occurring molecule distributed in virtually all body tissues and fluids [214]. It is naturally synthesized in the body during the metabolism of methionine to cysteine, taurine, glutathione and other polyamine compounds in the presence of methionine-adenosyl-transferase and adenosine-5'-triphosphate [215]. SAMe's predominant function is as a primary methyl group donor for a wide range of compounds including catecholamines, membrane phospholipids, fatty acids, nucleic acids, porphyrins, choline carnitine and creatinine. Following release of its methyl group, SAMe is converted to S-adenosyl-homocysteine which, in turn, acts as a competitive inhibitor of SAMe-mediated methylation reactions. An important function of SAMe involves methylation of certain phospholipids, particularly phosphatidylethanolamine, and proteins which aid in the maintenance/control of the fluidity and microviscosity of cell membranes. Intact SAM-e metabolism is also considered vital for myelin maintenance [216].

SAM-e is able to cross the blood-brain barrier. CSF levels of homovanillic acid and 5-hydroxyindolacetic acid increase in the brain following its administration [217]. While the bioavailability of oral administered SAM-e, as opposed to intravenous or intramuscular administration, is incomplete, with a significant first-pass effect and subsequent rapid liver metabolism [218], oral SAM-e does increase levels in blood [219] and CSF [220]. This is especially so when administered in an enteric-coated form, which is now the simplest, preferred method of administration.

SAMe provides neuroprotection against various aspects of neurotoxicity in normal and apolipoprotein E-deficient mice and in cultured neuronal cells deprived of dietary folate and vitamin E and subjected to iron overload. For instance, SAMe

(AdoMet; 1 mM) protects the stationary phase cells of saccharomyces cerevisiae against the killing effect of acid (10 mM HCl) by increased the cell survival of the acid stressed cells [221, 222].

It has been suggested that since SAMe plays an important function in several metabolic processes, its administration could influence the course of a variety of disorders. James et al. [223] evaluated plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism. Relative to the control children, those with autism had significantly lower baseline plasma concentrations of methionine, SAMe, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of *S*-adenosylhomocysteine, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAMe to *S*-adenosylhomocysteine) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism.

The intervention trial was effective in normalizing the metabolic imbalance in autistic children. An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism. SAMe was proposed as a treatment for aging [224], gastric illness [225], liver disease [226], migraine [227], Alzheimer's disease [228], epilepsy, multiple sclerosis, HIV-associated neurological implications and spinal cord disease [229]. Parenteral SAM-e, a methyl group donor, was shown to be an effective antidepressant (e.g. [230]).

Strous et al. [231] investigated the efficacy of SAMe in managing schizophrenia symptomatology in patients with the low activity catechol-O-methyltransferase enzyme activity polymorphism. Eighteen patients with chronic schizophrenia were randomly assigned to receive either SAMe (800 mg) or placebo for 8 weeks in a double-blind design. Results indicated some reduction in aggressive behavior and improved quality of life following SAMe administration. Female patients showed improvement of depressive symptoms. Clinical improvement did not correlate with serum SAMe levels. This preliminary pilot short-term study cautiously supports SAMe as an adjunct in management of aggressive behavior and quality of life impairment in schizophrenia. However, like all medications, SAMe may result in unwanted side-effects mostly mild to moderate in nature and of brief duration. Most side effects appear to be gastrointestinal in nature (nausea, vomiting, diarrhea, heartburn), but may include hypomanic switch (in depressed individuals with underlying bipolar disorder), anaphylaxis, dizziness, insomnia and headache [215].

12.5.4 Cannabinoids

Cannabidiol is a major constituent of the Cannabis sativa plant. The endocannabinoid system consists of cannabinoid receptors, their endogenous ligands and enzymes for synthesis and degradation of endocannabinoids and represents a local

messenger system within and between the nervous and immune system (e.g. [232, 233]). Two subtypes of G-protein coupled cannabinoid receptors (CB1 and CB2) have been cloned and several putative endogenous ligands (endocannabinoids) have been detected during the past 15 years [234].

Since the discovery of an endogenous cannabinoid system, research into the pharmacology and therapeutic potential of cannabinoids has steadily increased. Although it is now apparent that cannabinoids have neuroprotective properties, many of the mechanisms involved in the process have yet to be characterized. Cannabinoids have antioxidant, anti-inflammatory, anti-excitotoxic, and anxiolytic-like properties that allow them to afford neuroprotection in different neurodegenerative disorders [234–237]. Cannabinoids have been shown to protect against neurotoxicity in a number of different cellular, animal, and human experimental paradigms [238–241]. Cannabinoids produce neuroprotection by reducing intracellular calcium release from ryanodine-sensitive stores [242].

Delta(9)-tetrahydrocannabinol (Delta9-THC), the main Cannabis sativa component, increased serum BDNF levels in healthy controls but not light users of cannabis, which had lower basal BDNF levels [243]. The modulation of mediotemporal and ventrostriatal function by Delta9-THC may underlie the effects of Cannabis sativa on verbal learning and psychotic symptoms, respectively [244]. Delta9-THC induces anxiety and psychotic-like symptoms in healthy volunteers; these effects of delta9-tetrahydrocannabinol are significantly reduced by cannabidiol, which is devoid of the typical effects of the plant. Studies in animal models and in healthy volunteers clearly suggest an anxiolytic-like effect of cannabidiol [236].

Recent advances in knowledge about cannabinoid receptor function have renewed interest in the association between cannabis and psychosis (reviewed in [245–249]):

- Case series, autobiographical accounts, and surveys of cannabis users in the general population suggest an association between cannabis and psychosis.
- Cross-sectional studies document an association between cannabis use and psychotic symptoms, and longitudinal studies suggest that early exposure to cannabis confers a close to two-fold increase in the risk of developing schizophrenia.
- Pharmacological studies show that cannabinoids can induce a full range of transient positive, negative, and cognitive symptoms in healthy individuals that are similar to those seen in schizophrenia.
- There is considerable evidence that in individuals with an established psychotic disorder such as schizophrenia, exposure to cannabis can exacerbate symptoms, trigger relapse, and worsen the course of the illness. For instance, Delta9-THC in a 3-day, double-blind, randomized, placebo-controlled study (0, 2.5, and 5 mg intravenous) transiently increased learning and recall deficits; positive, negative, and general schizophrenia symptoms; perceptual alterations; akathisia, rigidity, and dyskinesia; deficits in vigilance; and plasma prolactin and cortisol [250]. However, Schwarcz et al. [251] reported improvement of symptoms of schizophrenia in a small group of patients who received the cannabinoid agonist dronabinol (synthetic Delta9-THC). They found that 4 of 6 treatment-refractory

patients with severe chronic schizophrenia but who had a self-reported history of improving with marijuana abuse improved with dronabinol. This improvement seems to have been a reduction of core psychotic symptoms in 3 of the 4 responders and not just nonspecific calming. These results complement the recent finding that the cannabinoid blocker rimonabant does not improve schizophrenic symptoms and suggest that the role of cannabinoids in psychosis may be more complex than previously thought.

- Only a very small proportion of the general population exposed to cannabis develops a psychotic illness. It is likely that cannabis exposure is a "component cause" that interacts with other factors to "cause" schizophrenia or other psychotic disorder, but is neither necessary nor sufficient to do so alone.
- Although there is evidence that cannabis use increases the risk of developing psychotic symptoms, the causal nature of this association remains unclear [252].
- It is unclear if research findings support the clinical opinion that cannabis use leads to worse outcomes in people with psychosis, or whether this impression is confounded by other factors [253]. Rathbone et al. [248] evaluated the effects of cannabis use on people with schizophrenia and schizophrenia-like illnesses. At present, there is insufficient evidence to support or refute the use of cannabis/cannabinoid compounds for people suffering with schizophrenia.

Thus, highlighting the association between schizophrenia and Cannabis sativa and the endogenous cannabinoid receptor system, respectively, there are two opposing relevant findings. On the one hand, there is substantial evidence that cannabis is an independent risk factor for psychosis that may lead to a worse outcome of the disease. This risk seems to be increased in genetically predisposed people and may depend on the amount of cannabis used. On the other hand, during the last few years, an endogenous cannabinoid receptor system has been discovered [254].

Medications that activate cannabinoid receptors are already available: these are Cesamet (nabilone), Marinol (dronabinol; Delta9-THC) and Sativex (Delta9-THC with cannabidiol). The first two of these agents can be prescribed to reduce chemotherapy-induced nausea and vomiting. Marinol can also be prescribed to stimulate appetite, while Sativex is prescribed for the symptomatic relief of neuropathic pain in adults with multiple sclerosis and as an adjunctive analgesic treatment for adult patients with advanced cancer [255]. One challenge now is to identify therapeutic targets for cannabinoid receptor agonists in psychiatry, next is to identify the factors that underlie individual vulnerability to cannabinoid-related psychosis and to elucidate the biological mechanisms underlying this risk.

12.5.5 Omega-3 Fatty Acids

The two main omega-3 fatty acids in fish oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have important biological functions in the CNS [256]:

- DHA is a major structural component of neuronal membranes, and changing the fatty acid composition of neuronal membranes leads to functional changes in the activity of receptors and other proteins embedded in the membrane phospholipid.
- EPA has important physiological functions that can affect neuronal activity.
- Omega-3 polyunsaturated fatty acids are important for cell protection after ischemia and also seem to play an important role in the activation of antiapoptotic signaling pathways [257];
- DHA pretreatment effectively reduces cell-associated methylmercury (MeHg)-induced neurotoxicity and prooxidant response from MeHg in both cerebellar astrocytes and neurons [258], improves functional outcome and reduces volume loss after hypoxia-ischemia in neonatal rats [259].

For schizophrenia or schizoaffective disorder patients treated with 3 g/day of ethyl EPA, improvement in residual symptoms and cognitive impairment was no greater than for those patients treated with placebo [260]. There are contradictory findings regarding therapeutic benefits of omega-3 fatty acids, particularly when EPA is added to existing antipsychotic therapy of schizophrenia patients [261–263].

12.5.6 Piracetam

Piracetam is a nootropic agent with potent neuroprotective properties that improves cognitive and mental abilities [264, 265]. The mechanism of action of piracetam is not well understood. It facilitates cholinergic and excitatory amine neurotransmission without specific binding to receptors [266]. Glutamate alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors mediate most of the excitatory neurotransmission in the CNS and also participate in forms of synaptic plasticity thought to underlie memory and learning, and the formation of neural networks during development [267]. Interestingly, the neurogenic activity of piracetam is mediated through the AMPA receptor [268]. Piracetam molecules surround the phospholipid polar heads in the cell membrane, forming mobile drug-phospholipid complexes. Consequently, they restore membrane integrity and fluidity, resulting in better cell membrane viscosity and improved cell function [269]. This mechanism works at different sites: neuronal, red cell, and platelet [270, 271].

Piracetam has been used experimentally or clinically to treat a wide range of diseases and conditions, mainly in treatment of organic brain syndrome, myoclonus, memory impairment, post-concussion syndrome, vertigo, alcohol withdrawal, cerebrovascular insufficiency, hypoxia, intoxications of different origins or mechanic brain injuries [272]. Piracetam has been used successfully in treatment of alcoholism, recovery of aphasia after stroke, improvement or reduction of deterioration in dementia and Alzheimer's disease (http://www.piracetam.com/index.htm).

Piracetam has increased reading comprehension, accuracy, memory and verbal learning in dyslexic children [265], it has improved mental performance in "aging, nondeteriorated individuals" suffering only from "middle-aged forgetfulness" [264].

Libov et al. [273] examined the efficacy of piracetam (4800 mg/day) in the treatment of tardive dyskinesia using an oral preparation in a 9-week, double-blind, crossover (4 weeks), placebo-controlled trial assessing 40 schizophrenia and schizoaffective patients. Authors concluded that piracetam appears to be effective in reducing symptoms of tardive dyskinesia. The specific mechanism by which piracetam may attenuate symptoms of tardive dyskinesia needs to be further evaluated.

12.5.7 Modafinil

Modafinil (2-[(Diphenylmethyl) sulfinyl] acetamide, Provigil) is a USA Food and Drug Administration-approved medication that promotes wakefulness. It is a novel cognitive enhancer, has neuroprotective effects [274], and selectively improves neuropsychological task performance in healthy volunteers and adult patients with attention deficit hyperactivity disorder. Modafinil exhibits robust effects on catecholamines, serotonin, glutamate, gamma amino-butyric acid, orexin, and histamine systems in the brain. It is associated with a number of neurochemical actions in the brain that may be related to cognitive processes [275].

Turner et al. [276] examined the potential of modafinil (200 mg) as a cognitive enhancer in schizophrenia among 20 chronic schizophrenia patients using a double-blind, randomized, placebo-controlled crossover design. Modafinil had some cognitive enhancing properties in schizophrenia similar to those observed in healthy adults and adult patients with attention deficit hyperactivity disorder.

Sevy et al. [277] examined 24 patients with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder who were randomly assigned to modafinil up to 200 mg a day or placebo as an adjunct therapy in an 8-week, double-blind, placebo-controlled study. At the end of the trial, fatigue improved in both groups, and there were no between group differences on changes in fatigue, symptoms, attention, working memory, or executive functioning. Lack of between group differences may be due to small sample size.

Saavedra-Velez et al. [278] reviewed available data on trials of modafinil published in English up to January 2008 (6 trials were identified). Authors concluded that while the available data suggest that modafinil is generally well tolerated and may have some efficacy in the treatment of antipsychotic-induced sedation and cognitive domains, the small sample sizes, contradictory results, and methodological differences between trials, especially with respect to cognitive testing, make it difficult to draw firm conclusions about the overall effectiveness of adjunctive modafinil in the treatment of schizophrenia. Therefore, further research is required to address the potential benefits and risks of co-administration of modafinil to patients with schizophrenia.

12.5.8 L-Theanine

L-Theanine (gamma-glutamylethylamide) is a unique amino acid present almost exclusively in the tea plant (*Camellia sinensis*), where it typically occurs in amounts estimated from 1 to 2% by dry weight [46, 279]. L-theanine is absorbed through the intestinal tract and is hydrolyzed to glutamic acid and ethylamine in the kidney.Peak plasma concentration was found 30 minutes after oral dosing [280]. When 200 mg of L-theanine was orally administered to rats, the plasma concentrations of L-theanine and ethylamine reached their highest levels about 0.5 and 2 h after administration, respectively. When theanine becomes catabolized in the liver [281, 282] or kidney [280] it decomposes into two components: glutamic acid and ethylamine. The main effects of theanine may be grouped as follows: neuroprotective, mood-enhancing, supporting the immune system, possessing anti-obesity and anti-tumor activity, hypotensive, and reducing liver injury effects resulting from alcohol.

The *neuroprotective effects* of L-theanine are the focus of considerable attention. For instance, animal studies indicate possible neuroprotective effects of L-theanine in the hippocampus through blockade of multiple glutamate receptor subtypes, NMDA, and AMPA receptors [283, 284]. L-theanine was found to inhibit ischemic delayed neuronal death in gerbils. Furthermore, L-theanine is effective in protecting nerve cells from injury caused by low levels of oxygen, a condition known as ischemia, which is also characterized by excessive glutamate release [283]. L-Theanine was also reported to stimulate the release of nerve growth factor, a protein needed by cholinergic brain cells for survival [285].

The antioxidant activity of L-theanine has been studied with regard to its effect on the oxidation of low-density lipoprotein. In vitro testing, using malondialdehyde as a marker of lipid peroxidation, demonstrated inhibition of low-density lipoprotein oxidation with L-theanine, although the effect was weaker than the potent antioxidant effect of green tea polyphenols [286]. L-Theanine attenuated the doxorubicin -induced adverse reactions involved in oxidative damage, due to increased glutamate and the recovery of glutathione levels in normal tissues [287].

L-Theanine bears structural similarity to glutamic acid and hence competes with it in binding to glutamate receptors, offering protection against glutamate neurotoxicity [288]. Nagasawa et al. [289] investigated the molecular mechanism underlying the neuroprotective effect of L-theanine using primary cultured rat cortical neurons. Their findings strongly support the notion that neuroprotection by L-theanine can be observed when it is administered orally in vivo.

Theanine may have *mood-modulating activity* and play a role in reducing emotional distress (*anti-stress activity*). This amino acid actually acts antagonistically against the stimulatory effects of caffeine on the nervous system [283, 288]. Research on human volunteers has demonstrated that L-theanine induces a sense of relaxation approximately 30–40 minutes after ingestion via at least two different mechanisms. First, this amino acid directly stimulates the brain alpha wave activity [290], creating a state of deep relaxation and mental alertness similar to what is achieved through meditation. Second, the relaxing effects of L-theanine partly depend on its ability to interact with the brain's glutamatergic system [291].

Whereas historically, L-theanine has been shown to have relaxing properties [284, 292], it also has a reputation for counteracting the anxious jitters associated with caffeine without interfering with its ability to fight fatigue or sharpen mental focus [283, 293], although, the anxiolytic effects of theanine have not been established scientifically in animal or human studies. The pharmacological effects of L-theanine reported in animals suggest that it may have some anxiolytic properties, given that both serotonin and GABA play a fundamental role in the neurobiology of anxiety and are molecular targets in the treatment of various anxiety disorders [294]. Supporting the preclinical pharmacological effects of L-theanine, one electrophysiological study using healthy human subjects reported possible relaxing effects of L-theanine (200 mg), as indicated by increased alpha activity in the occipital and parietal cortex [295].

L-Theanine has become a promising candidate for management of schizophrenia because L-theanine may influence neurotransmitters in the brain such as GABA, dopamine, and serotonin [296, 297], and ameliorate attention and learning [292], and emotional distress [298].

Ritsner et al. [299] conducted the first trial aimed to evaluate the efficacy and tolerability of L-theanine augmentation of antipsychotic treatment in patients with chronic schizophrenia or schizoaffective disorders. L-Theanine was added-on to ongoing antipsychotic treatment (400 mg/day) of 60 patients that participated in an 8-week, double-blind, randomized, placebo-controlled study (40 patients completed the study protocol). Compared with placebo, L-theanine augmentation is associated with reduction of anxiety, positive, and general psychopathology scores measured by Hamilton Scale for Anxiety (HAM-A) scale and by the PANSS three-dimensional model, respectively. According to the five-dimension model of psychopathology, L-theanine produced significant reductions on PANSS positive, and activation factor scores compared to placebo. The effect sizes for these differences ranged from modest to moderate (0.09–0.39). PANSS negative and CANTAB task scores, general functioning, side effect and quality of life measures were not affected by L-theanine augmentation. L-theanine was found to be a safe and well-tolerated medication. Thus, L-theanine augmentation to antipsychotic therapy can ameliorate positive, activation, and anxiety symptoms in schizophrenia and schizoaffective disorder patients. Further long-term studies of L-theanine are needed to substantiate the clinically significant benefits of L-theanine augmentation.

12.5.9 Retinoids (Bexarotene)

Vitamin A derivatives, retinoids, play a critical role in normal development and physiology by modulating cell growth, division, reproduction, differentiation, and immune function [300], they are involved in a complex signaling pathway that regulates gene expression and, in the CNS, controls neuronal differentiation and neural tube patterning [301]. Retinoids are also capable of inhibiting cell growth, inducing differentiation, and triggering apoptosis in a variety of tumor cell lines [302].

There are two types of retinoid nuclear hormone receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both belong to the corticosteroid receptor superfamily and co-exist in most cells. The alpha, beta, and gamma subtypes of the RARs and RXRs have distinct and conserved amino and carboxy terminal domains. Each receptor subtype has a specific pattern of expression during embryonal development and a different distribution in adult tissues. This differential expression of receptor subtypes is thought to regulate the expression of distinct sets of genes. Heterodimers of the RARs and RXRs bind and regulate a specific DNA sequence known as the retinoic acid response element, which is located in the promoter region of genes such as the *RAR-b2* gene (reviewed in [303]).

Rexinoid receptor (RAR, RXR) families have been shown to mediate genes associated with both growth and differentiation. Gorgun and Foss [304] explored the immunomodulatory effects of RAR and RXR rexinoids in human T- and B-cell leukemia cells and demonstrated that RXR rexinoids are capable of up-regulating high-affinity interleukin-2 receptor expression.

Several studies reported that retinoids are involved in neurodevelopment [305, 306] and regulation of genes thought to be important in the pathogenesis of schizophrenia [307–309]. Although a major functional implication of retinoic signaling has been repeatedly suggested in synaptic plasticity, learning and memory, sleep, schizophrenia, depression, Parkinson disease, and Alzheimer disease, the targets and the underlying mechanisms in the adult brain remain elusive (see reviews [46, 310]).

The retinoid hypothesis in schizophrenia, developed by Ann Goodman [311, 312] is supported by three independent lines of evidence:

- congenital anomalies similar to those caused by retinoid dysfunction, are found in schizophrenic patients and their relatives;
- the loci which have been suggestively linked to schizophrenia are the same as the genes of the retinoid cascade (convergent loci); and
- the transcriptional activation of dopamine D_2 receptors and numerous other schizophrenia candidate genes are regulated by retinoic acid [311].

Several recent reports at the molecular level now suggest that altered transport and lowered synthesis of retinoic acid may be fundamental mechanisms in schizophrenia [312]. Vitamin A (retinoid) deficiency induces selective memory impairment further supporting the hypothesis in that the fine regulation of retinoid-mediated gene expression is important for optimal brain and higher cognitive functions [313]. Animal experiments, which disrupt retinoid-signalling pathways, compromise the regulation of synaptic plasticity and related learning and memory behaviours [314–319]. These pathways have also been connected with the pathophysiology of Alzheimer's disease, schizophrenia and depression. Retinoid analogs have therefore been proposed as potential treatment for schizophrenia [311, 320].

Bexarotene, LGD1069 (Targretin) belongs to the group of synthetic medicines derived from vitamin A (retinoid). Its chemical name is 4-[1-(5,6,7,8-tetrahydro-3,

5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl] benzoic acid [321]. To date this medication has been exclusively used as treatment of neoplastic or dermatological diseases using a high daily dose (more than 300 mg/day) [322–324]. Adverse events potentially related to bexarotene include lipid abnormalities, hypothyroidism, headache, asthenia, rash, leucopenia, anemia, nausea, and increased risk of infection, peripheral edema, abdominal pain, dry skin, dizziness, hyperesthesia, hypoesthesia, and neuropathy [325].

Based on the retinoid hypothesis in schizophrenia, our group conducted a 6-week open label trial in two mental health centers [326]. It was assumed that the combined effect of antipsychotic agents and bexarotene would have a beneficial effect in treatment of psychopathological symptoms in chronic schizophrenia patients. Since high daily doses of bexarotene can produce numerous adverse effects, the first trial was aimed to examine safety and preliminary efficacy of a low daily dose (75 mg/day) of bexarotene in an open label pilot study. Twenty-five patients with chronic schizophrenia received a low dose of bexarotene (75 mg/day) augmentation. Significant improvement from baseline to endpoint was observed on the total PANSS score, general psychopathology, and on the positive and the dysphoric mood factor scores. Furthermore, a trend to a diminishing ESRS score was found. Low doses of bexarotene were well tolerated. Bexarotene was found to be a safe medication as measured by all laboratory parameters with the exception of increased total cholesterol serum levels. This short-term pilot study supports bexarotene as a potential valuable adjunct in management of schizophrenia. A double-blind controlled study is currently underway to replicate these preliminary results (ClinicalTrials.gov Identifier: NCT00141947).

12.6 Conclusions and Future Directions

Despite increases in our understanding of the pathophysiology and environmental and genetic influences, etiology oriented treatment of schizophrenia remains an elusive goal. The evidence of the “stressed brain” together with neurodegeneration (apoptosis, oxidative stress, excitotoxicity, stress sensitization, neurotrophic factor expression, and alteration of neurosteroids) in the pathophysiology of schizophrenia suggests that there may be treatment opportunities using neural protection. Beyond the use of antipsychotic agents, it will be of interest to examine whether agents that demonstrate neuroprotective properties in preclinical studies may prove to have neuroprotective effects in schizophrenia. In recent years several potential neuroprotective compounds for treatment of schizophrenia patients were investigated. There are at least three steps for searching and testing neuroprotective agents in clinical practice:

1. searching for candidates with neuroprotective properties using findings from basic research studies;

2. assessment of safety and efficacy of suitable candidates for augmentation to antipsychotic therapy in "add-on" randomized, double-blind, placebo-controlled studies; and
3. assessment of safety and efficacy of promising compounds in randomized, double-blind, placebo-controlled multicenter studies.

Clinical trials for the evaluation of neuroprotective agents pose unique challenges in terms of experimental design and data interpretation [89]. There are many methodological issues that have limited the clinical application of therapies that have shown effectiveness in various animal models of acute neurodegeneration. Inadequate preclinical pharmacological evaluation is most likely a major drawback [327]. In order to generate meaningful results, clinical trials on neuroprotective agents should ideally be designed to minimize the potential for bias and optimize the ability to detect the neuroprotective effect of a therapeutic intervention in as short a time as possible. Key issues for the design of clinical trials of neuroprotective therapies include identifying appropriate endpoints and determining the ideal timing of the intervention.

Pharmacotherapy needs to be thoroughly integrated with neuroprotection strategy, which may be a useful paradigm for the treatment of schizophrenia patients. Neuroprotection therapy may putatively have a significant impact on the subsequent course and outcome of schizophrenia. Several potential neuroprotective compounds, representing a wide range of mechanisms, are available and merit further investigation in schizophrenia.

Acknowledgments The author would like to express gratitude to my colleagues Drs. Anatoly Gibel, Yael Ratner, Professor Vladimir Lerner, and Professor Abraham Weizman for fruitful cooperation. Mrs. Rena Kurs provided outstanding editorial assistance. The author gratefully acknowledges the support of the team of clinical departments of Shaar Menashe Mental Health Center. Clinical trials with neuroprotective compounds were supported by generous grants from the Stanley Foundation.

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Chapter 13
Recombinant Human Erythropoietin: Novel Approach to Neuroprotection and Neuroregeneration in Schizophrenia

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Abstract Mainly due to modern imaging technology, schizophrenia is increasingly recognized as a developmental disease with an additional neurodegenerative component, reflected by cognitive decline and progressive loss of cortical gray matter. Even though the old term *dementia praecox* already implies the presence of a degenerative process, meaning loss of once acquired cognitive functions, the degenerative aspect of schizophrenia has been disregarded for decades. Dealing with a neurodegenerative disease automatically brings on the idea of employing neuroprotective/neurotrophic add-on strategies in its treatment. Erythropoietin (EPO) evolved as an ideal candidate compound for neuroprotection in various human brain diseases, but particularly in schizophrenia, due to its capability of combating a spectrum of pathophysiological processes operational during the progression of schizophrenic psychosis. In the nervous system, EPO acts not only anti-oxidative, anti-inflammatory, and anti-apoptotic, thereby antagonizing driving forces of neurodegeneration, but also in a neurotrophic and plasticity ameliorating fashion, thus targeting intrinsic problems of the schizophrenic phenotype. In fact, EPO improves cognitive functioning in mice and lastingly enhances hippocampal longterm potentiation among other features of neuronal plasticity, essential for learning and memory processes. EPO also prevents the development of slowly progressing global brain atrophy in a mouse model of chronic non-gliotic neurodegeneration and reduces haloperidol-induced cell death in primary hippocampal neuronal cultures. In preparation of a first trial on EPO in schizophrenia, we wondered whether EPO can penetrate an intact blood-brain-barrier. Using Indium^{111}-labeled EPO, we demonstrated that EPO enriched within brain tissue in healthy and even more so in schizophrenic individuals, most likely explained by the higher density of EPOR expression in frontal cortex and hippocampus of the latter. Based on all these grounds, we performed a double-blind, placebo-controlled, randomized multicenter trial. Treatment over 12 weeks with weekly high-dose intravenous EPO led to

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_13,

significant improvement of cognitive performance in chronic schizophrenic men. Moreover, it delayed progressive cortical gray matter loss in schizophrenia-relevant brain areas as demonstrated by voxel-based morphometric magnetic resonance imaging analysis. Encouraged by these findings, an EPO treatment trial including patients with first-episode schizophrenia has been planned.

Abbreviations

CNP-ase	cyclic nucleotide phosphodiesterase
EPO	erythropoietin
EPOR	erythropoietin receptor
GABA	gamma-aminobutyric acid
GAD67	glutamic acid decarboxylase 67
HIF	hypoxia inducible factor
MRI	magnetic resonance imaging
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
SPECT	single photon emission computed tomography
VBM	voxel-based morphometry
WCST	Wisconsin Card Sorting Test

13.1 Introduction

Erythropoietin (EPO) is a hematopoietic growth factor, which for decades has been known to be expressed in kidney and fetal liver. Its complex role during brain development and as a neuroprotective mediator in the adult nervous system, however, has only begun to be increasingly recognized over the last ten years [1–18]. The expression of EPO is mainly controlled by hypoxia inducible factors 1 and 2 (HIF-1, -2), but there may be other potential mechanisms of induction [14, 15, 19–22]. EPO is ideally suitable for translational approaches, having been in clinical use for over 20 years to treat patients suffering from anemia of varying genesis, where it has proven to be safe and very well tolerated [22, 23]. Its properties in the nervous system, verified in numerous basic research reports from different groups, around 120 preclinical studies [24–51] and several recent publications on clinical trials in acute brain diseases [52–55], make EPO to date the most promising candidate for an application in innovative neuroprotective / neuroregenerative treatment approaches.

In 1997, long before publication of the aforementioned papers, we had started out, based on our own preclinical data, to use EPO as a neuroprotective treatment approach in patients with ischemic stroke [52]. This successful approach was followed by first therapeutic trials in diseases as diverse as schizophrenia [56] and multiple sclerosis [57]. To this day we are still quite solitary pioneers in this field. Based on our work, about 40 clinical trials are currently conducted worldwide with EPO in neuroprotective indications [18], but published data about effectiveness are still scarce (e.g. [53–55]).

As evident by the different diseases that are heterogeneous regarding etiology and pathogenesis, the aim is not to target the underlying cause of a specific illness, but rather to modulate the determinants of the so-called final common pathway. These determinants cause disease progression, worsening of the symptoms and advancing deficiency in functioning. Examples are neuronal dysfunction including changes in plasticity, increased apoptosis of nerve cells, inflammation, oxidative stress, metabolic disturbance or vascular insufficiency. EPO shows properties that make it very effective with respect to many of these mechanisms of the final common pathway. EPO acts anti-apoptotic, anti-oxidant and anti-inflammatory, thereby antagonizing driving forces of neurodegeneration, but also in a neurotrophic and plasticity enhancing fashion, thus promoting neuroregeneration (for review see [5, 14, 16, 21, 58–64]) and targeting intrinsic problems of the schizophrenic phenotype [65–76].

In the nervous system and elsewhere, EPO operates via binding to specific receptors, EPOR, which are expressed during the intrauterine development many times higher as compared to the adult brain. Postnatal, a gradual reduction in EPOR expression is observed with low levels found in a healthy adult [5, 7, 13, 15, 77, 78]. However, this low expression changes rapidly to a high expression if harmful influences on the nervous system result in an "emergency situation" of nerve cells, e.g. hypoxia, ischemia, inflammation or degenerative processes [59, 78–81]. For instance after stroke, but also in neurodegenerative conditions like Alzheimer's disease, elevated EPOR expression was found in various cell populations (neurons, glia, endothelial cells) [59, 62, 82]. If, on the other hand, in an animal model of stroke, all endogenous EPO is caught away by intracerebroventricular administration of soluble EPOR, a dramatic increase in damage ensues [25]. Together, these observations served to identify EPO/EPOR as an important endogenous neuroprotective *standby system* in the adult brain.

13.2 Schizophrenia as a Neurodegenerative Disease

For many years, schools in psychiatry argued about a possible involvement of neurodegenerative processes in the pathophysiology of schizophrenia. Since until recently, astrogliosis was required as obligatory proof of neurodegeneration, the absence of astrogliosis in the brains of deceased schizophrenic patients [83] made neuropathologists conclude that degenerative processes in schizophrenia do not exist. It was, however, agreed upon neurodevelopmental anomalies being involved in the etiology and pathogenesis of schizophrenia, underlined by reduced dimensions of nerve cells including the neuropil, disturbances in migration patterns of neurons in the cerebral cortex or other cytoarchitectural anomalies [67, 84–89]. The exclusive interpretation of schizophrenia as a developmental disorder automatically questioned its importance as a brain disease. It also explains why neurobiological research in the area of schizophrenia is still in its infancy, when contrasted with other neurodegenerative diseases. This development is especially surprising, since well

over 100 years ago, based on the French literature of the early 19th century, Emil Kraepelin, the father of modern psychiatry, had included for schizophrenia the term "*dementia praecox*" into his classification system [90]. The very term "*dementia*" already implies the existence of a degenerative process, because it embodies the loss of a formerly acquired function. Today, based on the possibilities of modern imaging techniques, the neurodegenerative aspects of schizophrenia are undoubtedly recognized [91–94]. In 2001, Thompson and coworkers [95] published a prospective 5-year follow-up study of patients with juvenile onset of schizophrenia. This magnetic resonance imaging (MRI) study demonstrated for the first time, based on morphometric analysis of consecutive brain images, a progressive loss of cortical gray matter, starting in the parietal lobe during the onset of disease, and successively spreading onto the temporal and frontal lobes. This way, *proof-of-principle* was provided for neurodegenerative processes taking place in schizophrenia, possibly initiated by pre-existing neurodevelopmental disturbances that may lead to rapid decompensation of the system upon challenges, e.g. during puberty.

13.3 Development of an Animal Model for Neurodegeneration in Schizophrenia

These MRI findings prompted us to develop an animal model in which the neurodegenerative aspects of schizophrenia were to be modeled [46, 96]. In the design of our model, we acted on two main assumptions: (1) The parietal lobe is the crucial starting point for the described neurodegenerative process, and (2) a juvenile age (puberty with its massive plastic rearrangements in the brain) is prerequisite for the initiation of the process. Both assumptions proved to be fundamental. Although in schizophrenia, the *endogenous* cause for the commencement of degeneration in the parietal lobe around puberty is still far from clear, we imitated these features in our model. The onset of the degenerative process was *exogenously* induced by stereotactically setting a small defined cryolesion (application of –80°C cold for 60 seconds by a liquid nitrogen-filled, metal tipped cone) through the intact skull onto the right parietal lobe of juvenile (28 days old) mice. Indeed, progressive bilateral brain atrophy is triggered this way, accompanied by changes in behavior and dysfunctions of learning and memory as well as sensorimotor gating, similar to those found in schizophrenia (Figs. 13.1, 13.2, and 13.3) [46]. Interestingly, aging of lesioned animals provokes an augmentation of the cognitive deficits, pointing to an additive role of the aging process *per se* in the sense of a system decompensation (Fig. 13.2) [46].

Because the molecular/cellular basis of the observed global brain atrophy after unilateral parietal lesion was entirely unclear, we implemented a comprehensive follow-up project in which different cell types in the brain of cryolesioned mice were stereologically quantified and, simultaneously, tissue levels of markers of interest were determined (Table 13.1) [96]. Importantly, we found with all applied methods and all examined parameters, from imaging via histology to Western blot, that the

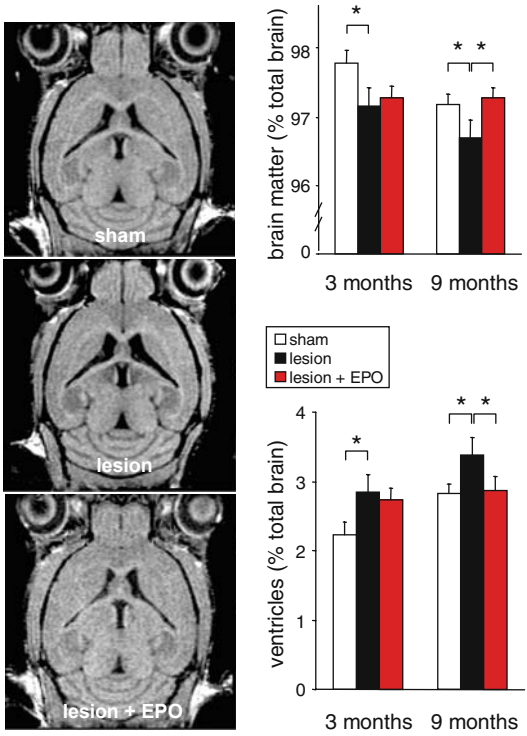

Fig. 13.1 Development of global brain atrophy after stereotactic cryolesion of the right parietal cortex in juvenile mice. Early EPO treatment can completely prevent brain matter loss. *Upper row*: Quantification of brain matter loss. *Lower row*: Illustration of brain atrophy by representative MRI images. Note the generally enlarged ventricles after lesion and the normal brain figuration (similar to sham-operated animals) in lesioned and EPO-treated animals (*p<0.05)

unilaterally applied lesion always induced *bilateral* alterations in the brain. After only a few hours, a bilateral rise of microglia numbers, which actually remained verifiable up to 12 months after the lesion, consistent with a chronically persisting mild inflammatory process, was found. While the overall number of neurons and also of astrocytes in the cingulate gyrus and hippocampus stayed unaltered,

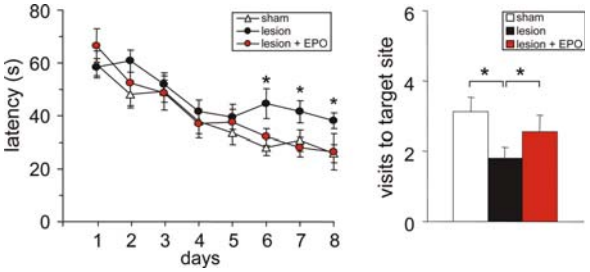

Fig. 13.2 Learning curve in *Morris Water Maze Test* including representation of the *Probe Trial* (*bar chart*) in sham-operated, lesioned and lesioned+EPO-treated animals 9 months after parietal cryolesion. While there are no differences detectable among treatment groups after 3 months (data not shown), the significantly inferior learning capacity of lesioned animals becomes evident after 9 months. EPO treatment can completely prevent this consequence of the lesion (*p<0.05)

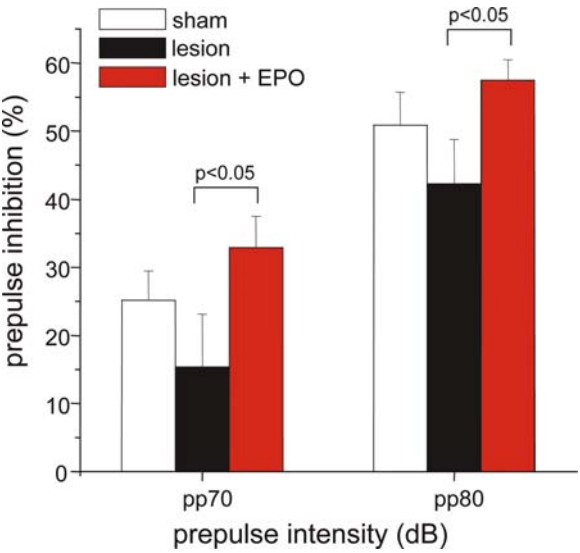

Fig. 13.3 The prepulse inhibition (PPI) of the startle response to acoustic stimulation, attributed to as readout of sensorimotor gating, is reduced at 3 months after juvenile parietal cryolesion and preserved under early post-lesion EPO treatment. The responsiveness of this network disturbance to EPO treatment is particularly interesting since PPI is known to be altered in schizophrenia

Table 13.1 Hippocampal histopathology in the murine juvenile parietal lesion model and in schizophrenia shows remarkable similarities. The effect of EPO on histopathological readouts of schizophrenia remains to be determined

Juvenile parietal lesion model (mouse) [96]	Schizophrenia (human)
Lack of astrogliosis *(experimental uncoupling of neurodegeneration and gliosis)*	Lack of astrogliosis [83] *(reason for questioning neurodegeneration in schizophrenia until recently)*
Early onset and persistent microglia activation	Global microglia activation [76]
Decrease in oligodendrocyte numbers/myelin-associated proteins	Decrease in oligodendrocytes / myelin-related genes [69, 71–73, 75]
Relative increase in determinants of GABA-ergic neurotransmission	Disturbances in GABA-ergic interneurons / GABA-synthesizing enzymes [66, 68, 74]
Reduction of a presynaptic protein, synapsin1	Reduced synapsins [65, 70]
Prevented by early EPO intervention	**Prevention by early EPO intervention?**

pointing to the existence of a non-gliotic neurodegeneration (as assumed in the case of schizophrenia), we found a quantitative shift in neuronal subpopulations. Parvalbumin-positive inhibitory GABA (gamma-aminobutyric acid) -ergic interneurons as well as the expression of the GABA-synthesizing enzyme GAD67 (glutamic acid decarboxylase 67) were augmented bilaterally in the hippocampus. As a further consequence of the parietal lesion, a reduction of the presynaptic protein synapsin1 was noticeable in hippocampus, consistent with an impairment of synapse function and neuroplasticity. A reduced expression of the myelin protein CNP-ase (cyclic nucleotide phosphodiesterase) does not only mirror the decline in the number of oligodendrocytes, but may also indicate a contribution of myelin loss to the observed

brain atrophy. It is especially noteworthy that an early two-week treatment with high-dose EPO (starting immediately after the lesion) is able to completely impede all described changes, comprising global brain atrophy and behavioral abnormalities as well as morphological/histological consequences of the juvenile parietal lesion [96].

Three main conclusions can be drawn from these findings:

(1) *Unilateral* minor parietal lesion in juvenile mice resulted in a *bilateral* non-gliotic neurodegeneration, which can be prevented by early EPO treatment.
(2) The observed non-gliotic neurodegeneration obviously imitates characteristics of neurodegeneration in schizophrenia [65, 66, 68–76, 83].
(3) For the lesion to initiate the neurodegenerative process, a vulnerable time point (puberty) and a vulnerable region (parietal lobe) are essential. A lesion in another position (e.g. occipital lobe) or at a later age (e.g. at 3 or 12 months) does not show a similar global effect on the brain (Table 13.1) [46, 96].

13.4 Preparing for a Neuroprotective/Neuroregenerative Approach in Schizophrenia

Once neurodegenerative processes in schizophrenia are assumed to co-determine disease progression and outcome, neuroprotective/neuroregenerative approaches in schizophrenia suggest themselves. Such kind of an approach is completely new in this disease. Stimulated by the potent effects of EPO in our animal model with respect to prevention of brain atrophy, behavioral changes and non-gliotic neurodegeneration [46, 96], we initiated as early as 2001 a neuroprotective study using EPO in schizophrenic patients [78]. Initially, the most important question was whether EPO as a big molecule of more than 30000 Dalton would be capable of penetrating the blood-brain-barrier, which is intact in most schizophrenic patients. We addressed this question by administering Indium^{111}-labeled EPO intravenously to young male schizophrenic patients and to healthy controls. Subjects were then examined using SPECT (single photon emission computed tomography) to assess the distribution of EPO in the organism, especially in the brain. It turned out that schizophrenic patients exhibit a stronger accumulation of the Indium^{111}-signal in the brain as compared to healthy men. At the same time, however, also healthy control patients demonstrated a clear penetration of EPO via their intact blood-brain-barrier into brain tissue as seen by a significant signal enhancement over time (Fig. 13.4) [78]. Nevertheless, it is important to underline that the percentage of EPO penetrating into the brain amounts to only about 0.1–1% of the EPO administered peripherally. This is why neuroprotective indications necessitate high doses of intravenous EPO. Having obtained the above results, the next question arose of how the higher enrichment of Indium^{111}-labeled EPO in the brains of schizophrenic patients as compared to control subjects could be explained. The answer to this question was found upon *post mortem* examination of the brains of schizophrenic

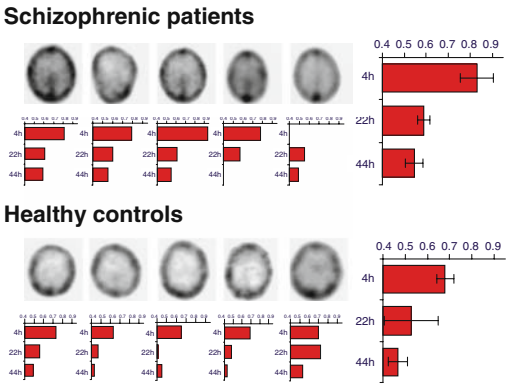

Fig. 13.4 Representative SPECT images after intravenous administration of Indium^{111}-labeled EPO to schizophrenic patients and healthy controls illustrate a higher intracerebral enrichment of the signal in the former. Individual bar charts display the relative signal at 4, 22 and 44 hours after EPO administration. On the *right side*, the mean values are given. Significant differences between the groups ($p < 0.05$) were found at the 4h and 44h time points

versus healthy persons. The former had a significantly higher EPOR expression in neurons as well as in glial cells of both hippocampus and cortex [78]. The reason for the augmentation of receptor expression in schizophrenia still remains unclear. In all likelihood, though, it can be attributed to the aforementioned "metabolic stress situation" of the respective brain cells, which is answered by an increase in EPOR expression and suggests the presence of an endogenous neuroprotective EPO system (possibly with a relative deficit of EPO). We next asked whether antipsychotics (as a criterion of distinction between patients and healthy controls, which acts supplemental to the disease itself) could possibly have influenced EPOR expression [78]. In fact, Pillai and Mahadik [97] reported on an increased expression of EPO and EPOR immunoreactivity in rat hippocampus and striatum upon 2–6 weeks of antipsychotic treatment, more pronounced and extended in olanzapine versus haloperidol-treated animals. Therefore, a role of antipsychotic treatment cannot be excluded even though the human brains examined in our study stemmed from patients who had died long before the broad introduction of newer antipsychotics to the clinic. Pillai and Mahadik [97] concluded from their work that second generation antipsychotics may, through the brain EPO system, exert neuroprotective effects. However, the question remains how EPO/EPOR upregulation upon antipsychotics occurs and whether it might be a sign of defense of the brain against metabolic stress (perhaps caused by certain antipsychotics) as delineated above. Along these lines, we detected in extensive analyses of primary rat hippocampal neurons that haloperidol-induced cell death is significantly reduced by EPO [78]. In any case, medication-induced or disease-induced, a higher responsiveness of the brain tissue to EPO due to the increased EPOR expression has to be assumed upon antipsychotic treatment and might be beneficial in schizophrenia.

13.5 *Proof-of-Principle* Trial: First Neuroprotective/ Neuroregenerative Add-On Treatment of Patients with Chronic Schizophrenia

The ground was prepared for the realization of the first multicenter, double-blind, randomized, placebo-controlled *proof-of-principle* study (phase IIb) with EPO as a neuroprotective/neuroregenerative *add-on* therapy in chronic schizophrenic patients [56]. Although patients suffering from a first-episode schizophrenic psychosis, i.e. experiencing the most aggressive stage of their disease with a dramatic cognitive decline and progressive brain atrophy, would probably have been the most suitable target population for demonstrating effects of neuroprotection, we decided to start out with chronically ill patients being aware of their disease and showing a stable cognitive deficit. The principal reasons for this decision came mainly from ethical considerations (first *proof-of-principle* study in schizophrenia, restricted capability of first-episode patients to giving informed consent and displaying longterm compliance). Added to these ethical considerations, though, came the hypothesis that principally every brain should possess a measurable potential of regeneration.

The total duration of our first *proof-of-principle* EPO schizophrenia trial extended to two years with an individual duration of 12 weeks. Chronic schizophrenic men with a defined cognitive deficit, stable medication and disease state, were treated for three months weekly *add-on* with an intravenous infusion of 40000 IU EPO versus placebo (solvent control). Primary outcome parameter was the change of cognitive functioning, comprising schizophrenia relevant measures, from baseline to the time point of cessation of therapy (week 12). A neuropsychological sum score, consisting of several subtests of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS [98]), i.e. delayed memory, semantic fluency and attention, as well as the Wisconsin Card Sorting Test (WCST-64 [99]), was determined before commencement of the treatment (at baseline) and then at weeks 2, 4 and 12 of study drug treatment. EPO- as well as placebo-treated patients improved in all evaluated categories, neuropsychology, psychopathology and social functioning. Patients receiving EPO, though, showed a significantly better outcome than patients receiving placebo with respect to their cognitive abilities (Fig. 13.5), and this improvement was unrelated to hemoglobin/hematocrit changes [56]. The cognitive outcome is of clear clinical relevance: From a qualitative perspective, the difference between EPO and placebo group in our trial can be compared with the results of Gold and colleagues [100] showing that the RBANS total score (also based on an intelligence quotient standard scale) differed by 16 points between unemployed and employed patients with schizophrenia. This means that in our study, a treatment of only 12 weeks yielded a difference that is over one third of the difference between the two groups in the study of Gold and colleagues [100] that differed with regard to employment, one of the most essential and stable functional outcome variables. Effects of EPO treatment on psychopathology or social functioning parameters were not yet detectable after this short treatment

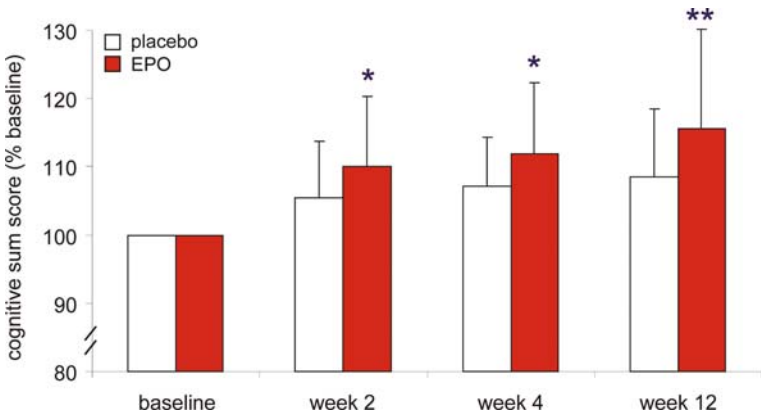

Fig. 13.5 Improvement of cognitive function, based on the individual performance before treatment (set to 100%) in EPO versus placebo patients with chronic schizophrenia. A difference can already be noted after 2 weeks and further increases until week 12 of treatment ($^{*}p<0.05$; $^{**}p=0.01$)

period in the chronic schizophrenic subjects of this study, i.e. patients who have been ill for more than 10 years.

Interestingly, the serum level of S100B, a glial damage marker, decreased under EPO administration [56]. The significance of this latter finding remains to be determined but seems interesting with respect to ample literature pointing to a role of S100B as a marker of disease severity and, in particular, of cognitive dysfunction in schizophrenia [101–103].

Surprisingly, in a currently conducted comprehensive voxel-based morphometric (VBM) analysis, in which brain dimensions of EPO versus placebo patients were compared before and after treatment, the EPO group as compared to the control group showed a reduced loss of gray matter in brain areas relevant for schizophrenia, including hippocampus, amygdala, nucleus accumbens, and several neocortical areas. This is amazing indeed since treatment extended to only 12 weeks. However, as gray matter loss *per annum* in schizophrenic patients amounts to about 0.5% (as compared to 0.2% in healthy people) [94], the measurability of a reduced decrease of brain substance within three months under neuroprotective treatment becomes plausible upon employing a very sensitive method like VBM (Wüstenberg et al., manuscript submitted).

EPO was safe and well tolerated upon this indication, dose, and treatment schedule [56]. The use of EPO as a neuroprotective/neuroregenerative treatment strategy in human brain disease, however, in particular when considering the high doses required to obtain sufficient levels within the brain in situations of widely intact blood-brain-barrier, requires meticulous and comprehensive safety management. EPO in this indication will never be a *laissez-faire treatment*. A close follow-up of patients at all times is mandatory, including clinical as well as laboratory examination combined with each and every EPO application [17, 18].

To conclude, EPO is the first compound leading to enhanced cognitive function in schizophrenia (and even chronic schizophrenic patients), associated with a reduction of gray matter loss. These findings do not present the closing, but just the beginning of a new development in the area of schizophrenia research. Because a therapeutic influence on cognition and brain atrophy has been unthinkable up to now, there should be, based on this *proof-of-concept* study, put high priority on attempts to even further improve this concept. Part of these attempts should be the exploration of different EPO doses but also of EPO variants, the modulation of treatment frequency and duration, the combination with various antipsychotic substances and certainly also the translation of these findings to the ultimate target population, i.e. patients with their first episode of a schizophrenic psychosis. The most pronounced changes with respect to cognition and gray matter occur at this early time point of disease. Therefore, it seems mandatory to examine exactly this target group with highest priority which is now, after a successful first trial, also ethically easier to justify. The intended study goal is outlined in Fig. 13.6. Should the efforts to reduce cognitive decline and gray matter loss in young patients with first-episode schizophrenia succeed, the result would bear considerable implications, not only individually, but also regarding society as a whole.

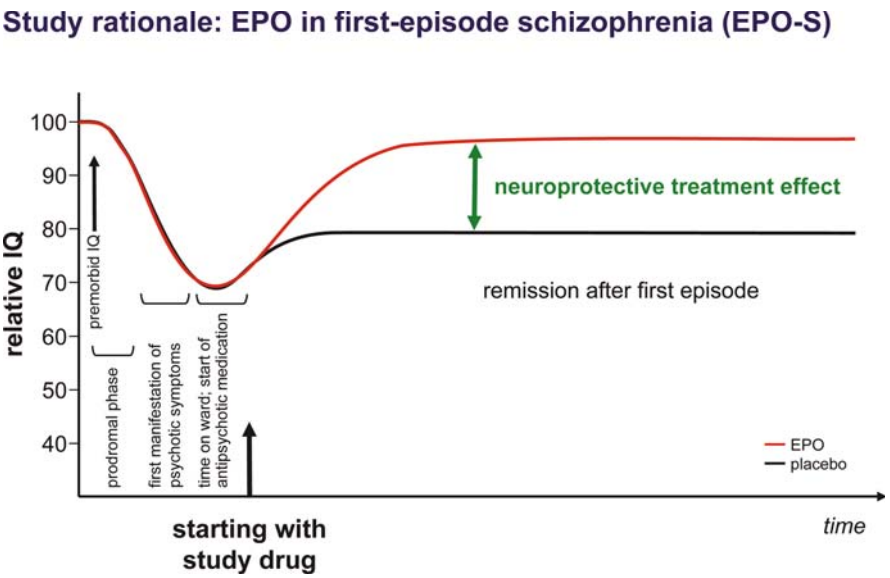

Fig. 13.6 Rationale of the planned EPO *add-on* trial in first-episode schizophrenic patients: Sketch of the spontaneous disease course with respect to cognitive performance and expected modification through neuroprotective treatment with EPO. Parallel to an improved recovery from cognitive decline in the acute disease state, the reduction in cortical gray matter is expected to be prevented/slowed down under EPO treatment

13.6 Back to the Mouse for Gaining Mechanistic Insight: Investigating EPO Mechanisms of Action with Respect to Cognitive Performance

Following our encouraging results even in chronic schizophrenic patients, we have not only planned a continuation of the clinical work with a *proof-of-principle* study in patients with first-episode schizophrenia, aiming to curtail cognitive decline and gray matter loss, but also went back to the mouse to examine which mechanisms could explain EPO effects on cognition [104, 105]. We conducted a great number of different experiments comprising behavioral tests in mice, in which higher brain functions were meticulously examined. In this first mechanistic approach, we focused on healthy young mice to exclude a potential interference with any disease variables. After a number of positive findings under EPO treatment in complex cognitive tests, e.g. the improvement of performance in the *Morris Water Maze Test* or in the *Five Choice Serial Reaction Time Task* [105], we selected for further exploration a simple model which delivered a rapid and robust, highly reproducible cognitive improvement of mice by EPO. We employed the so-called *Fear Conditioning*,in which mice are conditioned aversively by an electric shock featuring *context* and *cue*, i.e. a defined ambience and an acoustic key stimulus. If, in the wake of this conditioning, the mouse is either brought into the same context, or in a new context gets exposed to an acoustic aversion stimulus, this results in a prolonged *freezing*. This total standstill of all motor activity for a short time span points to a fear reaction of the animal which occurs at the time of recognizing / recalling the aversive stimulus. The duration of this *freezing* in comparison with the control group is interpreted as an achievement of memory. It turned out that in mice which had received high-dose EPO (5000 IU/kg body weight intraperitoneal) every other day for three weeks (starting from the age of 28 days), a significant improvement of memory was obtained which persisted for weeks after the end of treatment (Fig. 13.7). We examined hippocampal slices at the time point of maximal memory achievement and were able to show that longterm potentiation, a well recognized electrophysiological readout of neuronal plasticity in general and of learning and memory in particular, is highly significantly increased in slices derived from EPO-treated in comparison with slices from control animals. At the same time we observed an improvement in parameters of shortterm plasticity and a modulation of synaptic transmission, shifting the balance between excitatory and inhibitory activity (Fig. 13.8) [104].

All these changes are direct effects on nerve cells in the brain, independent of the hematopoietic effects of EPO. In hippocampal neurons, grown on multi-electrode arrays, and therefore open to the measurement of spontaneously developing electrical activity, chronic EPO treatment influences the electrical discharge emerging in the culture. EPO reduces in the developing neuronal networks the overall low-level activity (background noise), while enhancing the spiking activity, meaning the discharge potential of selected neuronal circuits in vitro [104]. Similarly, in the autaptic neuron culture model, i.e. on the level of single developing hippocampal nerve cells, EPO reduces the typical increase in excitatory synaptic transmission without changing the number of synapses. These observations may point to an

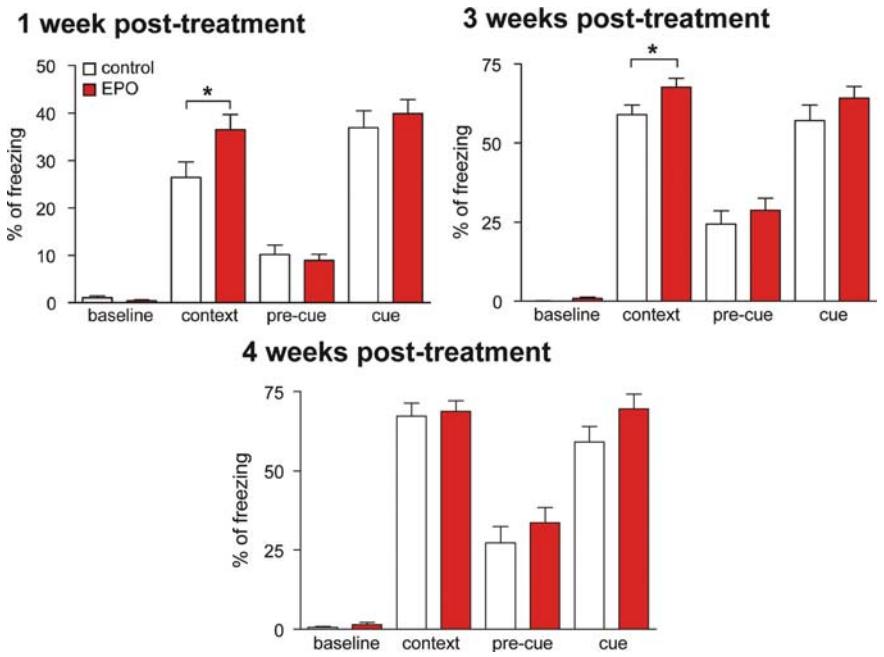

Fig. 13.7 In the *Fear Conditioning* paradigm, EPO treatment causes lasting improvement of hippocampal memory (*context*) in healthy young mice. Shown are the results of tests performed at 1, 3 or 4 weeks after three-week high-dose EPO treatment. Amygdala-associated learning processes (*cue*) remained unchanged in this set of experiments (*$p<0.05$)

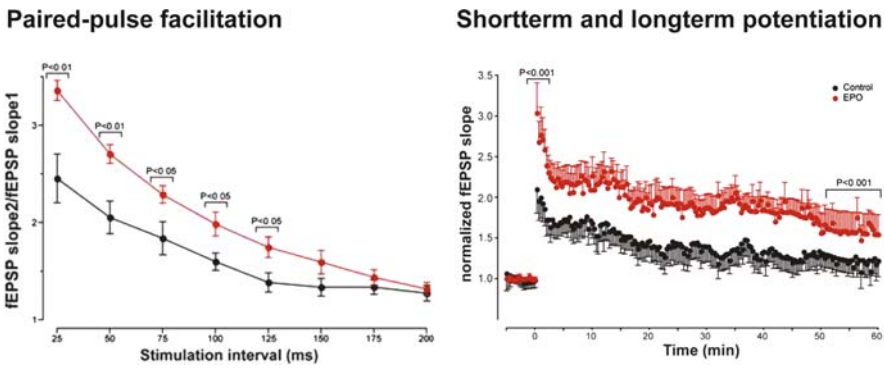

Fig. 13.8 EPO improves hippocampus-associated cognition via augmentation of shortterm plasticity (*paired-pulse facilitation*) as well as short- and longterm potentiation

EPO-induced focusing of neuronal activity, perhaps through functional silencing of synapses [104].

Taken together, EPO appears to influence hippocampus-dependent learning and memory processes via multifaceted modulation of neuronal plasticity including

changes in synaptic connectivity and, consequently, via modifying activity in relevant neuronal networks. These mechanisms of action of EPO have to be further examined and selectively translated to the treatment of neurological and psychiatric diseases.

13.7 Conclusions and Future Directions

Based on the results of a *proof-of-concept study*, EPO is at present the only compound that promises to lastingly reduce cognitive decline and cortical gray matter loss in schizophrenia. Logically, further basic research, trying to illuminate on the EPO mechanisms of action at the level of the neuron, further preclinical work, defining specific EPO targets, and further clinical research should follow along these lines, including additional phase II, and finally also phase III trials. For clinical EPO trials, however, industry as the primary beneficiary would have to provide funding. In the absence of continuous patent protection for the compound EPO, however, and in face of numerous competing EPO producers, the mainly "block buster oriented" big companies still hesitate to pursue new indications. Hopefully, this problem will be overcome, possibly through development of EPO variants [18], for instance by formulation of modified EPO molecules or other EPOR stimulating agents.

Acknowledgements This work has been supported by the Max Planck Society, by several private donations, as well as by the DFG Center for Molecular Physiology of the Brain (CMPB). The authors would like to thank Wiebke Timner for excellent editorial assistance.

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Chapter 14
Neuroprotective Agents in Mood Disorders: Pathophysiological and Therapeutic Implications

Giacomo Salvadore, Rodrigo Machado-Vieira, and Husseini K. Manji

Abstract Drug development for mood disorders such as major depressive disorder (MDD) and bipolar disorder (BPD) has undergone an important paradigm shift in the last decade due to our expanding knowledge of the pathophysiology and treatment of these illnesses. While previous research focused exclusively on abnormalities of the monoaminergic neurotransmitter systems, more recent preclinical and clinical studies have emphasized the role of impaired neuroplasticity and cellular resilience in mood disorders.

Also informing our thinking for drug development are the neurotrophic/neuroprotective properties associated with several agents currently used as treatments for mood disorders—including antidepressants, mood stabilizers, atypical antipsychotics, and a number of experimental agents currently under investigation. Many of these agents share some potent neurotrophic and neuroprotective properties, exert significant effects on signaling pathways that regulate cellular plasticity, and are believed to act directly on some of the core pathophysiological mechanisms underlying these devastating illnesses.

In this chapter, we discuss how drugs that modulate signaling pathways involved in regulating cell survival and cell death target some of the core pathophysiological mechanisms of BPD and MDD, with a particular emphasis on brain imaging and neuropathological abnormalities in mood disorders. Cellular and molecular mechanisms of action of agents with neurotrophic and neuroplastic properties are also discussed, with a special focus on the mood stabilizers lithium and valproate.

Abbreviations

ACC	Anterior cingulate cortex
ALS	Amyotrophic lateral sclerosis
APP	Amyloid peptide precursor protein
Bcl-2	B-cell lymphoma 2

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_14,

BDNF	Brain Derived Neurotrophic Factor
BPD	Bipolar disorder
Cho	Choline-containing compounds
CNS	central nervous system
Cr	phosphocreatine and creatine
CREB	Cyclic adenine monophospate response element-binding protein
DLPFC	Dorsolateral prefrontal cortex
ECT	Electroconvulsive therapy
ERK	Extracellular Signal-Regulated Kinase
GABA	Gamma-aminobutyric acid
GDNF	Glial cell line-derived neurotrophic factor
GM	Gray matter
GSK-3	Glycogen synthase kinase-3
^{1}H MRS	Proton magnetic resonance spectroscopy
HPA	hypothalamic-pituitary-adrenal
MAPK	Mitogen-activated protein kinases
MDD	Major depressive disorder
mI	myo-inositol
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAA	N-acetyl aspartate
NGF	Nerve Growth Factor
NMDA	N-methyl-D-aspartate
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
PI-3-K	Phosphatidylinositol-3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
SGPFC	Subgenual prefrontal cortex
SNP	Single nucleotide polymorphism
SSRIs	Selective serotonin reuptake inhibitors
VEGF	Vascular Endothelial Growth Factor

14.1 Introduction

Drug development for mood disorders has undergone an important paradigm shift in the last decade, based on our expanding knowledge of the pathophysiology and treatment of disorders such as major depressive disorder (MDD) and bipolar disorder (BPD). While previous research focused exclusively on abnormalities of the monoaminergic neurotransmitter systems, more recent preclinical and clinical studies have emphasized the role of impaired neuroplasticity and cellular resilience in mood disorders [1]. Furthermore, evidence suggests that modulating signaling pathways involved in neuroplasticity and cellular resilience might be one of

the main mechanisms of action underlying the clinical effects of medications currently used to treat mood disorders, including antidepressants and mood stabilizers such as lithium and valproate. Insights into the mechanisms of action of antidepressants and mood stabilizers have also allowed researchers to formulate new pathophysiological hypotheses that integrate preclinical findings with imaging data and neuropathological data from postmortem studies.

In this chapter we will first review the cellular and molecular mechanisms of action of agents with neurotrophic and neuroplastic properties, with a special focus on the mood stabilizers lithium and valproate. We then discuss imaging and neuropathological findings in patients with mood disorders, within a conceptual framework that highlights the role of neuroplasticity in the pathophysiology and treatment of these disorders.

14.2 The Molecular Mechanisms of Action of Lithium and Valproate

Mood stabilizers such as lithium and valproate are the most common treatment for BPD. Research into the effects of these structurally diverse agents suggests that they share some potent neurotrophic and neuroprotective properties [2, 3]. Lithium, for instance, exerts a wide range of biochemical and molecular effects by targeting diverse synapses and signal transduction pathways related to the activation of neurotrophic cascades in clinical and pre-clinical models of BPD. Most of these effects are associated with its chronic use at therapeutic doses, which represents the key clinical paradigm for evaluating the predictive validity of lithium and other mood stabilizers. Its diverse targets include cyclic adenine monophospate response element-binding protein (CREB), brain-derived neurotrophic factor (BDNF), B-cell lymphoma 2 (Bcl-2), mitogen-activated protein kinases (MAPK), and glycogen synthase kinase-3 (GSK-3) [4–6].

14.3 Lithium Upregulates Bcl-2 Levels in the Hippocampus and the Prefrontal Cortex (PFC): Importance of Bcl-2 in Preventing Apoptosis

Bcl-2 is a membrane-associated protein with antiapoptotic and neurotrophic properties [7]. Chronic treatment of rats with therapeutic doses of lithium or valproate upregulates Bcl-2 levels in the frontal cortex, an effect due primarily to a marked increase in the number of Bcl-2 immunoreactive cells in layers II and III of the anterior cingulate cortex [1, 8–10]. Interestingly, the importance of neurons in the ACC has recently been emphasized in neuroimaging studies of BPD, particularly because this area provides connections with other cortical regions, including the amygdala, and is a target for subcortical input [11]. Chronic lithium increases the number of Bcl-2 immunoreactive cells in the dentate gyrus and striatum [10]. Lithium was

also shown to increase Bcl-2 levels in C57BL/6 mice [9], in human neuroblastoma SH-SY5Y cells in vitro [1], and in rat cerebellar granule cells in vitro [9]. Overall, the data clearly show that chronic lithium robustly increases levels of the neuroprotective protein Bcl-2 in areas of rodent frontal cortex, hippocampus, and striatum in vivo, and in cultured cells of both rodent and human neuronal origin in vitro. Furthermore, at least in cultured cell systems, lithium reduces levels of the pro-apoptotic protein p53 [12].

Bcl-2 is expressed in the rodent and mammalian nervous system and is localized to the outer mitochondrial membrane, endoplasmic reticulum, and nuclear membrane. It is now clear that Bcl-2 is a protein that inhibits both apoptotic and necrotic cell death induced by diverse stimuli [13–15]. Several cellular mechanisms are involved in mediating Bcl-2's protective effects, including sequestering the proforms of caspases, inhibiting the effects of caspase activation, antioxidant effects, enhancing mitochondrial calcium uptake, and attenuating the release of calcium and cytochrome c from mitochondria (reviewed in [13, 14, 16, 17]). A role for Bcl-2 in protecting neurons from cell death is now supported by abundant evidence; Bcl-2 has been shown to protect neurons from a variety of insults in vitro including growth factor deprivation, glucocorticoids, ionizing radiation, and oxidant stressors such as hydrogen peroxide, *tert*-butylhydroperoxide, reactive oxygen species, and buthionine sulfoxamine [13, 14]. In addition to these potent in vitro effects, Bcl-2 also prevents cell death in numerous studies in vivo.

In the absence of pharmacological means of increasing central nervous system (CNS) Bcl-2 expression (until recently), all previous studies used transgenic mouse models or viral vector mediated delivery of the Bcl-2 gene into the CNS. In these models, Bcl-2 over-expression was shown to prevent motor neuron death induced by facial nerve axotomy and sciatic nerve axotomy, to save retinal ganglion cells from axotomy-induced death, to protect cells from the deleterious effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or focal ischemia, and to protect photoreceptor cells from two forms of inherited retinal degeneration; interestingly, neurons that survive ischemic lesions or traumatic brain injury in vivo show Bcl-2 upregulation [8, 15, 16, 18–21].

Overexpression of Bcl-2 has also recently been shown to prolong survival and attenuate motor neuron degeneration in a transgenic animal model of amyotrophic lateral sclerosis (ALS) [22]. Furthermore, not only does Bcl-2 overexpression protect against apoptotic and necrotic cell death, it can also promote *regeneration* of axons in the mammalian CNS, leading to the intriguing postulate that Bcl-2 acts as a major regulatory switch for a genetic program that controls the *growth* of CNS axons [18]. Because Bcl-2 has also recently been shown to promote neurite sprouting, increasing CNS Bcl-2 levels may represent a very effective therapeutic strategy for the treatment of many neurodegenerative diseases [18]. Recent studies in humans provide further evidence for a major role of Bcl-2 in mood disorders pathophysiology. A genetic variation in the Bcl-2 rs956572 single nucleotide polymorphism (SNP) is significantly associated with bipolar disorder and results in significantly lower protein and mRNA levels conferring less resistance to stress-induced apoptosis [23] . Noteworthy, this same risk SNP is also associated with decreased gray

matter volume in the ventral striatum in healthy subjects, an area known to play key roles in the neurobiology of reward processes and emotion regulation, and in the pathophysiology of mood disorders [24]. These findings are remarkable, as they converge with preclinical findings that Bcl-2 functions to enhance neuronal viability and might extend this evidence to humans.

14.4 Lithium and Valproate Also Regulate GSK-3 Pathway

Another major pathway likely to be involved in lithium's neuroprotective effects is GSK-3, a major regulator of apoptosis and cellular plasticity and resilience (see [4] for a review). GSK-3 is a serine/threonine kinase that regulates different cellular processes, including apoptosis. Generally, increased activity of GSK-3 is pro-apoptotic, whereas inhibiting GSK-3 attenuates or prevents apoptosis (reviewed in [4, 25]). Additionally, GSK-3 is deactivated through various signaling pathways also related to neuroprotection (e.g., the Wnt pathway, protein kinase A (PKA), protein kinase C (PKC), and the phosphatidylinositol-3-kinase (PI-3-K) pathway). Targets of GSK-3 involve transcription factors (β-catenin, CREB, c-Jun), proteins bound to microtubules (tau,microtubule-associated protein 1B, kinesin light chain), cell-cycle mediators (cyclin D, human ninein), and regulators of metabolism (glycogen synthase, pyruvate dehydrogenase) [26, 27].

While older literature suggested that lithium interacted with glycogen synthase, it was not until 1996, when Klein and Melton made the seminal observation that lithium inhibited the action of GSK-3 that the direct inhibition of this enzyme by lithium was identified [28]. GSK-3 is now known to regulate diverse functions in the adult mammalian brain, and to exert major cytoprotective effects (reviewed in [5, 29, 30]). Indeed GSK-3 is one of the kinases responsible for the aberrantly hyper-phosphorylated form of the microtubule-associated protein tau, a major constituent of neurofibrillary tangles [31]. GSK-3 also plays a major role in amyloid deposition [32]; thus GSK-3 inhibition regulates the two major pathways implicated in long-term disease progression in degenerative diseases.

14.5 Lithium and Valproate's Effects on the ERK/MAPK Pathway

Neurotrophic factors, such as Brain Derived Neurotrophic Factor (BDNF), Vascular Endothelial Growth Factor (VEGF) and Nerve Growth Factor (NGF) regulate some major intracellular cascades which influence cell survival and apoptosis. The cascade reactions of the two major cell survival pathways -the extracellular regulated kinase [33] MAPK signaling pathway and the phosphatidylinositol 3-kinase (PI3K–Akt) pathways- are known to play a critical role in the regulation of cell survival and growth of neurons during development and the survival and function of adult neurons, as well as modulate synaptic transmission and plasticity. RSK is another

kinase that also activates transcription factors that positively influence survival, and its upstream target, the Extracellular Signal-Regulated Kinase [33], is activated by Raf and MAPK. In vitro and in vivo studies have shown that the transcription factor, CREB and BDNF act together as important mediators of the neuroprotective and therapeutic effects of lithium and valproate.

Dysregulation at any target in the BDNF-ERK-CREB elements (such as by unrelenting cellular stress) could possibly explain the consequent atrophic processes in a selective subpopulation of vulnerable neurons and/or distal neurons and might underlie some of the structural and neurochemical abnormalities reported in imaging studies in BPD. Chronic stress (i.e., foot-shock, an animal model of depression) in rats results in ERK1/2 hyperphosphorylation in dendrites of the higher prefrontal cortical layers of rat brains, with concomitant phospho-CREB reduction in several cortical regions including the frontal cortex [34]. Because CREB is phosphorylated and activated by phospho-ERK1/2 directly, this phospho-CREB decrease indicates that chronic stress could downregulate CREB phosphorylation indirectly, leading to subsequent decrease of the transcription of neurotrophic genes such as BDNF.

Lithium's effects on the phosphorylation and activity of CREB have been investigated in several preclinical studies, with mixed results [35–37]. Postmortem studies in BD subjects have demonstrated reduced CREB phosphorylation in lithium-treated BD subjects [38, 39], but the instability of phosphorylated proteins postmortem is well known and a concern in such reports. CREB is also modulated by the mitogen-activated protein kinase (MAPK) signaling pathway, another target of lithium's effects. Thus, it is difficult to distinguish whether lithium's actions result from either CREB or MAPK signaling pathways.

Regarding BDNF regulation by lithium, its chronic administration increases BDNF expression in the rodent brain [35, 40], particularly in the hippocampus [41] and frontal cortex [42]. Specifically, a recent study found that chronic treatment of cultured rat cortical neurons with therapeutic concentrations of lithium selectively increases the levels of exon IV-containing BDNF mRNA, and the activity of BDNF promoter IV [43]. Finally, a recent microarray gene expression study of the molecular pharmacology of lithium on mouse brain mRNA further supports BDNF upregulation by lithium [44]; however, another recent study yielded conflicting results [45]. It has also been suggested that the neuroprotective effect of lithium in cortical neurons requires BDNF expression [46], a finding that emphasizes lithium's critical role in neuroprotection by directly upregulating neurotrophic factors levels.

Chronic treatment with lithium exerts major regulatory effects also on other neurotrophins, including NGF and glial cell line-derived neurotrophic factor [47]. Hellweg and colleagues showed that chronic (14 days) but not acute (one day) administration of lithium in adult rats significantly increases NGF concentrations in the frontal cortex (+23%), limbic forebrain (+47%), hippocampus (+72%), and amygdala (74%) [48]. Lithium was also shown to increase serum and hippocampal NT-3 levels in an animal model of mania [49]. Recent studies have also pointed out for the potential role of vascular endothelial growth factor

(VEGF) in the neuroprotective effects of lithium. VEGF has been implicated in neuronal survival, neuroprotection, regeneration, growth, differentiation and has been considered a neurotrophic factor. A recent study found that lithium upregulates VEGF in brain endothelial cells and astrocytes [50]; and that chronic treatment with lithium significantly attenuates the stress-induced decrease in VEGF expression in the hippocampus in stressed animals [51]. However, the role of VEGF in the mechanisms of action of mood stabilizers needs to be further clarified, as valproate treatment is associated with reduced levels of VEGF instead [52, 53].

14.6 Lithium's Neuroprotective Effects in Preclinical Paradigms

In view of lithium's major effects on Bcl-2, GSK-3 and ERK/MAPK cascade, it is not surprising that recent studies have investigated lithium and valproate's potential neuroprotective effects in a variety of preclinical paradigms, demonstrating robust neuroprotective properties against a variety of insults (reviewed in [1, 30, 54]). Notably, lithium pretreatment protects cultured brain neurons from glutamate-induced, N-methyl-D-aspartate (NMDA) receptor-mediated apoptosis (reviewed in [54]). Excessive NMDA throughput is likely involved in stress-induced hippocampal atrophy, and has been implicated in the pathogenesis of a variety of neurodegenerative diseases such as stroke, Huntington's disease, ALS, spinal cord injury, brain trauma and cerebellar degeneration. In cultured neurons, lithium-induced neuroprotection against glutamate excitotoxicity occurs within the therapeutic concentration range of this drug and requires five to six days of pretreatment for maximal effects. This neuroprotection by lithium requires BDNF induction and activation of its receptor TrkB, and is associated with upregulation of the anti-apoptotic protein Bcl-2, downregulation of the pro-apoptotic proteins p53 and Bax, and inhibition of caspase-3. Treatment of cultured neurons with other GSK-3 inhibitors or transfection with GSK-3 siRNA mimics lithium's neuroprotective effects [55], again suggesting that GSK-3 plays a critical role in mediating neuroprotection. Lithium also exerts beneficial effects in a number of animal models of neurodegenerative diseases. For example, pre- or post-insult treatment with lithium suppresses cerebral ischemia-induced brain infarction, caspase-3 activation, and neurological deficits in rats, and these neuroprotective effects are associated with induction of heat shock protein 70 (Hsp70) and decreased expression of Bax [56, 57].

Other studies have demonstrated that lithium has neuroprotective effects in animal and cellular models of Alzheimer's disease, Huntington's disease, Parkinson's disease, retinal degeneration, spinal cord injury, and HIV infection (reviewed in [54]). Notably, Phiel and colleagues [32] found that therapeutic concentrations of lithium acted on GSK-3 to block the production of Aβ peptides by interfering with amyloid peptide precursor protein (APP) cleavage at the gamma-secretase step. Lithium also blocked the accumulation of Aβ peptides in the brains of mice that overproduce APP. Similarly, lithium administration has been shown to significantly

lower levels of phosphorylation at several epitopes of tau known to be hyperphosphorylated in Alzheimer's disease and to significantly reduce levels of aggregated, insoluble tau [58]. Furthermore, levels of aggregated tau correlated strongly with degree of axonal degeneration, and lithium-treated mice showed less degeneration if lithium administration began during the early stages of tangle development. Most recently, it was demonstrated that lithium is neuroprotective in APP transgenic mice [59]; mice treated with lithium displayed improved performance in the water maze paradigm, as well as decreased tau phosphorylation and preserved dendritic structure in the frontal cortex and hippocampus [59]. Chronic lithium treatment also protects against neurodegeneration and improves spatial learning deficits in rats perfused with Aβ fibrils [60]. The evidence supporting the neuroprotective and neurotrophic effects of lithium is summarized in Table 14.1.

14.7 Neuroprotective Effects of Valproate

Valproate induces hippocampal neurogenesis and promotes neuronal maturation in mice (reviewed in [61]). Moreover, chronic treatment with valproate increases Bcl-2 expression in the frontal cortex, the striatum, and the hippocampus [1, 9]. In view of the important role that the extracellular receptor coupled kinase signaling cascade plays in mediating long-term neuroplastic events, a series of studies was undertaken to investigate the effects of lithium and valproate on this signaling cascade [62, 63]. These studies showed that lithium and valproate, at therapeutically relevant concentrations, robustly activate the ERK mitogen-activated protein kinase (MAPK) cascade in human neuroblastoma SH-SY5Y cells [62, 63]. Recent follow-up studies showed that, similar to the effects observed in neuroblastoma cells in vitro, chronic lithium and valproate also robustly increased the levels of activated ERK in brain areas implicated in the pathophysiology and treatment of BPD: the ACC and the hippocampus [62].

Valproate also has neuroprotective properties against oxidative stress [64], intracerebral hemorrhage [65], and glutamate-induced neurotoxicity [66]. Other downstream targets of valproate include the GSK-3 signaling cascade, on which valproate exerts an inhibitory effect that might be mediated by both direct and indirect mechanisms [4], and that might thus be relevant to its neurotrophic properties. Notably, a recent study showed that valproate upregulates the expression of glial cell line-derived neurotrophic factor (GDNF) as well as BDNF from astrocytes [62], suggesting that neuronal-glial cell interplay might be a major target for current and future drug development in BPD. BDNF is a secretory neurotrophin that is critical for neuronal survival and differentiation and might also be a critical regulator of plasticity and cell resilience in adult neurons and glia [67]. Extensive experimental evidence has implicated BDNF in affective disorders, anxiety disorders, and the mechanism of action of antidepressants (see below and [68] for an excellent review). The evidence supporting the neuroprotective and neurotrophic effects of valproate is summarized in Table 14.1.

Table 14.1 Neurotrophic and neuroprotective effects of lithium, valproate and antidepressants

Lithium

Demonstrates the following effects in preclinical studies
- Is neuroprotective against glutamate and NMDA toxicity, calcium toxicity, thapsigargin toxicity, β-amyloid toxicity, aging-induced cell death, growth factor and serum deprivation, glucose deprivation, and other insults (radiations, ischemia)
- Promotes hippocampal neurogenesis
- Increases Bcl-2 in the frontal cortex, the striatum, and the hippocampus
- Activates ERK/MAP kinase pathway
- Inhibits GSK-3 in vitro and in vivo

Demonstrates the following effects in human brain:
- Increases gray matter volume in lithium-treated patients with BPD; the effect is more pronounced in treatment responders
- Increases N-acetylaspartate (NAA) levels
- Larger anterior cingulate and prefrontal cortex volumes in lithium-treated patients with BPD
- Protects against reduced glial numbers or glia:neuron ratio in the amygdala

Valproate

Demonstrates the following effects in preclinical studies
- Is neuroprotective against oxidative stress, intracerebral hemorrhage and glutamate-mediated excitotoxicity
- Induces hippocampal neurogenesis and promotes neuronal maturation
- Increases Bcl-2 in the frontal cortex, the striatum, and the hippocampus
- Activates ERK/MAP kinase pathway
- Inhibits GSK-3 in vitro and in vivo
- Upregulates GDNF and BDNF in astrocytes

Demonstrates the following effects in human brain:
- No evidence about NAA increase or volumetric changes following valproate administration

Antidepressants

Demonstrates the following effects in preclinical studies
- Are neuroprotective against lipopolysaccharide- and Fas ligand-induced apoptosis
- Induce hippocampal neurogenesis
- Increase Bcl-2 mRNA and protein levels in the hippocampus
- Activate ERK/MAP kinase pathway
- Upregulate VEGF in the hippocampus
- Upregulate CREB and BDNF in the PFC and hippocampus

Demonstrates the following effects in human brain:
- Increase hippocampal NAA levels
- Increase occipital GABA levels
- Might prevent against gray matter loss in relevant brain areas (e.g., hippocampus, OFC)

Bcl-2: B-cell lymphoma 2; BDNF: Brain Derived Neurotrophic Factor; CREB: Cyclic adenine monophospate response element-binding protein; Dx: diagnosis; ERK: Extracellular Signal-Regulated Kinase; GABA: Gamma-aminobutyric acid; GDNF: Glial cell line-derived neurotrophic factor; GSK-3: Glycogen synthase kinase-3; MAPK: Mitogen-activated protein kinases; NAA: N-acetyl aspartate; NMDA: N-methyl-D-aspartate; OFC: Orbitofrontal cortex; PFC: Prefrontal cortex; VEGF: Vascular Endothelial Growth Factor

14.8 Neurotrophic and Neuroprotective Effects of Antidepressants

Research conducted over the last decade has highlighted the fact that some of the clinical benefits of antidepressants may stem from their neurotrophic properties. For instance, several studies demonstrated that chronic treatment with antidepressants or even electroconvulsive therapy (ECT) upregulated the expression of CREB and increased BDNF mRNA in the PFC and the hippocampus within a time frame consistent with the delayed onset of antidepressant action [69–71]. This observation, along with the fact that stress decreases BDNF levels in the hippocampus and produces depressive-like behaviors in preclinical paradigms, led researchers to formulate a "neurotrophin hypothesis of depression" [71]. According to this hypothesis, stress and antidepressants exert opposing effects on neurotrophin levels in areas of the brain belonging to mood regulating circuitry; stress might lead to cellular loss and a consequent volumetric reduction in patients with mood disorders by decreasing levels of BDNF and other neurotrophins. In contrast, antidepressants such as the selective serotonin reuptake inhibitors (SSRIs) would exert their clinical effects by upregulating neurotrophin expression and promoting the survival and proliferation of new neurons. Lending support to this hypothesis is the finding that interventions that selectively disrupt neurogenesis are also able to block the behavioral effects of antidepressants [72]. More recently, several studies have emphasized the role of VEGF in the mechanism of action of antidepressants, as VEGF upregulation following treatment with SSRIs is critical for antidepressant-induced neurogenesis in the hippocampus [73] and for the behavioural effects of antidepressants require the integrity of the VEGF signaling pathway [73].

In addition, by activating neurotrophic intracellular cascades, chronic treatment with antidepressants can also prevent lipopolysaccharide-induced apoptosis [74, 75] and promote cell survival in a rat model of cerebral ischemia [76]. Finally, the SSRI fluoxetine upregulates Bcl-2 mRNA and protein levels in the hippocampus, prevents Fas ligand-induced apoptosis, promotes neurogenesis [77], and activates the ERK and p38 MAPK cascades [78], providing further evidence for the neuroprotective properties of antidepressants. The putative neuroprotective and neurotrophic effects of antidepressants are summarized in Table 14.1.

14.9 Neuroimaging Studies in Mood Disorders: Evidence of Impairments in Cellular Plasticity and Resilience

Plasticity is characterized as the biological ability to induce and sustain important adaptive changes to internal and external stimuli in order to maintain the physiological functioning of our central nervous system. These changes aim to strengthen the synaptic signal and its efficacy by directly regulating neurotransmission (including receptor subunit phosphorylation and surface expression), intracellular signaling cascades in pre- and post-synaptic proteins, and gene expression of genes implicated

in cellular growth and survival as well as in synaptic transmission; cumulatively, this allows for the physiological remodeling of axonal and dendritic architecture. As discussed in greater detail in the following sections, loss of glial cells, synaptic contacts, and dendritic branches might underlie the volumetric reductions seen in patients with mood disorders. Neuroimaging and neuropathological studies are valuable methods to study the pathophysiology of mood disorders and the effects of treatments with neuroprotective/neurotrophic properties, given the putative ability of some of these agents to reverse some of the core cellular abnormalities underlying MDD and BPD.

14.9.1 Structural Findings

While whole brain gray matter volumes do not differ between patients with mood disorders and healthy controls [79, 80], discrete brain areas belonging to the frontolimbic cortex show volumetric reductions in patients with MDD and BPD (see [81]. for a review) For example, reduced GM density in the ACC characterizes adult, first episode, and also pediatric and adolescent patients with MDD and BPD [82–88].

The portion of the ACC ventral to the genu of the corpus callosum, known as the subgenual prefrontal cortex (SGPFC) has been the particular object of investigation by different groups as a key area involved in the pathophysiology of MDD and BPD as well as, possibly, treatment response. Decreased SGPFC volumes in patients with MDD and BPD have been reported by several independent groups, especially in subjects with a positive family history [89–91] of mood disorders.

Volumetric and density abnormalities have also been identified in other areas of the PFC including the ventral and the ventromedial PFC, the orbitofrontal cortex (OFC), the posterior cingulate cortex, and the superior, middle, and inferior frontal gyri [80, 84, 92–95]. Other regions strongly implicated in the pathophysiology of severe mood disorders and that might be modulated by prolonged treatment with antidepressants or mood stabilizers include the amygdala and the hippocampus. Numerous studies have reported hippocampal structural abnormalities in patients with MDD; meta-analyses support the reliability of these observations [96, 97]. Chronic stress and hypothalamic-pituitary-adrenal (HPA) axis hyperactivity may directly contribute to these structural abnormalities; HPA axis hyperactivity is commonly seen in patients with MDD, and may cause hippocampal atrophy in animals as well as increase the vulnerability of neurons to various insults such as glutamatergic excitotoxicity [71]. The evidence regarding hippocampal structural involvement in BPD is more controversial, as the vast majority of the volumetric studies focused on this region found no differences between patients and controls [98–103], with few exceptions [104]. It is likely that these negative results are confounded by concurrent treatment with mood stabilizers at the time of volumetric acquisition or by prolonged past exposure to them.

The amygdala is another structure that belongs to the medial temporal lobe and processes information with emotional valence [105]. Despite abundant evidence of functional abnormalities in this region in patients with mood disorders, the data

from structural studies are somewhat contradictory; a major technical limitation that might explain the heterogeneity of the results across studies is that most of them were performed with low field strengths (1.5T), which do not allow accurate tracing of the boundaries of this region because of its proximity to other grey matter structure. Enlarged [106], unchanged [107–109], or diminished [110, 111] amygdalar volumes have been described in patients with MDD, while studies of patients with BPD suggest possible opposite changes compared to healthy controls in adult and pediatric populations. In fact, most of the data in adult patients points towards a pattern of amygdala enlargement [100, 102, 112, 113], while the findings in pediatric BPD suggest decreased amygdala volumes [99, 114–116]. However, it is possible that morphological remodeling through prolonged treatment with mood stabilizers might be responsible for the volumetric amygdalar increases seen in adults with BPD [117].

14.9.2 The Effects of Medication on Structural Abnormalities in Mood Disorders

Emerging evidence suggests that treatment with lithium, and to a lesser extent with other mood stabilizers, modulates GM volume in patients with BPD, possibly by virtue of its neurotrophic and neuroprotective properties [1]. Drevets and colleagues [89] first reported that reduced SGPFC volume in patients with familial mood disorders was apparent in subjects treated with SSRIs but not in those treated with lithium, thus suggesting that lithium might prevent the cellular atrophy underlying these volumetric abnormalities.

From this seminal observation, several other studies addressed the role of medications in preventing volumetric abnormalities in patients with BPD. For example, Moore and colleagues reported a 3% GM volumetric increase following four weeks of lithium treatment in 10 patients with BPD [118]. These results were replicated in a follow-up cross-sectional study that investigated total GM volumes in lithium-treated patients, drug-free patients, and healthy controls [119]. Lithium's neuroprotective/neurotrophic effects have been also studied in vivo in specific brain areas that are part of mood regulating circuitry. Recently, Moore and colleagues replicated the evidence of increased GM volume following chronic lithium treatment in a larger sample of patients with BPD (N=28) and suggested a possible relationship between GM increase in the PFC and treatment response [120]. Evidence of volumetric increase in lithium-treated compared to unmedicated patients or those receiving medications other than lithium has been observed in the ventral PFC [121], the hippocampus [117, 122], and the ACC [82]. However, no differences between lithium-treated and unmedicated patients have been reported in the medial temporal lobe structures [100, 121], the basal ganglia [123], the pituitary gland [124], the thalamus [125], or the corpus callosum [126]. It is important to note that all the studies mentioned above suffer from the limitation of having a cross-sectional design; thus, baseline differences in the overall severity of BPD

across lithium-treated and unmedicated patients groups, as well as other unknown factors, might confound these findings.

A few studies with appropriate longitudinal designs have provided further evidence of lithium's neurotrophic effects in vivo. Recently, Yucel and colleagues demonstrated that both short-term (one to eight weeks) and long-term (1.9 years) treatment with lithium treatment induced a volumetric increase in the hippocampus [127, 128]. To their advantage, these studies enrolled only drug-naïve patients at baseline, thus avoiding the possible confounding effects of past medication exposures. Furthermore, some indirect evidence suggests that medications other than lithium might also induce volumetric increases in patients with BPD [80, 129, 130]. It is also worth mentioning that it is still debated whether the volumetric increases following treatment with lithium are determined by increased tissue water rather than by its neurotrophic properties [131].

With regards to MDD, the structural effects of antidepressant medications have not been adequately investigated. Cross-sectional reports suggest that treatment with antidepressants might prevent hippocampal [132] and OFC volume loss [133] in patients with MDD. However, one longitudinal study found that prolonged treatment with SSRIs had no effect on hippocampal volume [134].

In summary, the evidence reviewed above suggests that mood stabilizers, particularly lithium, might be able to modulate cerebral volumes in areas that are important in BPD pathophysiology. Whether these biological effects translate into better prognosis, increased cognitive function, or other clinical benefits, is essentially unknown. Future studies with a longitudinal design and a special focus on the link between brain changes and these outcome clinical variables in patients with BPD, as well with MDD, are warranted to address this issue.

Findings from brain imaging studies which investigated the effects of mood stabilizers and antidepressants on gray matter volume are summarized in Table 14.2.

14.9.3 Magnetic Resonance Spectroscopy Findings

Magnetic resonance spectroscopy (MRS) is a noninvasive imaging technique that allows quantification of various metabolites and amino acid neurotransmitters in the brain. Proton spectroscopy (^{1}H MRS) is the most commonly used approach and allows quantification of N-acetyl aspartate (NAA), choline-containing compounds (Cho), myo-inositol, phosphocreatine and creatine (Cr), and the amino acid neurotransmitters Glx (a combined measure of glutamate and glutamine) and gamma-aminobutyric acid (GABA)—when an adequate magnetic field strength is used. Here we focus on the studies that investigated NAA and amino acid neurotransmitters, as the modulation of these metabolites through drugs with neurotrophic properties might underlie some of their therapeutic properties.

NAA is the second most abundant amino acid in the brain after glutamate and is found exclusively in neurons. Although its precise role has yet to be clearly elucidated, NAA has traditionally been considered a measure of neuronal integrity

Table 14.2 Brain structural effects of medications with neurotrophic properties in patients with mood disorders

Study	N	Mean age	Mood status	Dx	Med. status	Area	Findings
Bearden et al. [122]	33	34	Various mood states	26 BPD-I 7 BPD-II	12 drug-free, 21 on Li monotherapy	Hippocampus	Total hippocampal volume greater in Li-treated pts. vs. pts drug-free
Blumberg et al. [121]	37	31.5	Various mood states	BPD-I	14 drug-free, 23 on various meds (Li, AC, AD)	VLPFC	Smaller GMV in the VLPFC in drug–free pts. vs. pts. on meds
Brambilla et al. [123]	22	37	Various mood states	17 BPD-I 5 BPD-II	8 drug-free, 14 on Li monotherapy	Basal ganglia	No differences btw.Li-treated and drug-free pts
Brambilla et al. [119]	27	35	Various mood states	21 BPD-I 6 BPD-II	11 drug-free, 16 on Li monotherapy	SGPFC	No differences btw.Li-treated and drug-free pts
Brambilla et al. [100]	24	35	Various mood states	18 BPD-I 6 BPD-II	9 drug-free, 15 on Li monotherapy	Temporal lobe	No differences btw.Li-treated and drug-free pts
Caetano et al. [125]	25	34.4	Various mood states	20 BPD-I 5 BPD-II	11 drug-free, 14 on Li monotherapy	Thalamus	No differences btw.Li-treated and drug-free pts
Drevets et al. [89]	21	25	Various mood states	N/S	13 drug-free, 8 on meds	ACC, SGPFC	SGPFC vol. reduction apparent in patients treated with SSRIs and not in pt. treated with Li
Foland et al. [117]	49	~ 40	Various mood states	BPD-I	All on meds; 12 w Li, 37 w/o Li	Amygdala, hippocampus	Larger bilateral hippocampal and L amygdala volumes in Li-treated pts. vs. pts. w/o Li
Hwang et al. [130]	49	~ 32	N/S	35 BPD-I 14 BPD-II	21 drug-naïve, 28 on meds	Basal ganglia	Significant shape differences with HC in drug-naïve but not treated pts.

Table 14.2 (continued)

Study	N	Mean age	Mood status	Dx	Med. status	Area	Findings
Lavretsky et al. [133]	41	70.5	Depressed	MDD	30 drug-naïve, 11 with past AD exposure	PFC	Larger OFC volume in pt. with past AD exposure vs. drug-naïve
Moore et al. [118]	10	33	Depression	BPD-I	Drug-free at time of 1st scan	Total GMV	Li-tx determines a volumetric increase in total GMV
Moore et al. [120]	28	33	Depression or euthymia	23 BPD-I 5 BPD-II	Drug-free at time of 1st scan	Total GMV, SGPFC, PFC	Li-tx determines a volumetric increase in total GMV; only Li responders showed GMV increase in PFC and SGPFC
Nugent et al. [80]	36	37 (df) 41 (on-meds)	Depression	7 BPD-I 29 BPD-II	16 drug-free, 20 on different medications	Whole brain voxel-wise analysis	Unmedicated pts. show lower GMV in the PCC, and the left STG vs. medicated pts
Sassi et al. [124]	24	34.7	Various mood states	18 BPD-I 6 BPD-II	9 drug-free, 15 on Li monotherapy	Pituitary gland	No differences btw.Li-treated and drug-free pts
Sassi et al. [82]	27	35.1	Various mood states	21 BPD-I 6 BPD-II	11 drug-free, 16 on Li monotherapy	ACC	Left ACC volume greater in Li-treated pts. vs. pts drug-free
Sheline et al. [132]	38	51	Remitted	MDD	Not specified	Hippocampus	Hippocampal volume negatively correlated with time spent depressed while untreated
Vythilingam et al. [134]	22	41	Depressed	MDD	Drug-free at time of 1st scan	Hippocampus	No change in hippocampal volume after tx. With SSRIs

Table 14.2 (continued)

Study	N	Mean age	Mood status	Dx	Med. status	Area	Findings
Yucel et al. [127]	12	28.8	Various mood states	2 BPD-I 2 BPD-II 8 BPD-NOS	All drug-naïve at baseline	Hippocampus	Li-tx determines a volumetric increase in the hippocampus over time
Yucel et al. [128]	28	~ 25.5	Various mood states	7 BPD-I 10 BPD-II 11 BPD-NOS	All drug-naïve at baseline	Hippocampus	Li-tx determines a volumetric increase in the hippocampal head

ACC: anterior cingulate cortex; AD: antidepressant medications; BPD-I: bipolar disorder I; BPD-NOS: bipolar disorder not otherwise specified; BPD-II: bipolar disorder II; df: drug-free; Dx: diagnosis; GMV: gray matter volume; HC: healthy control subjects; Li: lithium; N/S: not specified; MDD: major depressive disorder; OFC: orbitofrontal cortex; PCC: posterior cingulate cortex; PFC: prefrontal cortex; SGPFC: subgenual prefrontal cortex; SSRIs: selective serotonin reuptake inhibitors; STG: superior temporal gyrus, tx: treatment; VLPFC: ventrolateral prefrontal cortex

and viability, possibly linked to mitochondrial energy metabolism [135, 136] and susceptible to increases following chronic treatment with drugs that have neuroprotective/neurotrophic properties (see following section). Studies of NAA in patients with MDD and BPD have produced mixed results; for example, most of the studies conducted in individuals with MDD found no significant differences compared to healthy controls across different brain regions, including the ACC, the dorsolateral PFC (DLPFC) [137–140], the hippocampus [141], and the basal ganglia [142].

In individuals with BPD, reduced NAA levels in the DLPFC [143, 144] and the medial OFC [145] have been described, although most studies found no significant differences compared to healthy controls in regions of the PFC [33, 146–148]. Notably, lithium and possibly other treatments may elevate NAA levels in patients with mood disorders [129, 149, 150]; thus, the negative results observed in patients with BPD might be driven mostly by medication effects. Results from studies that investigated the temporal lobe and the hippocampus in patients with BPD are more consistent. Four independent studies reported lower NAA/Cr and NAA/Cho levels in patients with BPD despite considerable methodological heterogeneity across these studies [146, 151–153].

Consistent with the emerging literature about the role of the amino acid neurotransmitter systems in the pathophysiology and treatment of mood disorders (reviewed in [154]), MRS studies conducted over the last decade have also reported widespread abnormalities in GABA, Glx and Glutamate levels in patients with MDD and BPD. Given the topic of this chapter, it is of particular interest that abnormalities in GABA and glutamate levels may be closely linked to cellular pathology involving the interplay between glia and neurons. This pathology, in turn, may be reversed by different agents with neuroprotective/neurotrophic properties (see next paragraph). Sanacora and colleagues [155] found significantly reduced GABA levels in the occipital cortex in unmedicated MDD patients compared to healthy control subjects, along with increased Glx levels in the same region [156]. Hasler and colleagues [157] recently showed that GABA and Glx concentrations were significantly reduced in the dorsomedial/dorsal anterolateral PFC in unmedicated patients with MDD. Notably, this study specifically assessed brain areas where reductions in glial cell density, number, and markers had been demonstrated in postmortem studies in MDD. Reduced Glx levels in MDD patients have also been described by other groups in the ACC [138] and the amygdala [158].

The only study that investigated GABA levels in patients with bipolar depression found reduced levels of this amino acid in the occipital cortex [159]. Regarding excitatory amino acids, three of four independent studies demonstrated Glx increases in patients with BPD compared to healthy controls in the PFC, despite heterogeneity across studies in terms of mood state, medication use, and observed region-of-interest [137, 147, 148, 160].

14.9.4 Medication Effects on ^{1}H MRS Measures in BPD and MDD

MRS findings provide further in vivo evidence regarding lithium's neuroprotective/neurotrophic effects. Moore and colleagues [149] first reported a 5% increase

in NAA levels after four weeks of lithium administration in 12 patients with BPD and nine healthy volunteers. Unfortunately, after this seminal report few adequately conducted studies have been performed to analyze longitudinal NAA changes after chronic lithium administration.

Friedman and colleagues [161] investigated lithium and valproate-induced changes in individuals with BPD whose symptoms were mostly depressive. Patients were scanned at baseline and then reassessed after chronic treatment with lithium (mean duration of treatment: 3.6 months) or valproate (mean duration of treatment: 1.4 months). Post-treatment GM NAA levels were not significantly different from baseline for either of the two mood stabilizers. It is possible that heterogeneity in terms of treatment duration and proportion of patients with BPD-II might be responsible for this result. Brambilla and colleagues [162] also failed to show NAA increases in the DLPFC in 12 healthy volunteers who were administered lithium for four weeks. This negative result, however, may be attributable to small sample size, slower lithium titration, and different voxel placement.

Higher NAA levels were observed in lithium-treated but not valproate-treated patients with BPD compared to control subjects in a cross-sectional report [163], while a similar cross-sectional study described higher hippocampal NAA levels in patients treated with valproate or valproate + quetiapine than drug-free patients [129].

Finally, lithium was shown to decrease gray matter (GM) Glx concentration after chronic treatment in patients with BPD [161]; given the emerging role of glutamatergic and GABAergic abnormalities in BPD, this finding is potentially very relevant because the modulation of amino acid neurotransmitters is likely to be directly related to the clinical effects of medications.

To date, the effects of antidepressants on MRS measures in MDD patients has not been adequately investigated. Chronic treatment with either citalopram or nortryptiline was shown to increase hippocampal NAA levels in patients with MDD [164]. In the same study, low baseline NAA levels predicted treatment response. This finding is noteworthy, as NAA increases might be directly related to the neurotrophic properties of antidepressants. In addition, recent evidence suggests that antidepressants might also be capable of reversing GABA abnormalities in patients with MDD; for instance, Sanacora and colleagues demonstrated that occipital cortex GABA concentrations increased after individuals with MDD were treated with either SSRIs [165] or ECT [166], but not with cognitive behavioral therapy [167]. ECT is also associated with increased left ACC glutamate/glutamine levels in ECT responders, but not in ECT-nonresponders [168]. In Table 14.3 we summarize the results from ^{1}H MRS studies which investigated putative markers of neurotrophic and neuroprotective effects of mood stabilizers and antidepressants.

In sum, emerging evidence suggests that MRS might be successfully used to study the in vivo effects of agents with neuroprotective and neurotrophic properties in patients with mood disorders. More rigorously conducted longitudinal studies are clearly needed; ideally, such studies would investigate not only the neurochemical effects of chronic treatments but also the clinical correlates of these changes along with potential biomarkers of treatment response. The use of new MRS biomarkers

Table 14.3 Brain neurochemical effects of medications with neurotrophic properties in patients with mood disorders

Study	N	Mean age	Current status	Dx	Med. status	Area	Metabolites of interest	Findings
Atmaca et al. [129]	12	29.8	Various mood state	BPD-I	10 Drug-naïve 10 on VPA 10 on VPA + QTP	Hippocampus	NAA, Cr, Cho	Lower NAA/Cre and NAA/Cho in drug-naïve vs. treated pts.
Block et al. [164]	13	36	Depressed	MDD	Drug-free at baseline, then citalopram or NTP	Hippocampus	NAA, Cr, Cho, Glx	No net tx. effect on any metabolyte ; increase in NAA and Cho correlated with clinical improvement
Brambilla et al. [162]	12	25	N/S	Healthy subjects	Drug-free at baseline, then Li	DLPFC	NAA, Cr, Cho, mI	No NAA increase following chronic Li tx.
Friedman et al. [161]	21	30.1	Depressed or mixed	8 BPD-I 13 BPD-II	12 on Li, 9 on VPA	Slice that encompasses several regions	NAA, Cr, Cho, Glx	Glx decrease and mI increase following Li but not VPA tx.
Moore et al. [149]	12	36.3	Depressed	11 BPD-I 1 BPD-II	Drug-free at baseline, then Li	Several brain regions	NAA	NAA increase following Li tx.
Pfedlerer et al. [168]	17	61	Depressed	MDD	Drug-free at baseline, then ECT	ACC	Glx	Normalization of Glx levels in ECT responders
Sanacora et al. [165]	11	39.2	Depressed	MDD	Drug-free at baseline, then SSRIs	Occipital cortex	GABA	GABA increase following tx. with SSRIs

Table 14.3 (continued)

Study	N	Mean age	Current status	Dx	Med. status	Area	Metabolites of interest	Findings
Sanacora et al. [166]	8	46	Depressed	MDD	Drug-free at baseline, then ECT	Occipital cortex	GABA	GABA increase following ECT
Sanacora et al. [167]	8	N/S	Depressed	MDD	Drug-free at baseline, then CBT	Occipital cortex	GABA	No change in GABA after CBT
Silverstone et al. [163]	25	~ 38	Euthymic	14 BPD-I 11 BPD-II	14 on Li,11 on VPA (+ other meds)	Frontal and temporal c.	NAA/Cr	NAA/Cr higher in Li-treated but not VPA-treated pts vs. HC

ACC: anterior cingulate cortex; BPD-I: bipolar disorder I; BPD-II: bipolar disorder II; c.: cortex; CBT: cognitive behavioral therapy; Cho: choline containing compounds; Cr: phosphocreatine and creatine; DLPFC: dorsolateral prefrontal cortex; ECT: electroconvulsive therapy; GABA: Gamma-aminobutyric acid; Li: lithium; MDD: major depressive disorder; NAA: N-acetyl aspartate; N/S: not specified; NTP: nortriptyiline; QTP: quetiapine; SSRIs: selective serotonin reuptake inhibitors; tx: treatment; VPA: valproate

of neural progenitor cells in vivo [169] might increase our understanding of the role of neurogenesis in the mechanism of action of antidepressants, even though future studies are needed to validate this method.

14.10 Neuropathological Studies

Postmortem studies are another valuable way to investigate the cellular determinants of volumetric and neurochemical abnormalities described in patients with mood disorders. Furthermore, histopathological changes might guide preclinical experiments that investigate the opposing effects of stress and neuroprotective drugs on cells and synapses.

The most consistent finding reported in postmortem studies in BPD and MDD is reduced density and number of glial cells, which have been reported in the ACC, the DLPFC, the OFC, and the amygdala [170–173]. Increased glial cell size and cell shape abnormalities have also been independently reported by different laboratories (reviewed in [173, 174]). Whether the glial cells involved in mood disorders are astrocytes or oligodendrocytes is currently unknown. In fact, early postmortem studies used Nissl staining, a technique that did not allow researchers to distinguish between the different kinds of glial cells. More recent postmortem studies performed with have found decreased glial acid fibrillar protein (GFAP) mRNA levels—a marker specific to astrocytes—and reduction of key oligodendrocyte-related and myelin-related genes in patients with BPD and MDD compared to unaffected controls [175, 176]; this suggests that more than one kind of glial cell is likely to be involved in the pathophysiology of mood disorders.

Several studies have also shown more subtle abnormalities in neurons (see [177] for a review). For instance, reduced density of large neuronal cells was observed in layers II, III, and V of the PFC [174]; whether these changes are related to glutamatergic excitotoxicity, stress, and related HPA-axis hyperactivation, insufficient glial support, or some other factor, is essentially unknown. The prefrontal layers where most of these neuronal abnormalities have been detected are associated with areas where pyramidal glutamatergic neurons give rise to long projections to other cortical associational regions, such as the striatum and thalamus.

In addition, immunohistochemical studies in patients with MDD and BPD have found decreased levels of calbindin and parvalbumin positive cells in the ACC, the hippocampus, the DLPFC, and the entorhinal cortex; taken together, the findings suggest GABAergic interneuron dysfunction [178–180]. Reduced synaptic markers have been also extensively shown in postmortem studies of BPD patients over the last decade and, to a lesser extent, in patients with MDD [181]. Fatemi and colleagues [182] found that patients with BPD but not MDD had reduced levels of the synaptosomal associated protein SNAP-25 in the hippocampus compared to healthy controls, although negative results regarding SNAP-25 have also been published, as well as evidence of increased SNAP-25 levels in the DLPFC of individuals with BPD [183]. Synaptic protein abnormalities in individuals with BPD were also documented by Eastwood and Harrison, who found decreased synaptophysin, complexin

II, and GAP-43 in the ACC of individuals with mood disorders [184]. The reduction in these synaptic markers was positively correlated with duration of illness, and was greater in subjects with a positive family history of mood disorders. The same group also demonstrated that patients with BPD had synaptic marker alterations in the hippocampus [185]. Finally, reduced dendritic spine density was observed in the subiculum of BPD subjects [186], providing further evidence that the volumetric reductions seen in BPD are likely due to synaptic pathology and neuropil reduction.

14.11 From Stress to Cellular Damage and Volumetric Changes: Pathophysiological Hypotheses

Patients with mood disorders show several structural and neurochemical abnormalities in areas associated with fronto-limbic circuitry. In the same regions where volumetric abnormalities have been described, postmortem studies demonstrated extensive neuropathological abnormalities involving neurons, glial cells, and synapses. Glial cells and astrocytes in particular, play a pivotal role in intrasynaptic glutamate re-uptake, and might protect neurons from glutamate-mediated excitotoxicity. Reduced glial cell number and function might lead to a less efficient glutamate-glutamine cycle between neurons and astrocytes, increased glutamate levels, and concomitant increases in cell death because of glutamatergic excitotoxic damage.

Indeed, whether the volumetric reductions seen in several brain areas in patients with mood disorders might be accounted for by neuron/glial cell decreases is a long-debated subject. Findings from postmortem studies conducted over the last decade have helped clarify this issue. Extensive evidence (reviewed above) of reduced synaptic markers involving the hippocampus, the ACC, and other brain regions suggests that the volumetric reductions seen in BPD and, perhaps, MDD, are more likely due to synaptic pathology and neuropil reduction. Stress, HPA axis hyperactivation, and dysregulated glutamatergic transmission might play a prominent role in determining these changes (see [187] for a review). Besides directly determining hippocampal atrophy, stress and glucocorticoids also reduce cellular resilience, making cells more vulnerable to various insults, such as glutamatergic excitotoxicity [188]. Stress leads to the release of more glutamate in the hippocampus, creating the possibility of excitotoxic damage and dendritic remodeling (see [189] for a comprehensive review). Stress also reduces hippocampal BDNF expression, which is critical for neuronal function and survival, and decreases neurogenesis (reviewed in [71]). However, further studies are needed to investigate the link between cellular pathology and volumetric abnormalities in mood disorders and their pathophysiological determinants.

14.12 Conclusions and Future Directions

Evidence from both animal and human studies of neuroprotective/neurotrophic agents in MDD and BPD suggest that these agents increase cellular resilience by

decreasing the cellular damage associated with stress and thus might reverse some of the core cellular and structural abnormalities associated with these disorders. Data from rodent studies show how chronic treatment with mood stabilizers and antidepressants upregulates the expression of synaptophysin and other molecules related to synaptic remodeling [190, 191], and increases hippocampal pyramidal spine synapse density [192], providing evidence that therapeutics used in the pharmacopeia of mood disorders induce a vast range of plastic synaptic changes. The activation of anti-apoptotic signaling cascades, along with the upregulation of neurotrophin levels and increased neurogenesis, might be responsible for these phenomena. Evidence of increased GM volume and markers associated with neuronal viability after chronic treatment with lithium, and possibly other drugs, creates the possibility of studying the putative neuroprotective and neurotrophic effects of these drugs in vivo.

The development and application of MRI systems with stronger fields will allow better definition of discrete brain structures and better spectral resolution of molecules related to the amino acid transmitter systems (GABA, glutamate, and glutamine) in order to investigate the changes induced by mood stabilizers and antidepressants. Finally, the development and implementation of MRS for measuring markers related to neurogenesis might help researchers investigate the neurotrophic effects of lithium, valproate, and SSRIs directly in humans, along with the potential clinical relevance of this phenomenon. Serial measurement of these markers in critical brain areas might also lead to better follow-up of patients, more personalized treatments, and more accurate prognostic judgments. Such work is key for expanding our ever-growing understanding of the biological underpinnings of these devastating disorders, as well as for informing the development of novel medications to treat them.

Acknowledgements The authors gratefully acknowledge the support of the Intramural Research Program of the National Institute of Mental Health. Ioline Henter provided outstanding editorial assistance.

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Chapter 15
Neuroprotection in Bipolar Depression

Chris B. Aiken

Natural forces within us are the true healers of disease

– Hippocrates

Abstract The past 10 years have seen a growth of therapeutic options for bipolar depression. Evidence for the clinical and neuroprotective effects of these treatments is reviewed in this chapter, including lamotrigine, pramipexole, modafinil, and atypical antipsychotics. Their neuroprotective profiles are compared to the better established effects of lithium and valproate, which include upregulation of brain-derived neurotrophic factor (BDNF) and B-cell lymphoma 2 (bcl-2) and inhibition of glycogen synthase kinase-3β (GSK-3β) and glutamate transmission.

The neuroprotective profile of antidepressants will also be examined to better understand their controversial role in bipolar depression. Treatments with efficacy in multiple phases of bipolar illness exert neuroprotective effects in multiple brain regions, including the anterior cingulate cortex, striatum, and hippocampus. Antidepressants, in contrast, have a more focal neuroprotective effect through BDNF in the hippocampus.

Finally, this chapter will explore both the clinical and neuroprotective effects of adjunctive and complimentary treatments for bipolar depression, including psychotherapy, omega-3 fatty acids, N-acetylcysteine, diet and exercise. Lastly, the neuroplasticity model will be explored as a tool for engaging patients in their recovery and improving medication adherence.

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_15,

Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
COX-2	Cyclooxygenase 2
CREB	cAMP-response element binding protein
BDNF	Brain-derived neurotrophic factor
Bcl-2	B-cell lymphoma 2
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
ERK1/2	Extracellular Signal-Regulated Kinases 1 and 2
FDA	Food and Drug Administration
GSK-3β	glycogen synthase kinase-3β
NAA	N-acetylaspartate
NMDA	N-methyl-D-aspartate
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

15.1 Introduction

Bipolar disorder is a recurrent illness in which manic or hypomanic states alternate with depressive episodes. The illness carries high rates of morbidity and mortality that are not fully explained by its core symptoms. Bipolar disorder is the sixth leading cause of disability in working-age adults worldwide, yet this functional impairment often persists after symptomatic recovery from an episode [1]. The mortality rate in bipolar disorder is higher than that predicted by the 20-fold increase in suicide risk it brings [2]. People with bipolar disorder die from natural causes at nearly twice the rate of the general population, which is similar to the mortality risk associated with nicotine [3].

Several features of bipolar disorder contribute to these epidemiologic findings. Depressive symptoms account for 30–60% of the lifespan and carry significant morbidity [4, 5]. Cognitive deficits also play a role, and have been proposed to explain the persistence of functional disability after recovery from an episode [1]. Finally, lifestyle factors such as poor habits of diet and exercise likely contribute to the elevated rates of comorbid cardiovascular disease, stroke, diabetes and respiratory illnesses observed in bipolar disorder [3].

Depression, cognition, diet and exercise are all associated with common neuroplastic changes. This chapter will review these findings and highlight both the common threads and inconsistencies along the way. All treatments whose clinincal benefits are supported by at least one randomized, placebo controlled trial in bipolar depression will be examined. Lastly, we will consider how the neuroprotective hypothesis can support an integrated approach to bipolar depression which engages patients in their recovery.

15.2 Lithium and Valproate

15.2.1 Clinical Studies

Lithium's clinical benefits in the treatment and prevention of bipolar depression are supported by more controlled trials than that of any other therapy [1]. Its broader role in depression is suggested by its efficacy as an augmentation agent in unipolar depression, and the research supporting this role also overshadows that of other augmentation strategies [6]. Valproate is primarily known for its antimanic properties [1], though several small, randomized placebo controlled trials have found it effective in both the acute treatment [7, 8] and prevention of bipolar depression [9].

15.2.2 Neuroprotective Properties

Among the available treatments for bipolar disorder, lithium and valproate have the most robust evidence supporting their neuroprotective properties. These effects are reviewed in more detail in Chapter 14 of this text and are summarized here. Both lithium and valproate modulate several neuroprotective pathways, including upregulation of brain-derived neurotrophic factor (BDNF) and B-cell lymphoma 2 (bcl-2) and inhibition of glycogen synthase kinase-3 (GSK-3β) and glutamate. These neuroprotective effects are seen in multiple brain regions implicated in the pathophysiology of bipolar disorder including the anterior cingulate cortex, striatum, and hippocampus.

15.3 Lamotrigine

15.3.1 Clinical Studies

Lamotrigine is unique among mood stabilizers in that its primary use is for the maintenance phase of bipolar disorder. Long-term studies, lasting up to 18 months, have found that lamotrigine delays the time to occurrence of all mood episodes, with more robust protection from depression than mania [10, 11]. Lamotrigine's tolerability makes it a good candidate for long-term use as a maintenance agent.

Compared to its maintenance effects, for which it is FDA-approved, lamotrigine's acute effects in bipolar depression are less clear. A meta-analysis of 5 randomized controlled trials of the acute (7–10 weeks) treatment of bipolar depression with lamotrigine found positive results in only one study, and then only on secondary outcome measures [12]. Results of the other four studies, which were previously unpublished, may have been obscured by a high placebo response rate. One other randomized, placebo-controlled trial, the LamLit study, has come to light

since the publication of this meta-analysis. In this study, lithium was augmented with either lamotrigine or placebo in 124 patients with bipolar depression over an 8 week period. Lamotrigine lead to significant improvements on the primary outcome measures [13].

15.3.2 Neuroprotective Properties

The primary mechanism of lamotrigine's neuroprotective effects appears to derive from blockade of voltage-sensitive sodium channels. Axonal accumulation of sodium ions can reverse the sodium-calcium exchanger, resulting in the toxic influx of calcium ions and build-up of glutamate at central excitatory synapses [14]. The role of this mechanism in lamotrigine's antidepressant effects is supported by an animal model of depression in which the drug veratrine was used to reverse lamotrigine's blockade of sodium channels. This reversal blunted the antidepressant effects of lamotrigine but not that of standard antidepressants [15].

Lamotrigine shares this neuroprotective mechanism with other treatments for mood disorders including lithium [16], valproate [17], riluzole [18] and ketamine [18]. Ketamine and riluzole both have pilot data in treatment-resistant unipolar depression [18], and riluzole has promising open-label data [19, 20] in bipolar depression. Another drug with similar properties to lamotrigine but which lacks clinical studies in bipolar disorder is sipatrigine [21].

Less robust evidence suggests that lamotrigine may share with lithium and valproate an effect on BDNF, bcl-2 and GSK-3β. Chronic treatment with lamotrigine significantly increased levels of BDNF and bcl-2 in the rat frontal cortex; this study found similar effects for the mood-stabilizer carbamazepine [22]. However, no effect on BDNF was found for lamotrigine in a separate study of human neuroblastoma SH-SY5Y [23]. At least one study has found that lamotrigine suppresses GSK-3β-facilitated cellular apoptosis [24].

Lamotrigine may also act as a neuroprotectant through the antioxidant glutathione. Glutathione is the major antioxidant in the brain and plays a key role in the cellular defense against oxidative damage. It is thought responsible for the antidepressant effects of N-acetylcysteine, which is discussed later in this chapter, and may play a role in the mechanisms of lithium, valproate and carbamazepine [25]. Lamotrigine's antioxidant effects were more pronounced than those of escitalopram and aripiprazole in a comparative study [26].

Lamotrigine has been found to protect cells from the effects of ischemia [27–32], axonal injury [33] and the neurotoxins dizocilpine [34], malonate [35], and 3-nitropropionic acid [36].

In an animal model of Parkinson's disease, lamotrigine limited dopaminergic neuronal death in the substantia nigra and promoted striatal dendritic sprouting after administration of the neurotoxin MPTP [37]. Lamotrigine protects against mitochondrial damage in animal models of Parkinson's disease [38, 39]; mitochondrial injury is also hypothesized to play a role in bipolar disorder [40].

15.4 Antipsychotics

15.4.1 Clinical Studies

Among the atypical antipsychotics, only olanzapine and quetiapine have support from randomized, placebo-controlled trials in bipolar depression, and only quetiapine has this support as monotherapy.

Quetiapine's benefits in bipolar depression derive from two large randomized, double blind, placebo-controlled trials of identical design: Bolder I and II. Combined, these trials involved 1,044 patients with bipolar I or II depression studied over an 8-week period [41, 42]. Quetiapine yielded a large effect size (0.8) in depression and was also beneficial for comorbid anxiety and rapid cycling [43].

By itself, olanzapine has marginal benefits in bipolar depression, but when combined with the antidepressant fluoxetine it brought about significant improvement over placebo in an 8 week, double-blind randomized controlled trial of 833 patients with bipolar I depression [44] that lead to the FDA-approval of this combination therapy for bipolar depression.

Other atypical antipsychotics do not have as clear a role in bipolar depression. Aripiprazole, though effective when combined with antidepressants in unipolar depression [45, 46], has shown negative results both for the acute treatment [47] and prevention [48] of bipolar depression in three randomized, placebo controlled trials. These studies did not examine whether aripiprazole, like olanzapine, could treat bipolar depression when combined with an antidepressant. Risperidone has been compared to other active treatments in bipolar depression in two small, randomized controlled trials. In these studies, risperidone was either marginally less effective [49] or showed no difference [50] from the other treatment arms, though the lack of a placebo arm makes it difficult to draw conclusions from this data. The remaining atypical agents, clozapine and ziprasidone, have never to my knowledge been evaluated in a controlled trial of bipolar depression. Likewise, the typical antipsychotics as a group lack controlled data in this condition [1].

15.4.2 Neuroprotective Properties

15.4.2.1 BDNF

Atypical antipsychotics elevate BDNF levels in animal models of depression but not in non-depressed animals. For example, immobilization stress is a model which leads to depression and reduced expression of BDNF. Acute treatment with quetiapine [51] and ziprasidone [52] attenuated the effects of this stress by increasing BDNF mRNA in the rat hippocampus, but did not upregulate BDNF in non-stressed animals under basal conditions [51]. Chronic administration of quetiapine also protected hippocampal neurons from the detrimental effects of this stress [53]. These effects of quetiapine on BDNF were replicated in animals whose BDNF levels had been reduced by exposure to MK-801, a glutamate NMDA receptor antagonist [51].

When animals are studied under basal conditions the results on BDNF are mixed. Among the positive results, olanzapine and clozapine upregulated BDNF mRNA expression in the rat hippocampus [54, 55] and, for clozapine, the rat frontal cortex [56]. Other studies found no change in BDNF after administration of clozapine [57, 58], olanzapine [58, 59] or aripiprazole [58]. The opposite effect, a decrease in BDNF, was found in the rat frontal cortex, occipital cortex, and hippocampus after administration of clozapine [60], olanzapine [61] or risperidone [62].

This last observation that atypical antipsychotics may reduce levels of BDNF is better understood in light of the findings for typical antipsychotics, which consistently downregulate expression of BDNF [52, 54, 60–63] in various brain regions including the frontal cortex and hippocampus. Dose effects may explain this similarity: in higher doses, the receptor profiles of atypical antipsychotics resemble those of typical antipsychotics. At lower doses, atypical antipsychotics may upregulate BDNF through 5-HT_2 receptor blockade; higher doses may downregulate BDNF through blockade of D_2 receptors. This hypothesis derives in part from the observation that BDNF is upregulated after 5-HT_2 blockade with ritanserin [64].

When we move to human studies, the results are still conflicting, though the limitations are greater. These studies relied on serum measures of BDNF, which crosses the blood-brain barrier [65] and correlates well with brain levels [66], but may not account for regional brain differences in BDNF after antipsychotic treatment. Regional differences are particularly relevant in schizophrenia, the population examined in most of these studies. In post-mortem studies of schizophrenia, BDNF is elevated in the anterior cingulate cortex and hippocampus [67, 68] and reduced in the prefrontal cortex [69].

A second limitation is the difficulty of separating the effects of treatment from those inherent to the illness. One study investigated this question by comparing serum BDNF levels of drug-naïve patients with schizophrenia to those on long-term antipsychotics. The results suggested that the low levels of serum BDNF which prior research had associated [70–72] with schizophrenia may instead be associated with antipsychotic treatment [73].

A third limitation of human research is that many of these studies included patients on both typical and atypical antipsychotics [74, 75], which would be expected to confound the results. Most studies found that antipsychotic treatment had no effect on serum BDNF levels [74–76] in schizophrenia, except one which found a positive association between BDNF and risperidone treatment only in the subset of patients who had responded to the drug [77].

The unique alterations of BDNF in schizophrenia make the translation of this research to bipolar disorders difficult. At least one human study did examine BDNF levels in bipolar disorder after treatment with an atypical antipsychotic. Though it found no change in serum BDNF levels, it included patients in the depressed and manic phase of the illness, and evaluated risperidone, which is not clearly known to treat bipolar depression [78].

15.4.2.2 N-acetylaspartate

N-acetylaspartate (NAA) is a marker of neuronal viability which is increased by lithium and depakote. A proton magnetic resonance spectroscopy study of NAA in bipolar subjects evaluated the combination of quetiapine and valproate for potentially synergistic effects on this marker. This study found that addition of quetiapine lead to increases in NAA beyond those seen with valproate alone, but only in post-hoc analysis [79]. Another study of bipolar adolescents in the manic phase found that successful treatment with olanzapine was associated with increases of NAA in the medial ventral prefrontal regions; this effect was not seen in olanzapine non-responders [80].

Chronic schizophrenia is known to result in reduced levels of NAA, a finding that appears to be inherent to the illness rather than the effects of treatment [81–83]. In human subjects with schizophrenia, short term treatment with typical and atypical antipsychotics increased measures of NAA in the dorsolateral prefrontal cortex but not in other brain regions [84].

15.4.2.3 GSK-3β, B-Catenin and Bcl-2

Antipsychotics may also share with lithium a neuroprotective effect through GSK-3β and β-catenin. These proteins play a critical role in brain development and have been implicated in the pathophysiology of bipolar disorder as well as Alzheimer's disease and schizophrenia [85, 86]. Several animal studies have found that both typical and atypical antipsychotics (haloperidol, clozapine and risperidone) modulate these proteins throughout the brain including the hippocampus, cortex, striatum, cerebellum, ventral midbrain, and striatum [85–87]. This modulation of GSK-3β was augmented when antidepressants (imipramine or fluoxetine) were added to an antipsychotic (risperidone), which is interesting in light of the synergistic clinical effects such combinations have in studies of depression [87].

The antiapoptotic protein bcl-2 is associated with the neuroprotective effects of lithium, and limited data suggest that atypical antipsychotics act through this mechanism as well. In an animal model, administration of olanzapine or clozapine upregulated bcl-2 in the frontal cortex and hippocampus [55, 88]. In human neuroblastoma SH-SY5Y cells, olanzapine but not haloperidol prevented the decrease in bcl-2 normally seen under conditions of serum-withdrawal [89].

15.4.2.4 Glutamate

Among the atypical agents, aripiprazole has been found to inhibit glutamate, an effect discussed in more detail in the section on lamotrigine. This effect may result from aripiprazole's partial agonism of 5-HT_{1A} and D_2 receptors (blockade of either of these receptors reversed its inhibitory effect on glutamate) [90].

15.4.2.5 Protection Against Neurotoxins and Ischemia

Atypical, but not typical, antipsychotics protect brain cells from cytotoxic and ischemic injury [91]. All of the atypical agents have at least some positive data in these studies, while investigations of haloperidol consistently showed no protective effects. Examples include animal models of ischemia [92, 93] and various cytotoxins including β-amyloid peptide [94], 1-methyl-4-phenylpyridinium [91], okadaic acid [95], phencyclidine [96, 97], methamphetamine [98], kainic acid [99], serum withdrawal [100] and oxidative stress from hydrogen peroxide [101].

In contrast to these antiapoptotic effects of atypical antipsychotics, long-term treatment with haloperidol is associated with an increase of neurotoxicity markers and pro-apoptotic proteins in animal models [102, 63, 103, 104].

15.5 Pramipexole

15.5.1 Clinical Studies

Pramipexole is a presynaptic dopamine agonist which is FDA-approved for the treatment of Parkinson's disease and restless leg syndrome. It is highly selective for the D_3 receptor and has less affinity for D_1, D_2 and D_4 [105]. The D_3 receptor is densely distributed in the mesolimbic system and thought to be involved in the motoric and anhedonic symptoms of depression [106].

Pramipexole's antidepressant effects were first seen in animal models [107–109] and naturalistic clinical trials where it was used as monotherapy [110] or augmentation in unipolar and bipolar patients [111–115]. The first randomized controlled trial to confirm these observations was undertaken in unipolar depression and compared pramipexole, fluoxetine and placebo in 174 subjects over an 8 week period. Pramipexole performed comparably to fluoxetine and significantly better than placebo, with an effect size of 0.6 [116].

In bipolar depression, pramipexole has two randomized controlled trials supporting its efficacy. The first involved 21 patients with treatment-resistant bipolar II depression who received pramipexole as an adjunct to valproate or lithium after failing or respond to the mood stabilizer alone. Pramipexole began to separate from placebo at three weeks, and brought about a significant reduction in depression by week six with a large effect size of 1.2–1.45 [106]. The second trial compared flexible dosing of pramipexole (average daily dose 1.7 mg) to placebo as an augmentation strategy for 22 outpatients with depression (68% bipolar I, 32% bipolar II) which was unresponsive to at least two antidepressant trials. All were on mood stabilizers and none on antipsychotics. Pramipexole brought about significantly greater improvements in depression compared to placebo on all measures except the primary outcome measure, with an effect size of 0.77 [117].

15.5.2 Neuroprotective Properties

Pramipexole shares with lithium and valproate a neuroprotective effect through the anti-apoptotic protein bcl-2. Preliminary data in bipolar subjects suggests that combining pramipexole with lithium or valproate has a synergistic effect on bcl-2 [106].

An in vitro study of mesencephalic cultures suggests that pramipexole also exerts neuroprotective effects through brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). This effect was shared by the related drug ropinirole, which is a less-selective D_3 agonist [118]. Ropinirole lacks controlled studies in depression, but showed promise in two naturalistic studies involving 18 patients with treatment-resistant bipolar and unipolar depression [119, 120].

Both ropinirole and pramipexole increase levels of the antioxidant glutathione in the brain, a mechanism shared with other treatments for bipolar depression [121, 122]. These medications have protective effects on dopaminergic neurons in cell cultures and in animal models of Parkinson's disease [123–126]. Pramipexole has also protected neurons from the cytotoxins tunicamycin [127] and 3-acetylpyridine [128].

15.6 Modafinil

15.6.1 Clinical Studies

Modafinil is a novel wakefulness-promoting agent which is primarily used for disorders of fatigue such as narcolepsy, obstructive sleep apnea and shift-work sleep disorder. Evidence for its benefits in mood disorders came first from studies of unipolar depression. Four randomized controlled trials, involving a total of 765 patients, have compared augmentation with modafinil to placebo in patients who were partial responders or had residual fatigue on antidepressant therapy. Modafinil augmentation improved mood and fatigue in two [129, 130] of these trials, and improved fatigue without significantly elevating mood in the other two trials [131, 132]. A separate trial examined monotherapy with modafinil in 66 patients with unipolar depression and atypical features (among which fatigue is a prominent symptom). This study showed positive effects in the open-label, acute treatment phase, but failed to separate from placebo during a 12 week continuation phase in which patients were randomly assigned to modafinil or placebo [133].

Support for the use of modafinil in bipolar depression comes from a single controlled trial which randomized 85 patients (44% bipolar I, 56% bipolar II) to modafinil or placebo for 6 weeks. Unlike the trials in unipolar subjects, this study found that modafinil improved depression without significantly impacting fatigue [134].

There remains, however, the possibility that modafinil can worsen mental symptoms in some patients. There are 3 case-reports of treatment-emergent mania with modafinil [135–137], although the rates of switching were equivalent between modafinil and placebo in the controlled trial of bipolar patients [134]. Finally, a study involving unipolar depression [138] was prematurely discontinued due to the emergence of suicidality in the modafinil group, although the rate of this event ($n = 2$) was not statistically significant.

15.6.2 Neuroprotective Properties

Research on the neuroprotective effects of modafinil is preliminary. Like other therapies for bipolar depression, it reduces glutamanergic toxicity [139, 140]. Modafinil was neuroprotective against ischemic damage and against the neurotoxic nerve gas soma [141]. Modafinil protected the nigrostriatal dopaminergic pathway [142–144] as well as striatal noradrenergic and serotonergic pathways [142] in animal models of Parkinson's disease.

15.7 Antidepressants

15.7.1 Clinical Studies

The role of antidepressants in the treatment of bipolar depression is an area of active controversy. This debate centers on two separate issues: their efficacy in depressed episodes and their potential to cause mania, mixed states and rapid cycling. This controversy has not diminished their popularity, for antidepressants continue to appear at or near the top of the most commonly prescribed medications for bipolar disorder, even as monotherapy, which is relatively contraindicated in bipolar [145, 146].

The strongest evidence for their role in bipolar depression only supports their use in combination with an atypical antipsychotic. This study, which was discussed previously in the section on antipsychotics, found that the combination of fluoxetine and olanzapine was effective in the acute phase of bipolar depression [47]. This kind of combination is a promising avenue for future research in bipolar depression. Although there is overlap in their neuroprotective mechanisms, limited data suggest that these mechanisms increase synergistically when antidepressants and atypical antipsychotics are combined. The clinical use of this combination is more robustly supported for unipolar depression, where they have been studied in at least 14 randomized controlled trials [147–151].

When antidepressants are used in bipolar depression with traditional mood stabilizers instead of atypical antipsychotics, clinical synergy is not observed. Two large randomized, placebo controlled trials did not support a role for antidepressant augmentation of traditional mood stabilizers in the acute treatment of bipolar

depression. Sachs et al. [152] found that augmentation with buproprion or paroxetine did not yield significant risks or benefits over placebo. Nemeroff et al. [153] found that that augmentation of lithium with imipramine or paroxetine was more effective than placebo only in patients who had low lithium levels.

It is possible that antidepressants benefit a subset of patients with bipolar disorder, but that these effects are lost in large randomized controlled trials due to biased or heterogeneous samples. Two studies, though limited in methodology, suggest that around 1 in 10 patients with bipolar disorder will benefit from an antidepressant. The first is a naturalistic study which found that 12% of 352 patients with bipolar depression responded acutely to antidepressant treatment, and tended to relapse if the antidepressant was discontinued within 12 months [154]. The second is a metaanalysis of seven randomized, controlled trials of long-term (>6 months) antidepressant therapy in bipolar, involving a total of 350 patients. The authors concluded that 1 in 11 patients with bipolar disorder would benefit from an antidepressant over placebo, but recommended against their use because the risk of causing harm (manic induction) was 1 in 7 [155].

Other studies have identified risk factors for cycle induction (including hypomania, mania, mixed states and rapid cycling) with antidepressant use. Though these studies are limited in methodology, they suggest an increased risk of harm with the following moderators: bipolar I subtype, presence of subsyndromal manic symptoms, recent mania or rapid cycling, short episode duration, past history of antidepressant-induced mania, multiple antidepressant trials, past history of substance abuse, family history of bipolarity, female gender, hyperthymic temperament and the short-allele of the serotonin transporter gene [156–160].

Overall, the available data suggest that a small subset of patients with bipolar disorder respond to antidepressant therapy, while another group is prone to mood destabilization on these drugs. For a third group, perhaps the majority of patients with bipolar disorder, antidepressants are neither effective nor harmful.

15.7.2 Neuroprotective Properties

The neuroprotective effects of antidepressants are reviewed in a previous chapter and include upregulation of BDNF and bcl-2 and inhibition of GSK-3β. Among these mechanisms, the role of BDNF in depression is supported by the most comprehensive model. Chronic stress leads to depression and down-regulation of hippocampal BDNF in several animal models. Antidepressants reverse these changes in BDNF along a time-course that coincides with the onset of their behavioral effects. Direct infusion of BDNF also has antidepressant effects.

Up-regulation of BDNF also occurs with treatments for bipolar depression, including lithium, lamotrigine, quetiapine and omega-3 fatty acids, as described elsewhere in this chapter. Bipolar depression [161, 162] is associated with more profound reductions in serum BDNF than those seen in unipolar depression [163]. On a genetic level, a polymorphism of the BDNF allele has been associated with

bipolar disorder, a finding confirmed by a metaanalysis of 14 studies [164] and further replicated by four studies published after this metaanalysis [165–168]. This gene has also been associated with early onset of the illness and rapid cycling [169]; other variants of the BDNF gene have been associated with lithium response [170].

It is unlikely that these neuroprotective mechanisms contribute to the manic-induction seen with antidepressants, as antimanic agents exert similar effects on BDNF, bcl-2 and GSK-3β. Bipolar mania, like bipolar depression, is associated with low levels of BDNF. These reductions correlate with the severity of mania [161, 171] and are normalized after successful treatment [172].

It remains an intriguing and incompletely explained finding that antidepressants and mood stabilizers share so many neuroprotective properties but have such different clinical profiles. One significant difference between the two classes of drugs is that the neuroprotective effects of antidepressants tend to be localized to the hippocampus, while those of mood stabilizers are found in broader brain regions including the anterior cingulate cortex, striatum, and hippocampus. It is possible that differences in their clinical profiles are better explained by factors other than neuroprotection.

15.8 N-acetylcysteine

15.8.1 Clinical Studies

N-acetylcysteine is a precursor of glutathione, the main antioxidant substrate in the body. N-acetylcysteine is deacetylated in the liver to form cysteine, the rate-limiting precursor of glutathione. Glutathione itself is not used as a therapeutic agent in psychiatry because, unlike its precursors, it does not cross the blood-brain barrier.

N-acetylcysteine was first tested in schizophrenia, where a placebo-controlled, randomized, double-blind trial found the treatment improved negative and depressive symptoms but not the positive, psychotic symptoms of the illness [17]. After these encouraging results, the same group conducted a trial in bipolar depression [17].

The study involved 75 subjects with bipolar disorder (82% bipolar I, 18% bipolar II) in the maintenance phase of the illness, most of whom had subsyndromal depression. N-acetylcysteine (1 gm twice daily) was studied over 24 weeks as an adjunct to usual medications. The treatment separated from placebo on ratings of depression, with a large effect size of 0.6–1.1, but did not change time to relapse into a mood episode. The study was followed by a 4 week wash-out phase, which revealed that the benefits of N-acetylcysteine were lost after its discontinuation.

15.8.2 Neuroprotective Properties

Oxidative stress has been theorized to play a role in the pathophysiology of bipolar disorder, depression and schizophrenia [43]. Most, but not all, studies of bipolar

patients have found increased markers of oxidative stress. Bipolar disorder is associated with reduced levels of antioxidant enzymes, including glutathione peroxidase, superoxide dismutase, and catalase; and genes encoding antioxidant enzymes are downregulated in the hippocampus of bipolar patients. Other markers of oxidation found in bipolar disorder include elevations of the free radical nitric oxide and markers of lipid peroxidation, including depletion of essential polyunsaturated fatty acids and elevations of thiobarbituric acid reactive substances [43].

Direct evidence of a link between glutathione and depression comes from an animal model of depression (inescapable shock). In this model, the behavioral depression was associated with depletion of glutathione, and replenishment of this antioxidant was seen following administration of various antidepressants (imipramine, maprotiline, fluvoxamine, trazodone) [175]. As discussed previously in this chapter, lamotrigine may elevate glutathione to a greater degree than antidepressants, and preliminary evidence suggests that the traditional mood stabilizers lithium, valproate and carbamazepine also raise this antioxidant.

Deficits of glutathione have been implicated in a number of neurodegenerative disorders including Parkinson's disease and schizophrenia [176]. The neuroprotective effects of this antioxidant have been demonstrated in a variety of animal models including vitamin deficiency [177], iron overload [177], axonal transaction [178–180], ischemia [181] and cytotoxic injury from cadmium [182] and 6-hydroxydopamine [183]. N-acetylcysteine protected dopaminergic pathways in animal models of Parkinson's disease [184–186]. It also protected against the degenerative process that lead to cerebral palsy, including lipopolysaccharide-induced white matter injury and hypomyelination, in an animal model of this disease [187].

15.9 Omega-3 Fatty Acids

15.9.1 Clinical Studies

Omega-3 fatty acids, or fish oil, are an important structural component of neural cell membranes and include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Low rates of fish consumption have been correlated with depression in multiple cross-national epidemiologic studies. Omega-3's appeared to share a common effect with lithium and valproate on signal transduction pathways, including protein kinase C [188].

These observations lead to the first clinical study of omega-3 fatty acids in bipolar disorder, which was a randomized controlled trial of 44 subjects who added omega-3 fatty acids (6.2 g/day of EPA and 3.4 g/day of DHA) or placebo to their standard therapy for 4 months. Omega-3 supplementation significantly delayed the time to a major episode, which was the primary outcome measure [189].

Further controlled trials of omega-3's in bipolar disorder used EPA without DHA, and yielded conflicting results. Supplementation with EPA was found to

improve bipolar depression in a randomized, double-blind, placebo controlled study involving 75 subjects treated over 12 weeks [188]. Two negative studies evaluated 6 g/day of EPA over a four month period in patients with bipolar depression ($n = 62$) or rapid cycling bipolar disorder ($n = 59$). Both of these studies used a randomized, double-blind, placebo controlled design [191].

Omega-3 fatty acids are also effective in controlled trials of unipolar depression. A recent metaanalysis of all randomized, placebo-controlled, double blind studies of omega-3 fatty acids in mood disorders identified 10 studies involving 329 patients. The pooled effect size of omega-3 fatty acids in unipolar and bipolar depression was moderate, 0.6 [191].

Although omega-3 fatty acids can be taken as a supplement, they can also be obtained from dietary sources such as oily fish (primarily salmon and sardines), walnuts and green leafy vegetables (e.g. spinach, kale). It is estimated that 10–20 ounces of salmon per week would supply an amount of omega-3 fatty acids comparable to that used to treat mood disorders (e.g. 1,000–2,000 mg/day of combined DHA+EPA. These figures are for farm-raised salmon; twice as much would need to be consumed for wild-salmon) [192].

15.9.2 Neuroprotective Properties

Research into the neuroprotective effects of omega-3 fatty acids has focused on dementia, Parkinson's disease and cerebral ischemia rather than mood disorders, but some of the mechanisms elucidated may have relevance to their effects on mood. Most of the studies involved DHA alone or combinations of DHA and EPA; studies of EPA alone have not found neuroprotective effects.

DHA is the precursor of Neuroprotectin-D_1, which promotes cell survival by downregulating apoptotic and inflammatory pathways, including GSK-3β, ERK1/2 and COX-2 [193, 194]. DHA, but not EPA, has also been found to modulate AMPA-mediated toxicity and reduce glutamate toxicity in hippocampal neurons [195, 196]. In rats, DHA augmented the upregulation of BDNF after exercise, as well as the cognitive effects of exercise, in a synergestic manner [197]. Supplementation with omega-3 fatty acids has also prevented the reduction of BDNF by traumatic brain injury [198].

Two small human studies have evaluated the neuroprotective effects of omega-3 fatty acids in bipolar disorder. Treatment with EPA was associated with elevation of NAA, a marker of neuronal integrity, in a 12-week trial of bipolar depression which employed a randomized, placebo-controlled design [199]. The combination of DHA and EPA brought about greater membrane fluidity, as detected by reductions in T(2) values, compared to controls in a 4-week study of women with bipolar disorder [200].

Other potential mechanisms of neuroprotection with DHA include antioxidant properties [198, 201] and modulation of glutamate receptors [202]. Omega-3 fatty acids have demonstrated neuroprotective properties in animal models of Parkinson's

disease [203], Alzheimer's disease [204, 205], cerebral ischemia [194, 206] and spinal cord injury [207, 208].

15.10 Diet

15.10.1 Clinical Studies

The clinical effects of diet have not been directly studied in bipolar disorder. Indirect evidence comes from naturalistic epidemiological studies of schizophrenia, which have found that a diet low in saturated fats and refined sugars is associated with improved clinical outcomes [209]. This diet was also associated with improvements in depression, self esteem and hostility independent of its effect on weight in two small, controlled trials of human subjects without mental illness [210, 211]. In a study of elderly humans, three months of caloric restriction by 30% was found to improve memory but not mood [212].

15.10.2 Neuroprotective Properties

A growing body of research suggests that dietary changes may be associated with neuroprotective effects similar to those seen with antidepressants. For example, low caloric intake has been found to increase levels of BDNF in the brain in mice [213]; a finding confirmed in a human study of obese subjects which measured serum BDNF [214]. A diet high in refined sugars and saturated fats has consistently been found to reduce hippocampal expression of BDNF in animals [215–217].

Foods rich in flavanoids hold potential for mood disorders although they have not been studied in this population. Multiple flavanoid-rich foods have been found to improve various measures of cognition in animals and humans, including Camellia sinensis (tea), Ginkgo biloba, cacao and blueberries [218]. Recently, blueberry consumption was found to also increase hippocampal BDNF levels in a 12-week study of rats who received 2% of their diet as blueberry supplementation [219].

15.11 Exercise

15.11.1 Clinical Studies

Physical exercise is well-supported as a treatment for unipolar depression, though little is known about its efficacy in bipolar depression. A recent metaanalysis from the Cochrane group identified 28 randomized controlled trials which compared exercise to standard treatment, no treatment or a placebo in adults. Pooling all the studies yielded a large effect size for exercise, but this effect fell to a moderate

and not clinically significant level when only the three methodologically robust trials were included. Positive results were found for exercise both as monotherapy and as augmentation of antidepressants [220].

Only one study has examined the effects of exercise in bipolar disorder, and it is limited by lack of randomization and retrospective design. This study included both manic and depressed inpatients and compared those who voluntarily participated in a walking program with those who did not. Although global measures of improvement were not significantly different between the two groups, specific measures of depression, anxiety, and stress had improved significantly in the exercise group [221].

Notably, only 25% of the 98 patients offered this walking program participated, which speaks to the difficulty of engaging patients with bipolar disorder in exercise. Though patients with bipolar disorder have poorer habits of diet and exercise than the general population, primary care physicians are less likely to discuss lifestyle changes with them [222]. Exercise tolerance is also lower in bipolar patients, even during euthymia, which may be because of the higher rates of smoking and obesity in bipolar disorder or to other, unknown factors [223].

Outside of its effects on mood, exercise has consistently produced positive effects on cognition which may be relevant to bipolar disorder. A metaanalysis of 18 fitness training studies in humans found consistent improvements in cognitive measures regardless of the patient population and type of exercise, although short duration exercise of less than 30 minutes did not produce significant benefits. Effect sizes were highest for executive functioning (0.7), followed by controlled tasks (0.5), spatial tasks (0.4) and speed tasks (0.3) [224]. A later metaanalysis of 37 studies confirmed these results, finding an overall effect size of 0.3 for exercise on cognition and positive results in 35 out of 37 studies [225]. Although studies of exercise in depressed subjects confirm these benefits on cognition [226, 227], it remains to be seen whether these findings will translate to patients with bipolar disorder.

15.11.2 Neuroprotective Properties

Exercise has consistently been found to increase levels of BDNF in animal studies [228–230]. This effect is similar to that observed with antidepressants, and administration of antidepressants to animals potentiates and accelerates the rise in BDNF after exercise [231–233]. Estrogen deficiency, on the other hand, has been found to blunt the benefits of exercise on BDNF [234].

These gains in BDNF are maintained equally well by exercise every other day or a daily routine [230]. Although BDNF levels eventually return to baseline after cessation of an exercise routine, there appears to be a priming mechanism whereby the levels rise more rapidly than expected upon resumption of the routine [230].

Other neuroprotective effects of exercise in animal studies include proliferation of hippocampal cells [235, 236], increases in cerebral capillary density [237],

prevention of ischemic damage [238], modulation of glutamate signaling [239], reduction of oxidative stress [240, 235], protection of Purkinje cells [241] and protection of striatal dopamine [242].

15.12 Psychotherapy

15.12.1 Clinical Studies

In the past decade, psychotherapy has emerged as an effective adjunct to the pharmacologic treatment of bipolar disorder. Two recent metaanalyses [243, 244] evaluated this body of research, which comprised 21 randomized controlled trials of which approximately half met the criteria for metaanalysis. Both papers concluded that psychosocial interventions are not clearly effective in the acute treatment of episodes but do have a role in the maintenance phase where they reduce relapse rates by approximately 40% [243]. No specific psychotherapy emerged as uniquely effective among those studied, which included cognitive behavioral therapy, family therapy, group psychoeducation and interpersonal social rhythm therapy.

15.12.2 Neuroprotective Properties

It is well established that chronic (but not acute) stress lowers hippocampal BDNF [169] and increases rates of relapse into both unipolar and bipolar depression [245–247]. For example, social isolation down-regulates BDNF, while enriched social environments have the opposite effect in animal studies [248–252]. It would therefore seem plausible that psychotherapies which enhance social adaptation and management of stress would also result in elevations of BDNF.

This hypothesis was not upheld in a study of unipolar depression, although that study did find an association between response to interpersonal psychotherapy and elevations of cAMP-response element binding protein (CREB), which is involved in neuroprotective signaling [253]. A separate study of panic disorder did find an association between response to cognitive behavioral therapy and increases in BDNF [254].

While these preliminary findings are intriguing, the neuroprotective properties of psychotherapy remain an area for future investigation. It is worth noting that these two studies examined the effects of *acute* psychotherapy on neuroprotective mechanisms, and these results may not translate to bipolar disorder where psychotherapy has a preventative rather than an acute effect. Maintenance psychotherapies are designed to foster behaviors that prevent episodes, such as regulation of sleep and social rhythms, reduction of substance abuse, improvement in insight and acceptance of the illness, and prevention and management of interpersonal and environmental stress.

It will be interesting to see if these psychotherapeutic strategies are associated with neuroprotective changes in bipolar disorder. For example, social rhythm therapy might modulate GSK-3β, a neuroprotective protein which appears to mediate the effects of lithium on circadian rhythm regulation [255–259].

15.13 Neuroplasticity and Psychoeducation

This final section discusses how the neuroplasticity model can engage patients in their recovery and is based on the author's experience. By illuminating common mechanisms between medications, diet and exercise, this model allows patients to view medication as part of an integrated treatment plan in which they play an active role. Photographic images of neuroprotection can enhance this message and counter the fears of toxicity which popular books attribute to psychiatric medications [260] (see www.moodtreatmentcenter.com/mindbrain for examples).

In contrast, the monoamine hypothesis has been understood by the lay public to mean that mental illness is a "chemical imbalance" which can only be cured by altering brain chemistry [261]. This often leads to the further misunderstanding that psychiatric medications will cause "chemical dependency" or are "chemical and unnatural". Another misconception derived from this model is that patients are passive participants in an illness which can only be treated through chemistry. Instead, patients can be educated that medications enhance the brain's natural healing mechanisms and that active lifestyle changes can further their effects.

In the words of one patient, who had learned about neuroprotection from a previous physician, "I used to stop my medications a lot because I didn't want to become dependent on them. Then a doctor told me they helped brain cells grow and since then I've never stopped taking them." Future studies might evaluate whether this mode of education leads to measurable changes in medication adherence.

15.14 Conclusions and Future Directions

Research over the past decade has widened the therapeutic armamentarium for bipolar depression beyond lithium. These developments occupy a middle ground in a spectrum between antimanic and antidepressant agents, as shown in Table 15.1.

As one moves down the medications in the table, the antimanic properties decrease and the antidepressant properties increase, with medications in the middle preferentially effective for bipolar depression and those at the bottom more selective for unipolar depression.

An exception to this pattern is the atypical antipsychotics, which possess both strong antimanic and strong antidepressant properties, and treat both unipolar

Table 15.1 Treatments for mood disorders categorized by clinical profile

Clinical profile	Examples
Antimanic agents with no effect in bipolar depression	Carbamazepine, typical antipsychotics, phenytoin
Antimanic agents with varying degrees of efficacy in bipolar depression	Lithium, valproate, atypical antipsychotics
Agents with efficacy in bipolar depression and mild antimanic effects	Lamotrigine, omega-3 fatty acids
Agents with efficacy in bipolar depression and possible promanic effects	Pramipexole, modafinil
Agents with mild or unclear effects in bipolar depression and promanic effects	Antidepressants

and bipolar depression, although the extent of their clinical effects varies widely within the class and often depends on a synergestic interaction with an antidepressant.

More questions than answers emerge from the neuroprotective properties of the agents in Table 15.1. As one moves down the spectrum from antimanic to antidepressant agents, the regional impact of the neuroprotective effects becomes increasingly focal and localized to the hippocampus. Agents with both antimanic and antidepressant effects, such as lithium, valproate and the atypical antipsychotics, impact a wider array of neuroprotective mechanisms throughout broader regions of the brain.

A question for future research is why the agents at the top of the spectrum, which treat mania but not depression, lack significant neuroprotective effects and may be neurotoxic. This chapter presented evidence of neurotoxicity with haloperidol, and there is research supporting both neuroprotective [22, 262, 263] and neurotoxic effects [264] with carbamazepine.

It is likely that bipolar depression is a heterogeneous condition and this may explain the variety of mechanisms in Table 15.1. In one model, bipolar depression is seen as the end result of chronic stress and depletion of BDNF, which is identical to the model of unipolar depression [169]. A minority of patients with bipolar depression may fit this model and benefit from upregulation of BDNF with antidepressants without a risk of cycle induction. In a different view, the primary defect in bipolar disorder is a cycling mechanism in which mania is the driving force and depression is a secondary consequence of this manic cycling [265]. Conceivably, patients whose depression fits this model may recover from antimanic agents alone. These models are not mutually exclusive, and both may operate within the same patient. Patients whose depression is driven by both models may benefit from treatments in the middle of Table 15.1, such as lithium, lamotrigine, quetiapine, and omega-3 fatty acids, which reduce mood cycling and address the neuroplastic changes associated with depression and chronic stress.

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Chapter 16
New Antiepileptic Drugs in Neuropsychiatric Disorders – Basic Mechanisms Related to Clinical Efficacy

Cecilie Johannessen Landmark and Svein I. Johannessen

Abstract This chapter will cover basic mechanisms of action and correlates to clinical efficacy of recent advances of new antiepileptic drugs (AEDs) in neuropsychiatric disorders as anxiety, schizophrenia, bipolar disorder and mania. New AEDs, including lamotrigine, levetiracetam, oxcarbazepine, pregabalin, tiagabine, topiramate, vigabatrin and zonisamide are investigated in preclinical models to reveal relevant mechanisms of action. Each AED may affect several targets and neuronal excitability by modulating ion channels, receptors, and intracellular signalling pathways. Pathophysiological pathways and possible targets for new AEDs and their potential neuroprotective role will be discussed. Furthermore, all these new AEDs are undergoing clinical trials in one or more of the neuropsychiatric disorders to prove their efficacy, and clinical findings will be correlated to basic findings. In addition, a number of new AEDs are in late-stage development, and several of these are investigated in psychiatric disorders as well, as valproate analogues and retigabine. In the clinical setting, therapeutic drug monitoring (TDM) of AEDs has made it possible to study the individual variations in drug utilization and to optimalize efficacy and tolerability. The experience with TDM of new AEDs in epilepsy is valuable for the extended use of these drugs in neuropsychiatric disorders. It is of major importance to improve our understanding of the relationship between mechanisms of action, pathological conditions and clinical efficacy for optimal pharmacological treatment.

Abbreviations

AED	Antiepileptic drug
CNS	Central nervous system
GABA	Gamma-amino butyric acid
GAD	Glutamic acid decarboxylase
NMDA	N-methyl-D-aspartate
TDM	Therapeutic drug monitoring

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_16,

16.1 Pharmacological and Neurochemical Background

Antiepileptic drugs (AEDs) are widely used in neuropsychiatric disorders, and new AEDs are also increasingly investigated in other disorders [1–3]. AEDs may be useful for treating, anxiety, schizophrenia, social phobia, myotonia, post-traumatic stress syndrome, alcohol and drug abuse and withdrawal, and even more conditions are being explored [1]. These disorders seem related to disturbed excitability in the CNS. Most of the new AEDs have several proposed mechanisms of action, in epilepsy as well as in other disorders. Current knowledge indicates that most AEDs have more than one mechanism of action, each of which may contribute to the therapeutic efficacy to a variable extent depending on the condition [4]. The main pharmacodynamic mechanisms responsible for the clinical efficacy of AEDs in these diseases include disturbance in GABAergic or glutamatergic neurotransmission or alteration of voltage-gated ion channels or intracellular signalling pathways [1]. The action at the molecular level is not completely understood, but some of the mechanisms of action of AEDs seem to be similar in epilepsy and in various neuropsychiatric disorders. Many of the AEDs have primarily been investigated in animal models for epilepsy, and only subsequent studies demonstrated their effects in other pathophysiological processes [5]. Results from these basic investigations are important to be implemented into new approaches for clinical studies to improve our understanding of rational pharmacological treatment of various neuropsychiatric disorders. In this chapter, new AEDs are defined as AEDs marketed after 1990 and include felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, rufinamide, stiripentol, tiagabine, topiramate, vigabatrin, and zonisamide.

The rationale for this chapter is to relate the various mechanisms of action of newer AEDs to pathophysiological mechanisms and clinical efficacy in neuropsychiatric disorders. Relevant new AEDs, relevant mechanisms of action, clinical efficacy and possible future drugs of importance are highlighted. Preclinical and clinical evidence will be discussed in relation to the various possible indications and recent findings regarding clinical efficacy.

16.2 Literature and Selection Criteria

This review is based on recent publications and Pub Med, Google Scholar searches and references from relevant articles on the activity of gabapentin, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, tiagabine, topiramate, vigabatrin and zonisamide in neuropsychiatric disorders. No relevant articles regarding felbamate, lacosamide, rufinamide, stiripentol were found, but they will be mentioned where they theoretically could be of interest. In addition, the following new AEDs, which are second generation to existing AEDs will be mentioned: eslicarbazepine, fluorofelbamate, the levetiracetam analogue brivaracetam and the valproic acid amide analogues valnoctamide, valrocemide and their analogues. Neuropsychiatric

disorders included are: anxiety, schizophrenia, bipolar disorder, in addition to indications for tardive dyskinesia, alcohol, drug dependence and abuse, cigarette dependence, and eating disorders.

Relevant published articles in peer reviewed recognized international journals in English, randomized clinical trials, open-label trials and case reports were included. Abstracts were included where the whole article was not assessable to obtain. Unpublished or non-English material and articles of limited values or out-of date results or use of methods were excluded. The searches were conducted in published articles from 1983 and up to date (July 2009). Articles published in recent years were preferred, and a large majority of the included articles were published from 2000 and onwards. Primary sources were preferred, but relevant review articles were also included.

16.3 Pathophysiological Processes and Possible Targets for AEDs

16.3.1 Rationale for Efficacy of AEDs in Neuropsychiatric Disorders

There are several possible common pathophysiological processes in the different neuropsychiatric disorders compared to epilepsy. The comorbidity of epilepsy and other episodic neurological and psychiatric disorders suggests that these conditions share one or more common etiologies [1]. Epidemiological evidence indicates that for instance epilepsy and depression are comorbid conditions, defined as the existence of one of these conditions increases the risk of the others [6].

The incidence of psychiatric diseases in patients with epilepsy is significantly higher than in the general population, depressive and anxiety disorders being the most frequent diagnosis [7–9]. Mood disorders in patients with epilepsy are underdiagnosed, but an average incidence of 30–40% is assumed [6, 9]. In a recent screening study with 85.000 patients included, bipolar symptoms occurred in 12% of community-based epilepsy patients, whereas 26% of the epilepsy patients carried a diagnosis of unipolar depression [10]. Affective disorders and epilepsy appear to partially share similar pathogenic mechanisms, and antidepressants and AEDs may share mechanisms of action [11, 12]. There seems to be a bidirectional relationship between depression and epilepsy, and that they share a common underlying mechanism [13]. In a recent study, lamotrigine reduced seizure frequency and improved depressive symptoms in patients suffering from both disorders [7]. Evidence suggests that these disorders may arise from a multiplicity of neurobiological abnormalities, but one component may arise from noradrenergic and serotonergic deficits [13]. A disorder in one individual may arise via different mechanisms than a phenomenologically similar disorder in another individual [13]. Both seizures and severe affective episodes may correlate with neuronal impairment due to excess of glutamate, excess of calcium or nitric oxide, and result in neurotoxic effects. It is of interest to know whether AEDs can exert

neuroprotective effects. More preclinical studies in other models evaluating the effects of AEDs on monoamine neurotransmitters are needed for a better insight into the pathophysiological processes.

16.3.2 Excitability in CNS and Targets for AEDs

The pathophysiological processes related to the synapse and regulating neuronal excitability may be divided into four main categories. AEDs may affect these processes by [1, 3]:

- Increasing GABAergic inhibitory neurotransmission
- Decreasing glutamatergic excitatory neurotransmission
- Blocking voltage-dependent sodium or calcium channels
- Affecting intracellular signalling pathways.

Pathophysiological processes and targets for the action of AEDs in the GABAergic and glutamatergic synapse are summarized in Fig. 16.1. The possible

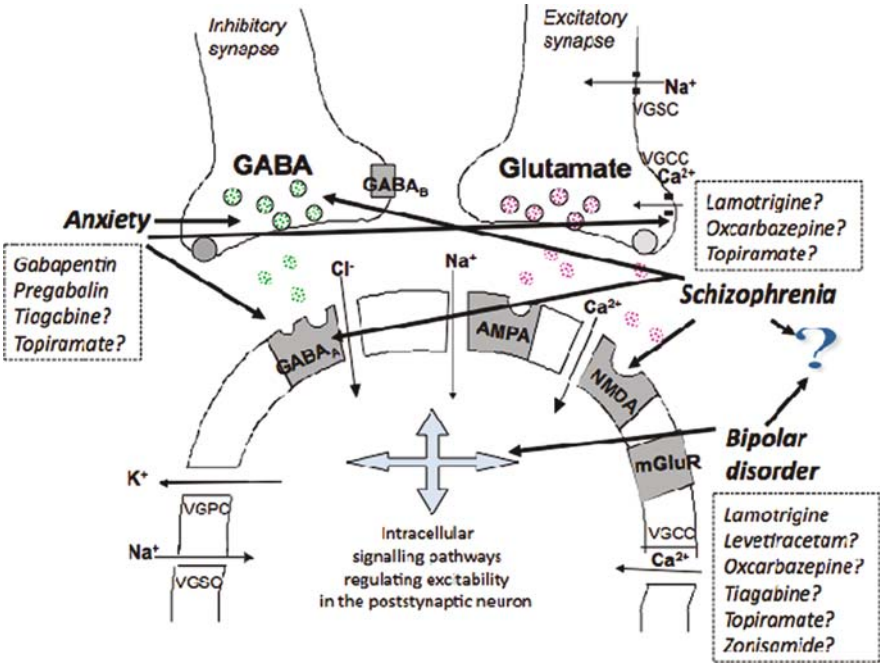

Fig. 16.1 Pathophysiological processes in the GABAergic and glutamatergic synapses and possible role of new antiepileptic drugs. Suggested involved pathophysiological processes and pharmacological actions of new AEDs in anxiety, schizophrenia and bipolar disorder in the glutamatergic and GABAergic synapses. VGSC = voltage-gated sodium channel, VGCC = voltage-gated calcium channel

Table 16.1 Approved or accepted or investigational use of new AEDs in neuropsychiatric disorders

New AEDs in neuropsychiatric disorders	Anxiety	Schizophrenia	Bipolar disorder	Other indications	Relevant references
Gabapentin	X			I^{1}	[86]
Lamotrigine		I	X		[48, 50, 60, 69, 79]
Levetiracetam	I		I	I^{2}	[31, 76]
Oxcarbazepine		I	I		[51, 64]
Pregabalin	X			I^{3}	[18, 26, 85]
Tiagabine	I		I		[27]
Topiramate	I	I	I	$I^{2,4}$	[33, 37, 56, 57, 73, 82, 83, 87]
Vigabatrin				I^{5}	[81]
Zonisamide			I	I^{1}	[89]

Clinical use of new AEDs in neuropsychiatric disorders, where X is accepted or approved indications and I relates to investigational use. I^{1} is investigational use in dependence of drugs of abuse (cocaine), I^{2} is investigational use for cigarette smoking, I^{3} is investigational use for tardive dyskinesia, I^{4} is investigational use in binge-eating disorder, I^{5} is investigational use in benzodiazepine dependence

mechanisms of action of AEDs related to the main pathophysiological processes in the neurological and psychiatric disorders are summarized in Table 16.1. Approved and investigational clinical use in these disorders, in European countries or in the U.S. are also summarized in Table 16.1. In addition to these mechanisms directly involved in excitatory and inhibitory neurotransmission by the amino acid neurotransmitters GABA and glutamate, indirect mechanisms may be involved, such as modulation of other neurotransmitters as monoamines or peptides. All mechanisms related to decreased excitability with special focus on a decrease in glutamatergic signalling, will have potential neuroprotective properties. Conclusive evidence of the importance of the effects of AEDs on other neurotransmitter systems is, however, lacking, although some studies exist [14]. Evidence for the efficacy and safety of AEDs, especially for the newer compounds, are still lacking in these disorders. There is a need for controlled studies with a larger number of patients and homogeneity of diagnosis to establish efficacy of the individual AEDs [2]. The neuropsychiatric disorders anxiety, schizophrenia and bipolar disorder will be discussed below, regarding preclinical and clinical evidence for the use of new AEDs.

16.3.3 Basic Findings in Relation to Clinical Efficacy of New AEDs

Recent findings regarding mechanisms of action of new AEDs in the synapse correlated to relevant pathophysiological mechanisms will be discussed in relation to

results from clinical studies in neuropsychiatric disorders. Focus will be on anxiety, schizophrenia and bipolar disorder, but recent advances in other application as in tardive dyskinesia, alcohol, cigarette and drug abuse and dependence, eating disorders and neuroprotection will also be reviewed.

16.3.4 Anxiety

Dysfunction of GABA transmission has been implicated in the pathophysiology of anxiety disorders [15, 16] (Fig. 16.1). Evidence supporting this resulted from clinical experience with benzodiazepines, followed by determination of mechanism of action, pharmacogenetic studies, and neuroimaging investigations of the GABA receptor subunit compositions [17, 18]. Several new AEDs including gabapentin, pregabalin, tiagabine and vigabatrin, which are all known to target metabolic pathways of GABA or GABA uptake, could possibly possess anxiolytic properties [18].

Even if the structure of gabapentin is similar to GABA, gabapentin and its derivative pregabalin do not seem to affect the $GABA_A$ receptor complex [19, 20]. Pregabalin, like gabapentin, is a ligand of α2δ(1 and 2) voltage-activated calcium channel (VGCC) subunits and are involved in reduced neuronal excitability. It remains to be determined, however, whether this effect is sufficient to account for the broad-spectrum activity of gabapentin and pregabalin [19, 20]. The exocytosis of glutamate is decreased, but it has also been suggested that aspartate, noradrenalin and substance P also may be reduced, as seen from in vitro studies [21–23]. The subunits are major binding proteins for pregabalin in neocortex, hippocampus, amygdala, and spinal cord, as demonstrated in genetically modified mice [24].

In anxiety disorders, randomized controlled trials have proven the efficacy of gabapentin and pregabalin in the treatment of social anxiety disorder, and pregabalin in the treatment of generalized anxiety disorder [19, 25]. Pregabalin was approved for genearlized anxiety disorder by the European Medicine Agency (EMEA) in 2005. A recent study showed the efficacy of pregabalin in generalized anxiety disorder in a four-week multicenter, double blind, placebo controlled trial [26]. Pregabalin was used in doses of 300, 450 and 600 mg per day, and it was shown that 300 and 600 mg daily, demonstrated efficacy over placebo (89–93 patients in each group), in decreasing psychotic and somatic symptoms of generalized anxiety disorder. It was pointed out that 300 mg daily had the best efficacy and tolerability profile.

Tiagabine has demonstrated efficacy and good tolerability in generalized anxiety disorder in two randomized trials (Table 16.1) [17, 18, 27]. Tiagabine and vigabatrin are mechanism based designed drugs that inhibit GABA reuptake (GAT-1) and the GABA metabolism (via GABA transaminase inhibition), respectively. These two drugs are the only AEDs with one specific mechanism of action. Both these drugs cause an increase in synaptic GABA availability and enhance inhibitory GABAergic neurotransmission. The use of vigabatrin in epilepsy has diminished during the

last years since vigabatrin, (but not tiagabine), has a risk of more than 30% of the patients developing serious irreversible visual field defects by possible damage of photoreceptors in the retina [28–30]. Consequently, the possible clinical effects of vigabatrin in other disorders than epilepsy have not been further investigated (except from one study found, see Table 16.1).

Levetiracetam may be effective for patients with epilepsy and also anxiety and depression, as shown in an open study [31]. Levetiracetam may decrease glutamate exocytosis by the inhibition of synaptic vesicle protein 2A (SV2A), and it has also demonstrated to enhance chloride currents in the $GABA_A$ receptor [17, 32]. Topiramate may also be effective in social phobia, as demonstrated in one open study [33]. Topiramate is a drug with several proposed mechanisms of action, including modulation of $GABA_A$ and glutamate receptors, inhibition of voltage-gated sodium channels, modulation of voltage-gated potassium and calcium channels, and carbonic anhydrase inhibition [34–37].

Since the various $GABA_A$ receptor subtypes are different in their specific regional and cellular localization, they consequently serve distinct neuronal circuits and functions. New subtype-selective drugs in development for e.g. the α_3-subunit in $GABA_A$ receptors could be used in anxiety, in addition to insomnia, memory enhancement, and psychiatric disorders [38]. Further investigations that include more of the new AEDs would be of importance for the evaluation of drug treatment alternatives in anxiety, an area with a large patient population.

16.3.5 Schizophrenia

The main focus in the neurochemical background of the pathophysiology of schizophrenia is the monoamines dopamine and serotonin. Nevertheless, the amino acid neurotransmitters GABA and glutamate are important modulators of monoaminergic neuronal pathways. In the present review glutamatergic and GABAergic neurotransmission is focused upon to delimit the approach to the common mechanisms for several of the disorders, that several AEDs affect glutamatergic and GABAergic neurotransmission [39–43]. Mechanisms affecting excitability in the GABAergic and glutamatergic synapses may play a role in the pathophysiology of schizophrenia (Fig. 16.1). GABAergic hypofunction has been postulated based on several findings. A deficiency in an isoform of glutamate decarboxylase (GAD_{67}), the enzyme responsible for the conversion of glutamate to GABA, has been implicated in the pathogenesis of schizophrenia, and reduced amounts of the enzyme are found post-mortem in prefrontal areas of schizophrenic patient [44, 45]. Histone deacetylases are negative regulators of gene expression, which could down-regulate reelin, a neuronal migration factor has mechanisms affecting GABAergic activity [46].

Both GABA and glutamate contribute to the control of dopaminergic activity in the brain that is thought to be hyperactive in schizophrenia [39, 43, 47]. Recently, a deficit in the α_3-subunit in $GABA_A$ receptors was linked to dopaminergic hyperfunction, which is considered to be a contributing factor to the development of

schizophrenia [38]. The glutamate hypothesis as a basis of understanding the neurochemical background for schizophrenia suggests involvement of a hypofunction in glutamatergic NMDA receptors, possibly on critical GABAergic interneurons [39, 43, 47].

AEDs with proved efficacy in bipolar disorder, valproate, lamotrigine and carbamazepine, have been investigated in schizophrenia as well, and they may be efficacious in the treatment of neuroleptic-resistant schizophrenia [40]. The use of lamotrigine (up to 200 mg per day) as adjuvant treatment with neuroleptics in patients with schizophrenia and obsessive-compulsive disorder may be advantageous but needs further investigation [48]. Two randomized controlled studies demonstrated, however, that lamotrigine was not advantageous as add-on therapy to atypical antipsychotics in patients with refractory psychosis [48, 49]. Results from a recent study with patients suffering from schizophrenia or schizoaffective disorder and obsessive compulsive disorder suggested that they may benefit from lamotrigine treatment [50]. Further investigations of lamotrigine will be needed to evaluate its role in schizophrenia.

Recent data regarding in-patients with mood or schizoaffective diagnosis treated with the carbamazepine derivative oxcarbazepine or valproate demonstrated a similar efficacy of the two drugs (Table 16.1) [51]. Oxcarbazepine, however, seemed to be more effective than valproate on negative symptoms like social withdrawal [51]. Inhibition of neuronal excitability by inhibition of voltage-gated sodium channels, and modulation of intracellular signalling pathways have been suggested as relevant mechanisms [1, 52] (see the next section regarding bipolar disorder). Valproate has several mechanisms that affect GABAergic activity, such as potent inhibition of histone deacetylases, increase in GAD activity and other effects on enzymes related to the synthesis and metabolism of GABA, such as α-ketoglutarate and succinic semialdehyde dehydrogenase [53, 54]. It remains to be investigated if derivatives of valproate in development could possess similar effects.

Topiramate has been demonstrated to possess many molecular activities as previously described, including selective antagonism at the glutamatergic kainate receptor [37, 55]. Antagonism of glutamate receptors is regarded as protective against excitotoxicity. Recently, in a randomized, double blind, placebo-controlled, crossover trial, it has been shown to be an effective adjuvant treatment in reducing general psychopathological symptoms in treatment-resistant schizophrenia (Table 16.1) [56]. The effect could probably be coupled to its antagonistic effect on glutamatergic kainate receptors [56]. In a study with 44 male patients with aggression and borderline personality disorder, topiramate (up to 250 mg daily) was effective in reducing aggressive behaviour and well tolerated in a 18-month follow-up [57]. No basic mechanisms were suggested to explain its efficacy. Of the new AEDs, only lamotrigine, oxcarbazepine and topiramate have been investigated in schizophrenia, and further studies of these drugs and other newer AEDs are encouraged together with the use of neuroleptics. A recent review concludes that a mood-stabilizing AED as adjunctive therapy may have some benefits for patients treated for schizophrenia, but further investigations in larger patient populations will be needed [58].

16.3.6 Bipolar Disorder

Bipolar disorder is a severe, chronic and potentially life-threatening illness of recurrent episodes, i.e. mania, hypomania, depression and mixed states (concomitant manic and depressive symptoms), and rapid cycling (four or more episodes per year) [1]. It is subdivided into bipolar I with classical manic and depressive phases, and bipolar II with hypomanic and depressive episodes. Several pathophysiological mechanisms have been postulated to be involved in the development of bipolar disorder. Dysfunction of dopaminergic neurons has been implicated in several neuropsychiatric disorders, including bipolar disorder [47].

Interference with intracellular mediators and signalling pathways and decreased glutamatergic neurotransmission also seem to be important mechanisms in the pathophysiology of bipolar disorder, which will be focused upon in more detail (Table 16.1). The inositol theory is considered to be of major importance. This involves a depletion of inositol caused by a reduction in the recycling of inositol-containing metabolites required for signal transduction [1]. Lithium, which was first investigated, inhibited the enzymatic degradation of inositol phosphates to free intracellular inositol [1]. A clinical finding that correlates between bipolar disorder and inositol levels is that patients with bipolar depression seem to show improvement in symptoms following inositol augmentation of lithium, valproate or lamotrigine [59, 60]. The effects of AEDs are usually compared to the effects on inositol levels by lithium, which may be regarded as a milestone in the inositol hypothesis of bipolar disorder [1]. It has been suggested that a common mechanism for lithium, carbamazepine and valproate involves inositol depletion by inhibition of the collapse of sensory neuron growth cones and increase in the growth cone area, effects which are reversed by inositol [61]. Many target proteins and signalling pathways have been proposed as important for their clinical efficacy in bipolar disorder, even though many of the studies are preclinical. In addition, evidence is now pointing that atrophy and glial death are involved in mood disorders, and that mood-stabilizing agents may exert beneficial neuroprotective actions by attenuating or reversing disease-related impairments in neuronal plasticity, neurogenesis or cell survival [62, 63].

Kindling is considered an animal model of epileptogenesis, and transferred to bipolar disorder, this mechanism might explain how events trigger affective episodes, but patients may relapse in the absence of any obvious stress factor. Many AEDs have demonstrated anti-kindling effects in vitro and may explain why they exert efficacy in both disorders [52]. Several AEDs exert their action on voltage-gated ion channels, and the net effect is action in the direction of decreased excitability, and they are established as effective mood-stabilizers [52]. Calcium is involved in cellular plasticity, as a reduction of intracellular calcium concentration may be of major importance in neuroprotection by avoiding cell destruction and finally, cell death [52]. Other factors involved in cell survival pathways contributing to neuroprotection are bcl-2, brain-derived neurotrophic factor, mitogen-activated protein kinases, and cyclic adenine monophosphate element-binding protein [62]. Another postulated mechanism involved in the pathophysiology of bipolar disorder

is modulation of the expression of the early inducible genes for c-fos and c-jun and thus, promoting the expression of various intracellular regulatory proteins, where valproate, carbamazepine, and lithium share most of the effects at the level of protein kinase C [63].

Oxcarbazepine has in a double-blind randomized trial been shown to be equally effective as carbamazepine, but with improved tolerability in bipolar disorder type I and II [64]. Lamotrigine has demonstrated efficacy in bipolar depression and is effective in prevention of depression relapse in a rodent model [65]. It has no antimanic activity, however, in placebo-controlled studies [66–69]. A recent study with lamotrigine monotherapy in 182 patients with rapid cycling bipolar disorder showed that patients treated with lamotrigine were 1.8 times more likely than the placebo group to achieve euthymia following six months [69]. Lamotrigine and carbamazepine seem to affect mood disorders reliably, while GABAergic drugs influence mood negatively, and one hypothesis is that the two drugs affect structures in the limbic system, e.g. amygdala, known to be related to emotion [70]. This effect could be caused by modulation of monoamines, as lamotrigine dose-dependently decreases extracellular serotonin and dopamine in rats, or decreases the exocytosis of glutamate [70–72]. Lamotrigine inhibits high-voltage-gated calcium channels responsible for neurotransmitter exocytosis [34, 35]. Monotherapy with lamotrigine is superior to gabapentin monotherapy and placebo in bipolar disorder, and at present, there is lacking evidence for the use of gabapentin in maintenance therapy of bipolar disorder or rapid cycling [67].

There are some reports on topiramate and zonisamide suggesting potential use as mood stabilizers [73, 74]. A newly published open-label study with 35 patients with bipolar disorder suggested that zonisamide could be efficacious as adjunctive treatment, as it showed modest benefit in global improvement, even though larger studies will be needed [74]. Both drugs are regarded as broad-spectrum drugs with multiple mechanisms of action resulting in reduction in excitatory neurotransmission, which may be the main goal. In mood-related disorders, valproate, carbamazepine and lamotrigine are valuable drugs, but that there is no clear, as evidence to support the use of the newer AEDs oxcarbazepine, vigabatrin, tiagabine, topiramate, zonisamide and levetiracetam in these disorders [12].

16.4 Other Potential Applications for AEDs

16.4.1 Tardive Dyskinesia

Tardive dyskinesia are often irreversible adverse effects of long-term use of antipsychotics. In the treatment of tardive dyskinesia there is a potential for using valproate as a pharmacological tool, since it has GABA-potentiating properties, and GABAergic hypofunction in the basal ganglia is stated as an important mechanism underlying the pathophysiology [75]. A finding that supports this hypothesis is that

valproate inhibits reserpine-induced orofacial movements in rats, an animal model for tardive dyskinesia [75]. It remains to be investigated whether derivatives of valproate may posess the same properties. Furthermore, the effect of levetiracetam (500–3000 mg/day) was studied in a randomized, double blind placebo controlled study with 50 patients for 12 weeks [76]. The efficacy was demonstrated as improvement in the abnormal involuntary movement scale (AIMS), and the score declined by 43.5% in the treated group compared to 18.7% for placebo, and patients that continued treatment in an open phase showed further improvement. The mechanism for the effect has been suggested to involve reduced neuronal hyper synchronicity in the basal ganglia due to enhancement of GABA turnover in the striatum and substantia nigra and enhanced nitric oxide production [76]. These effects were speculated to inhibit neuronal loss in striatum due to excitatory and oxidative toxicity initiated by dopaminergic blockade by antipsychotics at D2-receptors located on cortico-striatal glutamatergic terminals [76]. This hypothesis would be in accordance with the suggested pathophysiological processes involved as described above, and possible neuroprotective properties.

16.4.2 Alcohol and Cigarette Dependence and Drug Abuse

Early reports suggest the use of new AEDs in conditions of drug and alcohol abuse and withdrawal: topiramate for opiate and alcohol withdrawal, vigabatrin for methamphetamine and cocaine addiction, and lamotrigine for patients with bipolar disorder and cocaine dependence [1, 77–81]. Recently, several new studies have been conducted to elucidate this interesting field. Topiramate (up to 75 mg per day) was effective in abstinence-related nicotine withdrawal in 40 cigarette smokers, as it possibly enhances both withdrawal and reward related to its antiglutamaterigc properties [82]. Topiramate is also currently investigated in a randomized controlled study with 180 patients with depression for tobacco dependence, and the study will be ongoing to 2011 [83].

In a preclinical study, the effect of levetiracetam (50 or 200 mg/kg) acutely or repeatedly (100 mg/day for 14 days) was studied in rabbits in combination with alcohol (0.8 g/kg 60 minutes after levetiracetam administration) by EEG recordings to measure the possible pharmacodynamic interaction [84]. The study demonstrated a synergistic effect of levetiracetam and ethanol, and that the drug decreased the sensitivity of ethanol in hippocampus, which may be of importance in the treatment of alcohol addiction. It was suggested that the interaction was likely to occur because of their similar effects on neurotransmitter systems as with GABA, and further that inhibition of N-type of calcium channels would reduce intraneuronal calcium levels and decrease glutamatergic neurotransmission, which could also possess neuroprotective effects [84]. The results will need to be confirmed clinically.

Pregabalin (225–900 mg daily) demonstrated efficacy in the discontinuation of long-term benzodiazepine use in 15 patients in an open-label study over three

months, where all patients discontinued successfully in 3–14 weeks with a significant reduction in anxiety and improvement in cognitive functioning [85]. The findings were suggested to be related to the inhibitory effect of pregabalin at the voltage-sensitive calcium channels at excitatory terminals and decrease in the release of glutamate, noradrenaline, substance P and consecuently a dampening of the firing rate of post-synaptic neurons. The findings will need to be confirmed in a larger and randomized controlled study. The related compound gabapentin did not, however, show promising results in the treatment of craving in cocaine-dependence in patients [86]. Possible mechanisms were not discussed in this study, but cocaine affects monoaminergic pathways and mainly dopamine in the nucleus accumbens. It seems that the different modes of action of benzodiazepines and cocaine may not be affected in the same way by pregabalin and gabapentin.

16.4.3 Eating Disorders

Topiramate may also be useful in bulimia and binge eating disorder [87, 88]. Zonisamide (100–600 mg per day) demonstrated efficacy in binge eating disorder compared to placebo in a study with 60 obese patients over 16 weeks, but it was not well tolerated, as almost 25% of the patients discontinued in the zonisamide group because of adverse events [89]. The involved mechanisms of action of the drugs in these disorders are unclear but effects on serotonergic or dopaminergic neurotransmission may be involved, as they are major mediators in craving and appetite, respectively. Reduction in glutamatergic neurotransmission has also been shown to reduce feeding behaviour, and mechanisms involving all three neurotransmitters are possible [89]. Alternative explanations include a direct effect on weight loss related to gastrointestinal side effects or inhibition of carboanhydrase-mediated de novo lipogenesis [89]. These mechansisms may be involved for both topiramate and zonisamide.

16.4.4 Neuroprotection

Disturbances in excitability seem to be involved in the development of all neuropsychiatric disorders mentioned, schizophrenia, anxiety affective disorder, bipolar disorders, and others. Neuroprotection is therefore an important field, also in the neuropsychiatric setting (see Chapter 1 in this book). In vitro studies have demonstrated that there are multiple mechanisms underlying the neuroprotective effects of several AEDs, including topiramate, levetiracetam, and zonisamide as in ischemia [90]. Zonisamide scavenges nitric oxide and other free radicals in vitro, inhibits lipid peroxidation and free-radical-induced DNA damage, and could therefore provide neuroprotective effects [91]. These drugs may decrease hyperexcitability by affecting glutamatergic transmission.

16.5 New AEDs in Development

Derivatives of established AEDs are undergoing development and clinical trials in non-epileptic CNS disorders as bipolar disorder. These follow-up compounds have to be more potent, safer, and possess favourable pharmacokinetics to become a successful second-generation of AEDs [92, 93]. These drugs include the carbamazepine analogues eslicarbazepine acetate (BIA-2-093) and 10-hydroxy carbazepine (MHD), the valproate derivatives valrocemide and isovaleramide (NPS 1776), the gabapentin derivative XP13512, the levetiracetam derivatives brivaracetam (ucb 34714), and the felbamate derivative fluorofelbamate [92, 94, 95]. Fluorofelbamate protects against chemically induced ischemia in cultured hippocampal neurons and hypoxic injury in the CA1 region in hippocampal slices, and in vivo protection against hypoxia and ischemic damage in animal models [96]. In addition, the valproate derivative valnoctamide is undergoing a phase II double blind controlled trial as a non-teratogenic alternative to valproate for women with bipolar disorder [97]. Investigations of the clinical relevant mechanisms of these second-generation substances are still ongoing. It is important that these new substances are evaluated in non-epileptic disorders to map their therapeutic efficacy.

Retigabine is among a new class of antiepileptic compounds that selectively and potently opens voltage-gated KCNQ2/3 and KCNQ3/5 potassium channels which are involved in the control of neuronal excitability [98–100]. It is expected that retigabine will be marketed for the use in epilepsy in the near future. In a rodent model of mania, retigabine (1 mg/kg) had antimanic-like effect like lamotrigine and lithium, and that it may have effect in bipolar disorder as well [101]. It was suggested that the effect was mediated by reduction in excessive striatal dopamine neurotransmission.

Future AEDs for potential use in both epilepsy and other disorders may include drugs acting on new targets in the synapse, such as metabotropic GABAergic and glutamatergic receptors. Furthermore, development of selective drugs acting on specific subtypes of receptors and ion channels, which may be altered in pathophysiological processes, seems as a promising research field [2].

16.6 Clinical Pharmacological Considerations

Pharmacological variability among patients involving differences in pharmacodynamic, kinetic, and genetic factors is of major importance in neuropsychiatric disorders, as efficacy and tolerability often are difficult to evaluate and separate. For new AEDs, as for the older drugs, monitoring of the treatment is also important in psychiatric disorders, as it has been in epilepsy for several decades. Therapeutic drug monitoring (TDM) is defined as the measurement and clinical use of drug concentrations in body fluids, usually serum or plasma, to adjust each patient's individual drug dosage and tailor the treatment to each patient [102, 103]. Recently, new terminology in this field has been implied, as individual reference ranges should be used

instead of therapeutic ranges or reference ranges, whereby each patient is his/her own control, and the right dosage may be adjusted according to clinical judgement of optimal efficacy and tolerability at a given serum concentration.

There are several reasons why TDM is benefitial for patients using AEDs. For most AEDs, there are large inter- and intra-individual variations between dosage and measured serum concentrations. There may be variations in pharmacokinetic parameters due to age-dependent alterations in absorption, distribution, metabolism and excretion, involving changes in protein binding, clearance and half-life. AEDs, antidepressants and neuroleptics are susceptible for pharmacodynamic and –kinetic interactions, which may have serious consequences for the patients [104–107]. It should be noted, however, that new AEDs are less susceptible to pharmacokinetic interactions than the older drugs as phenytoin, phenobarbital, carbamazepine, and valproate. TDM is therefore of importance in these patient groups [102, 103]. Furthermore, non-adherence is a problem, and in bipolar disorder, more than 60% of bipolar patients are at least partially non-adherent to medications [108]. In anxiety, depression, and schizophrenia, non-adherence rates between 30 and 65% have been reported, depending on methodological criteria [109–111].

16.7 Conclusions and Future Directions

- AEDs are broad spectrum drugs with several proposed mechanisms of action, and they may affect common pathophysiological processes in epilepsy and bipolar disorder. Several neurobiological mechanisms disturb neuronal excitability and seem to be involved in various pathophysiological processes involving an imbalance in GABAergic and glutamatergic neurotransmission, and AEDs affect them in various ways. In anxiety, GABAergic mechanisms are investigated most thoroughly, and in schizophrenia, disturbance of glutamatergic and GABAergic neurotransmission is involved. In bipolar disorder intracellular signalling pathways are focused upon.
- A number of new AEDs seem to be promising regarding effect and safety in the future treatment of anxiety, schizophrenia and bipolar disorder. New AEDs that seem to enhance GABAergic neurotransmission, like tiagabine, pregabalin, gabapentin and possibly levetiracetam, seem to have a role in anxiety. The role of AEDs in schizophrenia will need further investigations, especially for lamotrigine, oxcarbazepine and topiramate. In bipolar disorder, lamotrigine is the new AED that appears to have a clear role based on their effect on intracellular pathways. In addition, most other new AEDs are investigated for their potential efficacy. A better understanding of the mechanisms of action of AEDs in different disorders will be provided by increased knowledge of the underlying molecular deficits in each disorder, and clinical investigations that prove efficacy of the AEDs in various disorders. Future clinical application of new AEDs and derivatives in neuropsychiatric disorders also include tardive dyskinesia, drug, cigarette and alcohol dependence, eating disorders, and neuroprotection.

- In the clinical setting, the implementation of TDM of new AEDs will add valuable experience from epilepsy to more extended use in neuropsychiatry, since pharmacological variability is extensive, many patients use polytherapy with potential for interactions, and compliance is often poor in these patient populations. It is of major importance to improve our understanding of the relationship between mechanisms of action, pathological conditions and clinical efficacy for optimalized pharmacological treatment.

Acknowledgement The authors have no conflict of interest regarding the manuscript.

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Chapter 17
The Neuroprotective Efficacy of Vitamins

Chanoch Miodownik and Vladimir Lerner

With the progress of understanding the illnesses' reasons, it becomes clearer that to prevent maladies is much easier, than to treat them.

(I.I. Mechnikoff)

Abstract It has been known for a long time that vitamins are essential nutrients for humans and animals. These substances are important for regular cell function, growth and development. Relatively small amounts of vitamins are needed to perform vital functions. As a rule vitamins promote the actions of enzymes in order to improve its efficiency and in this role they are called coenzymes.

There are 13 essential vitamins vitamin A, vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), pantothenic acid, biotin, vitamin B_6 (pyridoxine), folate (folic acid, vitamin B_9), vitamin B_{12}, vitamin C, vitamin D, vitamin E, and vitamin K, which are needed for normal functioning of mammalians' life.

Normal neurosystem functioning depends on its structural and functional perfection. During life, the human body is exposed to many elements, which create free radicals. These free radicals are known to date as distractive agents for many biological systems include the neurosystem. Free radicals are atoms or groups of atoms with unpaired number of electrons and can be formed when oxygen interacts with certain molecules. Once these highly reactive radicals are formed, they can start a chain reaction – "domino effect", which produces membranes damage. To prevent this damage, the body has an antioxidation defense system. Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidative agents are intimately involved in the prevention of cellular damage – the common pathway for cancer, aging, and a variety of diseases. The antioxidant defense system is important in maintaining cellular homeostasis and preventing oxidative stress.

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_17,

According to the present invention, antioxidants like vitamins and other antioxidative agents may be considered as further active components because antioxidants inhibit free radical distractive activities. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cell membrane to neutralize oxygen radicals and thereby protect the membrane.

Although there are several enzyme systems within the body that scavenge free radicals, the principle vitamin antioxidants are vitamin E, beta-carotene, vitamin C and vitamins from the B group.

Vitamins C, E and K are known to protect neurons from oxidative damage in stroke and in other neurodegenerative conditions. B vitamins are critically important in maintaining the normal functions of the brain. Deficiency in B vitamins results in a predictable sequence of different neurological and psychiatric disturbances.

This chapter is focused on evidence from clinical and basic science studies supporting a role of several vitamins as potential neuroprotective compounds. Neuroprotective effects of them as add-on therapies merit further investigations in schizophrenia and mood disorders.

Abbreviations

AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
ATP	Adenosine Triphosphate
BARS	Barnes Akathisia Rating Scale
BPRS	Brief Psychiatric Rating Scale
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DFE	dietary folate equivalent
7-DHC	7-dehydrocholesterol
DNA	Deoxyribonucleic Acid
GABA	Gamma-Aminobutyric Acid
5-HT	Serotonin
L-5HTP	L-5-hydroxytryptophan
IU	International Unit
LT	Lithium-Induced Tremor
mcg	micrograms
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NIA	Neuroleptic-Induced Akathisia
NIH	National Institutes of Health
nM/L	Nanomoles per Liter
NMDA	N-methyl-D-aspartic Acid
PANSS	Positive and Negative Syndrome Scale
PD	Parkinson's Disease
PLP	Pyridoxal 5′-Phosphate
PSNL	Partial Sciatic Nerve Ligation

RAE	Retinol Activity Equivalents
RDA	Recommended Dietary Allowance
RNA	Ribonucleic Acid
SAS	Simpson-Angus Scale
SGAs	Second Generation Agents
SNCI	Sciatic Nerve Crush Injury
SSRIs	Selective Serotonin Reuptake Inhibitors
TD	Tardive Dyskinesia
TH	Thermal Hyperalgesia
t.i.d.	three times a day
TMP	Thiamin Monophosphate
TTP	Thiamin Triphosphate
TPP	Thiamin Pyrophosphate
U.S.	United States of America
USDA	United States Department of Agriculture
UVB	Ultraviolet-B

17.1 Introduction

Vitamins are known as essential nutrients for human beings. It is natural substances, which are found in living plants and animals.

In 1905, an English scientist, William Fletcher was the first researcher who found that removing special factors from food induce diseases. Consistent with this idea he was researching the causes of the disease Beriberi. He discovered that eating unpolished rice prevented Beriberi while on the other hand eating polished rice did not. William Fletcher believed that the husk of the rice contained something that prevented the disease. He suggested the assumption that there were special nutrients which were mandatory important for normal life [1, 2].

Almost parallel, another English biochemist Sir Frederick Gowland Hopkins (1861–1947) also came into conclusion that there are some food factors, which are important to healthy life.

The word "vitamine" was coined at the Lister Institute in London by the Polish biochemist Kazimierz Funk commonly anglicized as Casimir Funk (1884–1967) in 1912. He was generally credited with the first formulation of the concept of vitamins, which he called vital amines or "Vitamines". Vita in Latin is life and the –amine suffix is for amine; at the time it was thought that all vitamins were amines. In 1920, British biochemist Sir Jack Cecil Drummond (1891–1952) proposed that the final "e" should be dropped, to deemphasize the "amine" reference, after the discovery that vitamin C had no amine component, and the name has been "vitamin" ever since [2].

The reason the alphabet soup of vitamins seems to skip from E to the rarely-mentioned K is that most of the "letters" were reclassified, as with fatty acids, discarded as false leads, or renamed because of their relationship to "vitamin B",

which became a "complex" of vitamins. Vitamin G, Riboflavin, for example, is now known as B_2. Throughout the early 1900s, scientists developed a way to isolate and identify a number of vitamins by depriving animals of them. Initially, lipid from fish oil was used to cure rickets in rats, and the fat-soluble nutrient was called "antirachitic A". The irony here is that the first "vitamin" bioactivity ever isolated, which cured rickets, was initially called vitamin A, this bioactivity is now called vitamin D, which is itself subject to the semantic debate that it is not truly a vitamin because it is a steroid derivative. What we now call "vitamin A" was identified in fish oil because it was inactivated by ultraviolet light. Most of what we now recognize as the water-soluble organic micronutrients were initially referred to as just one entity, "vitamin B" [3].

There are 13 essential vitamins divided to two types: nine water-soluble (8 B vitamins and vitamin C) and four fat-soluble (A, D, E and K). The body needs vitamins to stay healthy and a varied diet usually gives you all the vitamins you need. Vitamins do not provide energy (calories) directly, but they do help regulate energy-producing processes. With the exception of vitamin D and K, vitamins cannot be synthesized by the human body and must be obtained from the diet. Vitamins have to come from food because they are not manufactured or formed by the body [4, 5].

Each vitamin has a special role to play within the body, helping to regulate the processes such as cell growth and repair, reproduction and digestion. Most vitamins take part as coenzymes in biochemical reactions, a fact which makes it significant component in the central nervous system and derived mental health. Some vitamins have antioxidative effect. Oxidation is defined as the loss of at least one electron when two or more substances interact. This phrase is quite broad definition contains different substances from metal to living tissue. Oxidation can sometimes produce reactive substances known as free radicals that can cause oxidative stress or damage to the cells.

Free radicals are atoms or groups of atoms with an unpaired number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. Free radicals may play a role in heart disease, cancer and other diseases and disturbances lead to neurocognitive impairment and movement disorders. Since oxidation is a naturally occurring process within the body, there should be a homeostatic mechanism in order to maintain a balanced body state for keeping health. To prevent free radical damage the body has a defense system of antioxidants [6, 7].

Antioxidants are substances that may protect cells against the effects of free radicals and terminate the chain reaction before vital molecules will be damaged.

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines [8].

Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are lutein, lycopene, beta-carotene – precursor of vitamin A, vitamins B, C and E. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied from outside in diet.

Antioxidants are found in many foods. These include fruits and vegetables, nuts, grains, some kinds of meat, poultry and fish. These molecules are capable of slowing or preventing the oxidation of other molecules. This action has a great importance since oxidation can produce free radicals, which may destroy living cells all over the human body. The oxidation reaction can damage neurons too and induce clinical symptoms in different body systems, for example cardiovascular [6, 7].

Since antioxidative agents have the ability to stop the free radicals chain of damage, its absence may lead to many difficult neurologic disturbances such as a stroke, neurodegenerative diseases and peripheral neuropathies. On the base of these scientific facts, a new modern attitude was developed. It is called the neuroprotective approach.

The goal of the neuroprotection approach is to limit neuronal dysfunction or death after injury of central nervous system and attempt to maintain the highest possible integrity of cellular interactions in the brain resulting in an undisturbed neural function (see also Chapter 1 in this book).

Within last decades, new technologies, such as magnetic resonance imaging (MRI) and spectroscopy (MRS), brought into the psychiatric world an opportunity to look at the living brain in any detail, and certainly at its metabolism. This advance opened the gate to a new understanding about the overlap findings between neuropathology and mental disorders.

The connection between the psychopathology and neuro-cellular and structural damage produced hypotheses about supplementation of some substances, which will work as neuroprotective agents.

There is a wide range of neuroprotection products available that can potentially be used in more than one disorder, since many of the underlying mechanisms of damage to neural tissues are similar.

The underlying process, which can explain the pathogenesis of mental disorders, is not yet well understood. There are no clear biological markers of disease in brain tissue of patients with chronic mental disturbances, in contrast to what is found in brain of patients with dementia.

Almost every nutritional supplement was suspected to be a neuroprotective agent, however some of them have well-marked properties. Partial list of these substances contains vitamin A, vitamins B, C and E, omega-3, nootropil, L-theanine and others.

Since there are many substances, which have neuroprotective action, we decided to be focused only on vitamins with this ability. The source of data are scientific evidence based materials, our and others empirical experiences. Some information is collected from animal experiments.

It is important to take in account that every individual should be responsible for his deeds and be aware to possible toxic effects especially while taking mega-doses

of vitamins. Some vitamins may imitate it selves as innocent agents but may play a dangerous role if you are not aware to it.

17.2 Vitamin A

History and structure. The discovery of vitamin A may have stemmed from research dating back to 1906, indicating that factors other than carbohydrates, proteins, and fats were necessary to keep cattle healthy [9]. In 1913 two independent research groups Elmer McCollum and Marguerite Davis from the University of Wisconsin-Madison and Thomas B. Osborne and Lafayette B. Mendel from Yale University reported a similar observation with rats fed "the ether extract of egg or of butter" [10, 11]. Both papers appeared in the same volume of the journal. McCollum and Davis were credited for the discovery of the first accessory food substance to be recognized as a vitamin. Since "water-soluble factor B" (Vitamin B) had recently been discovered, the researchers chose the name "fat-soluble factor A" (vitamin A) [9]. Both teams had shown by controlled animal experiments that certain fats contain a factor essential for nutrition, whereas others do not [10, 11]. Vitamin A was first synthesized in 1947 by two Dutch chemists, David Adriaan van Dorp (1915–1995) and Jozef Ferdinand Arens (1914–2001).

"Fat-soluble A" was first believed to be a single vitamin capable of curing xerophthalmia and rickets. Cod-liver oil was first used as a therapeutic agent in the 1770s. The beneficial effect of fish liver oils in the treatment of rickets, osteomalacia, generalized malnourishment, and certain eye conditions was widely recognized by the middle of the 19th century, but no satisfactory explanation accounted for its superiority over other edible fats (Fig. 17.1) [2].

Sources of vitamin A. Vitamin A is widespread in the daily regular food both from vegetarian and from animal origins. Main sources are liver (beef, pork, chicken, turkey, and fish), carrots, broccoli leaves, sweet potatoes, kale, butter, spinach, leafy vegetables, pumpkin, collard greens, cantaloupe melon, eggs, apricots, papaya, mango, peas, broccoli, and winter squash.

Vitamin A is found in colorful fruits and vegetables. It is called provitamin A carotenoid and it can be converted in the body into retinol, one of the most active forms of vitamin A. Free retinol is not generally found in foods. Plants contain carotenoids, some of which are precursors for vitamin A (e.g., alpha-carotene, beta-carotene, and beta-cryptoxanthin). Yellow and orange vegetables contain significant

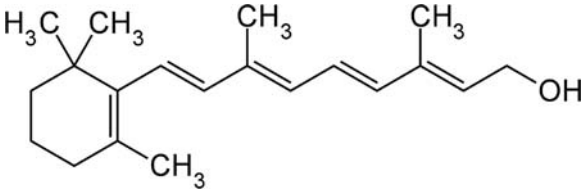

Fig. 17.1 Vitamin A – 3D structure

quantities of carotenoids. Green vegetables also contain carotenoids, though the pigment is masked by the green pigment of chlorophyll. Retinyl palmitate is a precursor and a storage form of retinol, which is found in foods from animal origin [12].

Retinol can be converted into retinal and retinoic acid (other active forms of vitamin A) in the body [12]. In the United States, approximately 26% of vitamin A is consumed by men, and 34% is consumed by women in form of provitamin A carotenoid [13]. Common provitamin A carotenoids found in foods that come from plants are beta-carotene, alpha-carotene, and beta-cryptoxanthin [14]. Among these, beta-carotene is most efficiently converted into retinol [13, 15–17]. Alpha-carotene and beta-cryptoxanthin are also converted to vitamin A, but only half as efficiently as beta-carotene [13].

Different dietary sources of vitamin A have different potencies. For example, beta-carotene is less easily absorbed than retinol and must be converted to retinal and retinol by the body. The most recent international standard of measure for vitamin A is retinol activity equivalents (RAE), which represent vitamin A activity as retinol. Two micrograms (mcg) of beta-carotene in oil provided as a supplement can be converted by the body to 1 mcg of retinol giving it an RAE ratio of 2:1. However, 12 mcg of beta-carotene from foods are required to provide the body with 1 mcg of retinol, giving dietary beta-carotene an RAE ratio of 12:1. A number of good food sources of vitamin A are listed in the table below along with their vitamin A content in micrograms of retinol activity equivalents (mcg RAE). In those foods where retinol activity comes mainly from provitamin A carotenoids, the carotenoid content and the retinol activity equivalents are presented in Table 17.1 [13].

Neuroprotective effect. Vitamin A (retinol) and its derivatives, referred to generically as "retinoids", play an essential role in the normal development of many organ tissues of all vertebrates. It participates in important processes, such as normal vision, reproduction, embryonic development, cell and tissue differentiation and immunological functions. Vitamin A influences the cellular life cycle by controlling the expression of some genes, which regulate cell proliferation, differentiation and apoptosis.

Retinoid and retinoid-associated signaling plays an essential role in normal neurodevelopment and appears to remain active in the adult CNS. Sato and colleagues evaluated on animal model the neuroprotective potential of vitamin A (all-trans retinol), and its geometric isomers, all-trans retinoic acid and 9-cis retinoic acid in stroke. Vitamin A (retinol) and its derivatives were administered as two intra-peritoneal injections immediately prior to and following ischemia. A reduction in infarct volume was observed with all-trans retinol, in a dose-dependent manner: maximum protection was observed with a 10 mg/kg dose. A similar protective profile was observed with all-trans retinol, but not the stereo-isomer 9-cis retinoic acid. Administration of the derivatives 1 h following ischemia did not produce significant protection. Taken together these data suggest a possible use of vitamin A derivatives as an acute neuroprotective strategy for stroke [18].

The complex molecular pathways that mediate the effects of vitamin A and its derivatives are increasingly recognized as a component of the repair capacity that

Table 17.1 The content of retinol and vitamin A in different food sources [13]

Food	Serving	Vitamin A, RAE (mcg)	Vitamin A, IU	Retinol, mcg	Retinol, IU
Cod liver oil	1 teaspoon	1,350	4,500	1,350	4,500
Fortified breakfast cereals	1 serving	150–230	500–767	150–230	500–767
Egg	1 large	91	303	89	296
Butter	1 tablespoon	97	323	95	317
Whole milk	1 cup (8 fl oz.)	68	227	68	227
2% fat milk (vitamin A added)	1 cup (8 fl oz)	134	447	134	447
Nonfat milk (vitamin A added)	1 cup (8 fl oz.)	149	497	149	497
Sweet potato, canned	1/2 cup, mashed	555	1,848	0	0
Sweet potato, baked	1/2 cup	961	3,203	0	0
Pumpkin, canned	1/2 cup	953	3,177	0	0
Carrot (raw)	1/2 cup, chopped	538	1,793	0	0
Cantaloupe	1/2 medium melon	467	1,555	0	0
Mango	1 fruit	79	263	0	0
Spinach	1/2 cup, cooked	472	1,572	0	0
Broccoli	1/2 cup, cooked	60	200	0	0
Kale	1/2 cup, cooked	443	1,475	0	0
Collards	1/2 cup, cooked	386	1,285	0	0
Squash, butternut	1/2 cup, cooked	572	1,907	0	0

The Dietary Reference Intake (DRI) Recommended Daily Amount (RDA) for Vitamin A for a 25-year old male is 900 micrograms/day, or 3,000 IU. Vitamin A plays a role in a variety of functions throughout the body, such as vision, gene transcription, immune function, embryonic development and reproduction, bone metabolism, hematopoiesis, skin health, reducing risk of heart disease, and antioxidant activity

could be activated to induce protection and regeneration in the mature nervous tissue. Malaspina and Michael-Titus have reviewed evidence, which supports the hypothesis of an activation of retinoid-associated signaling molecular pathways in the mature nervous tissue and its significance in the context of neurodegenerative, trauma-induced and psychiatric disorders, at spinal and supra-spinal levels. The authors summarize the potential therapeutic avenues based on the modulation of retinoid targets undergoing reactivation under conditions of acute injury and chronic degeneration in the central nervous system, and discuss some of the unresolved issues linked to this treatment strategy [19].

In last years, a new theoretical attitude to pathogenesis of schizophrenia emerged. The new theory, produced by Ann Goodman, is called retinoid hypothesis of schizophrenia which based on its involvement in neurodevelopment [20–23] during embryonic life and regulation of genes thought to be important in the pathogenesis of schizophrenia [24–27]. This retinoid theory is supported by three independent lines of evidence: (1) congenital anomalies similar to those caused by retinoid dysfunction, are found in schizophrenic patients and their relatives; (2) the loci

which have been suggestively linked to schizophrenia are the same as the genes of the retinoid cascade (convergent loci); and (3) the transcriptional activation of the dopamine D_2 receptors and numerous other schizophrenia candidate genes are regulated by retinoic acid [28]. Several recent reports at the molecular level now suggest that altered transport and lowered synthesis of retinoic acid may be fundamental mechanisms in schizophrenia [29]. Vitamin A (retinoid) deficiency induces selective memory impairment further supporting the hypothesis in that the fine regulation of retinoid-mediated gene expression is important for optimal brain and higher cognition functions [30]. Animal experiments, which disrupt retinoid-signalling pathways, compromise the regulation of synaptic plasticity and related learning and memory behaviours [31]. These pathways have also been connected with the pathophysiology of Alzheimer's disease, schizophrenia and depression [32–36].

Lerner and colleagues performed an open study with a low dose (75 mg/day) of a synthetic retinoid specifically selective for retinoid X receptors – bexarotene in chronic schizophrenia patients. The researchers assumed that bexarotene augmentation to ongoing antipsychotic treatment might have a beneficial effect in the antipsychotic treatment of schizophrenia patients. The results of this study demonstrated a significant improvement of patients' conditions according to PANSS scores. The authors suggest that further studies are needed in order to examine the efficacy of bexarotene in a double blind mode with greater sample size. Anyhow the importance of this study is its possible validation of the retinoid theory, and the connection of vitamin A with the etiology of schizophrenia. In view of the probable interaction of bexarotene with the retinoid metabolizing enzymes, it is suggested that future studies should be directed to an examination of the effects of another retinoid analog that may not produce concomitant side effects [37].

17.3 Vitamins B Group

Deficiencies in the vitamins B: thiamine (B_1), B_6, folate (vitamin B_9) and B_{12} for a long time have been recognized as contributors to cognitive decline. In the evaluation of dementia and memory disorders, deficiencies in these vitamins, if present, should always be treated first before drugs like cholinesterase inhibitors are instated. Patients with these deficiencies often respond favorably to vitamin replacement, showing improved short-term memory and language abilities. Furthermore, it is now thought that people with even slightly lower levels of these vitamins go on to develop Alzheimer's more often than people with normal levels [38]. All, researchers seem to agree that a healthy diet high in the B vitamins and folate is at least protective against cognitive decline [39].

Although these data seem to be convincing, it is too general because the term "vitamins B" contains a group of some agents mentioned in the beginning of this paragraph. We would like to relate individually to each of them and its neuroprotective activity.

17.3.1 Vitamin B_1 (Thiamine)

History and structure. Vitamin B_1, also called thiamine or thiamin, is one of 8 B vitamins previously known as aneurine. It was the first of the water-soluble vitamins to be described [40] so it is named B_1, leading to the discovery of more such trace compounds essential for survival and to the notion of vitamin. Although beriberi disease was described by the ancient Chinese (2697 BC) [41], the relationship with thiamin deficiency was recognized by chance only in 1937. The beriberi syndrome consisted of a series of symptoms that were particularly noxious in samurai Japan: sheepishness, that is, inattention or stupor (not a desired quality in samurai), outbursts of anger (themselves potentially fatal if directed to a member of a higher class), lethargy, loss of appetite, tingling in the fingers and toes, labored breathing, quickened pulse, and, finally, death. Healthy soldiers in fighting form could fall ill and die in as little as two hours. Death usually occurred two hours after the midday meal, and it was gruesome. Unfortunately, nations, like people, forget the lessons learned in history, including the lessons of the history of vitamins. By 1860, Japan was once again faced with a beriberi crisis [4].

In 1878 alone, the Dutch lost 690 native laborers, and the Dutch colonial health officer on Sumatra recorded, "On average, transport by ship to Java ... has taken ten lives per ship." In 1879, so many had died of beriberi there was a shortage of gravediggers to bury them. The government in The Hague sent a young bacteriologist, Christiaan Eijkman (1858–1830) future the Nobel Prize in Physiology or Medicine 1929, to solve the problem. For eleven years, he did the things bacteriologist do, trying to find the germ that caused the disease [2].

White rice was considered a better food than brown rice in Eijkman's time, and brown rice was usually fed to the chickens. Recently, however, Dr. Eijkman's servants had had too much white rice and fed it to the poultry. Dr. Eijkman quickly concluded he had seen the first-ever case of beriberi in chickens. The cure for beriberi, Dr. Eijkman was sure, was as simple as finding what the poison was in white rice. Not aware that he was writing the first chapter in the history of vitamins, for the next five years Eijkman experimented with every conceivable variation of rice as chicken feed [2].

He served the chicken rice in stone pots. Then he tried wooden pots, metal pots, and coconut shells. He boiled the rice in well water, in stream water, and in distilled water. He gave the chickens white rice, brown rice, red rice, blue rice, and black rice, husked rice and unhusked rice, and every possible combination thereof.

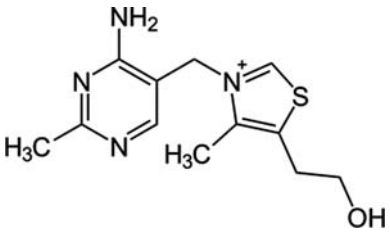

Fig. 17.2 Structure of Vitamin B_1 (Thiamin)

It was not an amazing discovery to the scientists of the day because they did not get a chance to read about it. Dr. Eijkman published his results in a paper in Dutch read all across Dutch Batavia, by perhaps a dozen persons, and was allowed to go home.

Thiamin occurs in the human body as free thiamin and as various phosphorylated forms: thiamin monophosphate (TMP), thiamin triphosphate (TTP), and thiamin pyrophosphate (TPP), which is also known as thiamin diphosphate [4].

Like other B complex vitamins, thiamin is considered as an "anti-stress" vitamin because it may strengthen the immune system and improve the body's ability to withstand stressful conditions.

Thiamin is found in both plants and animals and plays a crucial role in certain metabolic reactions. Adenosine triphosphate (ATP) is the basic energy molecular unit in every living cell. In the process of ATP production, thiamin plays an important role to form an active coenzyme, thiamine pyrophosphate.

Sources of vitamin B_1. A varied diet should provide most individuals with adequate thiamin amount to prevent deficiency. In the U.S., the average dietary thiamin intake for young adult men is about 2 and 1.2 mg/day for young adult women. A survey of people over the age of 60 found an average dietary thiamin intake of 1.4 mg/day for men and 1.1 mg/day for women (Food and Nutrition Board. Whole grain cereals, legumes (e.g., beans and lentils), nuts, lean pork, and yeast are rich sources of thiamin [42]. Because most of the thiamin is lost during the production of white flour and polished (milled) rice, white rice and foods made from white flour (e.g., bread and pasta) are fortified with thiamin in many Western countries. A number of thiamine-rich foods are listed in the Table 17.2 along with their thiamin content in milligrams (mg).

Table 17.2 The content of thiamin in different food sources [41]

Food	Serving	Thiamin (mg)
Lentils (cooked)	1/2 cup	0.17
Peas (cooked)	1/2 cup	0.21
Long grain brown rice (cooked)	1 cup	0.19
Long grain white rice, enriched (cooked)	1 cup	0.26
Long grain white rice, unenriched (cooked)	1 cup	0.04
Whole wheat bread	1 slice	0.10
White bread, enriched	1 slice	0.11
Fortified breakfast cereal	1 cup	0.5–2.0
Wheat germ breakfast cereal	1 cup	4.47
Pork, lean (cooked)	3 ounces*	0.72
Brazil nuts	1 ounce	0.18
Pecans	1 ounce	0.19
Spinach (cooked)	1/2 cup	0.09
Orange	1 fruit	0.10
Cantaloupe	1/2 fruit	0.11
Milk	1 cup	0.10
Egg (cooked)	1 large	0.03

*3 ounces of meat is a serving about the size of a deck of cards

Neuroprotective effect. Thiamin plays a key role in the maintenance of brain function. Thiamin diphosphate is a cofactor for several enzymes involved in glucose metabolism whereas thiamin triphosphate has distinct properties at the neuronal membrane. Thiamin metabolism in the brain is compartmented between neurons and neighbouring glial cells. Thiamin deficiency is commonly encountered in severe malnutrition associated with chronic alcoholism, results in Wernicke's encephalopathy (the Wernicke–Korsakoff syndrome). Magnetic resonance imaging reveals bilateral ventricular enlargement, mammillary body atrophy and cerebellar degeneration indicative of selective neuronal loss that is characteristic of Wernicke's encephalopathy. Several mechanisms have been proposed to explain this selective loss of neurons including a cerebral energy deficit resulting from reductions in activity of thiamin diphosphate-dependent enzymes, oxidative stress and N-methyl-D-aspartate receptor-mediated excitotoxicity.

For chronic alcohol consumers, thiamin is mandatory neuroprotective agent in order to prevent the irreversible Wernicke–Korsakoff syndrome. There is very little information regarding the usefulness of supplementing B vitamins in people with normal levels of the vitamins. For instance, one recent study found that there is simply not enough evidence to warrant thiamine supplementation in dementia unless the person is severely thiamine deficient [43].

17.3.2 Vitamin B_6 (Pyridoxine)

History and structure. Vitamin B_6 was discovered in 1926 by Hungarian scientist Paul Gyorgy (1893–1976), who worked in Heidelberg University (Germany). He found that there is a substance capable of curing a characteristic skin disease in rats (dermatitis acrodynia). The factor is then called the rat anti-acrodynia factor, deficiency of which causes the so-called "rat-pellagra". This substance was called "adermin" [44]. Later it was discovered that vitamin B_6 deficiency may cause niacin deficiency as a result of impaired tryptophan metabolism [45, 46]. In 1939 American researchers gave it the name that now stands – "pyridoxine." This name was a result of its chemical structure – a pyridine ring (5 carbons and 1 nitrogen) and 3 hydroxyl groups – 2-methyl, 3-hydroxy, 5-hydroxymethyl-pyridine. The name pyridoxine has been used as a generic term especially in clinical context. Most commercial labels list it as "pyridoxine hydrochloride."

Vitamin B_6 is present in the human body as six vitamers, which all share a 2-methyl-3-hydroxypyridine structure but differ in the nature of the C_4 and C_5 substituents [47].

These compounds are metabolically interchangeable, namely pyridoxine or pyridoxol (the alcohol) (Fig. 17.3), pyridoxal (the aldehyde) (Fig. 17.4) and pyridoxamine (the amine) (Fig. 17.5). All three of these C4 variants can exist with the C5 substituent as a hydroxymethyl group or with this group esterified to phosphate (e.g. pyridoxal 5′-phosphate). In plants the C5 hydroxymethyl group of pyridoxine can be esterified to glucose, forming pyridoxine-5′-β-D-glucoside [48].

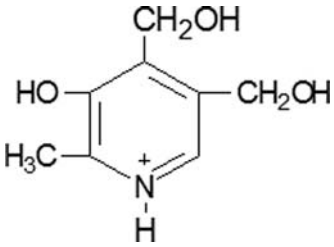

Fig. 17.3 Structure of pyridoxine

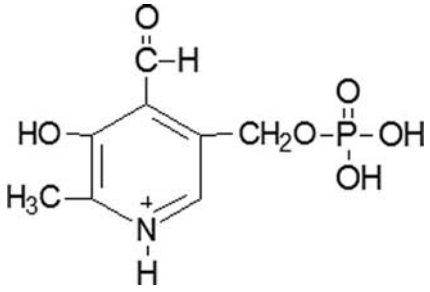

Fig. 17.4 Structure of pyridoxal

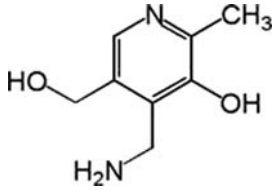

Fig. 17.5 Structure of pyridoxamine

The two B_6 vitamers that have been used therapeutically are pyridoxine (hydrochloride) and pyridoxal 5′-phosphate (PLP).

Involvement of vitamin B_6 in organs' functions is extended. Its deficiency can cause a broad spectrum of psychiatric symptoms. The mechanism of action is not yet clear enough, but many investigators came to the conclusion that vitamin B_6 can be helpful in conditions such as autism [49–52], Alzheimer's disease [53], hyperactivity and learning disability [54], anxiety disorder [55, 56], premenstrual syndrome [57], schizophrenia [58, 59], and tardive dyskinesia [60–63].

Symptoms of vitamin B_6 deficiency include weakness, mental confusion, irritability and nervousness, insomnia, poor coordination in walking, hyperactivity, convulsions, abnormal electroencephalogram, declining blood lymphocytes and white blood cells, anemia, and skin lesions.

Sources of vitamin B_6. Vitamin B_6 is mainly bound to protein in food. Pyridoxol is found especially in plants, whereas pyridoxal and pyridoxamine are principally found in animal tissues. Excellent sources of pyridoxine are chicken, turkey, beef liver, pork, calf and eggs. Good sources include ham and fish (tuna, trout, halibut, herring, and salmon), nuts (peanuts, walnuts), bread, corn and whole grain cereals. Generally, vegetables and fruits are rather poor sources of vitamin B_6,

Table 17.3 Food sources of vitamin B_6 [65]

Food	Milligrams (mg) per serving	% DV*
Ready-to-eat cereal, 100% fortified, 3/4 c	2.00	100
Potato, Baked, flesh and skin, 1 medium	0.70	35
Banana, raw, 1 medium	0.68	34
Garbanzo beans, canned, 1/2 c	0.57	30
Chicken breast, meat only, cooked, 1/2 breast	0.52	25
Ready-to-eat cereal, 25% fortified, 3/4 c	0.50	25
Oatmeal, instant, fortified, 1 packet	0.42	20
Pork loin, lean only, cooked, 3 oz	0.42	20
Roast beef, eye of round, lean only, cooked, 3 oz	0.32	15
Trout, rainbow, cooked, 3 oz	0.29	15
Sunflower seeds, kernels, dry roasted, 1 oz	0.23	10
Spinach, frozen, cooked, 1/2 c	0.14	8
Tomato juice, canned, 6 oz	0.20	10
Avocado, raw, sliced, 1/2 cup	0.20	10
Salmon, Sockeye, cooked, 3 oz	0.19	10
Tuna, canned in water, drained solids, 3 oz	0.18	10
Wheat bran, crude or unprocessed, 1/4 c	0.18	10
Peanut butter, smooth, 2 Tbs.	0.15	8
Walnuts, English/Persian, 1 oz	0.15	8
Soybeans, green, boiled, drained, 1/2 c	0.05	2
Lima beans, frozen, cooked, drained, 1/2 c	0.10	6

*DV = Daily Value. DVs are reference numbers based on the Recommended Dietary Allowance (RDA). They were developed to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for vitamin B_6 is 2.0 milligrams (mg). The percent DV (%DV) listed on the nutrition facts panel of food labels tells you what percentage of the DV is provided in one serving. Percent DVs are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs. Foods that provide lower percentages of the DV also contribute to a healthful diet

although there are products in these food classes which contain considerable amounts of pyridoxine, such as beans and cauliflower, bananas and raisins [64]. The basic sources of vitamin B_6 and each element's daily value are presented in Table 17.3 [65].

Vitamin B_6 is involved in more bodily functions than almost any other single nutrient. It affects both physical and mental health. Once digested, pyridoxine is converted rapidly to pyridoxal phosphate which acts as a co-enzyme in a whole host of biochemical reactions [66, 67]. Our interest in this chapter will be concentrated on vitamin B_6 in the nervous system in which it is required for normal brain function as a neuroprotective agent. Vitamin B_6 is involved in the synthesis of RNA and DNA, which contain the genetic instructions for the reproduction of all cells and for normal cellular growth.

Although vitamin B_6 is not synthesized in the brain, it readily enters the cerebral spinal fluid and brain from the plasma. Once within the CSF, B_6 can enter brain cells. The holoenzyme needed to convert cystathionine to cysteine contains B_6 in its pyridoxal phosphate form. It is relatively easy to deplete brain B_6. Any substance

that impedes or inhibits the metabolic functions of vitamin B_6 in humans could have some very serious consequences.

Vitamin B_6 serves as a coenzyme of many enzymes involved in the metabolism of amino acids. It plays an important role in protein, carbohydrate and lipid metabolism. It helps in the transformation of amino acids to form active biogenic amines such as histamine, serotonin, dopamine, adrenaline and the formation of nicotinic acid. Furthermore, it removes an acid group from glutamic acid (glutamate) to form gamma-aminobutyric acid (GABA). Subsequent studies have found that GABA acts on the presynaptic nerve terminals to inhibit the release of excitatory neurotransmitters and thereby having a calming effect [68].

Morani and Bodhank [69] evaluated the neuroprotective actions of pyridoxine hydrochloride 100 mg/kg for 30 days, in wistar rats with partial sciatic nerve ligation (PSNL) and sciatic nerve crush injury (SNCI) and thermal hyperalgesia (TH) models. The authors found that pyridoxine hydrochloride is effective against TH in PSNL and SNCI models of mononeuropathy in rats which opens a possibility of exploring the potential of pyridoxine hydrochloride in the treatment of heat or crush injuries. It is important to note that this latest study deals with the neuroprotective influence of pyridoxine on peripheral nervous system. Dakshinamurti and colleagues examined neuroprotective ability of vitamin B_6 in mice as addition to antiepileptic drugs. They found that pyridoxine has anti-seizure and neuroprotective actions mediated through mechanisms similar to those targeted therapeutic strategies [70].

Neuroprotective effect on Schizophrenia. On the beginning of the seventies of the XX century few publications raised in the psychiatric literature dealing with the use of pyridoxine's efficacy in schizophrenia [58, 59]. Since studies in those days were not methodically well built as controlled trials and there was no systematically use of standard rating scales, the results were described mostly as clinical impression and not as monitored scientific data. As many innovations in medicine, the first publications were of clinical case descriptions. For example the case of a 15 year old woman presenting with psychotic symptoms secondary to homocysteinuria who responded to a combination of folic acid and pyridoxine [71]. Petrie and colleges found that pyridoxine has greater therapeutic effects in chronic schizophrenic patients [72]. Another case of "An unusual schizophrenic illness responsive to pyridoxine HCl (B_6) subsequent to "henothiazines and butyrophenone toxicities" was described in 1983 by Sidney Brooks and coworkers [59]. These authors reported about 18-year old male with schizophrenic symptoms who was successfully treated only with 500 mg/day of pyridoxine after a lot of somatic and neurological symptoms induced by two different kinds of neuroleptic medications. Dose decreasing of vitamin B_6 led to deterioration of his mental symptoms while returning to previous dose improved the patient's state back to original balance condition. On the next step, Bucci in 1970 started an opened study in which he added 50 mg t.i.d. of pyridoxine to the treatment of 15 chronic schizophrenic patients without changing any other component of what they have received. The results were divided into first period after 6 weeks when the positive effect was subjective only, but on the second period after 8–10 weeks, the researcher had the impression of clinical improvement of affect, motivation and occupational ability [58].

The neuroprotective effect regarding any mental illness should be examined by its connection to detected variable. Scanning the literature of the connection between vitamin B_6 and schizophrenia reveals two kinds of reports and studies. One direction is examining the potential influence of the vitamin on basic positive and negative symptoms of the illness. The other direction is looking for a resolution of various side effects induced by psychotropic drugs used for treating these symptoms. We mean conventional neuroleptics, drugs that are in use for many years before the days of the new generation drugs. In this upcoming part of this chapter, we would like to summarize the scientific information about the neuroprotective effect of vitamin B_6 including our experience in this field.

As mentioned above, the problem with most previous studies and reports was absence of control groups and data were based on general impression, but not on standard scientific tools [58, 59, 63, 72, 73]. There are some anecdotal reports (including ours) regarding the reduction of psychotic symptoms after vitamin B_6 supplementation in patients suffering from schizophrenia or organic mental disorder [58, 63, 73–75], but only three studies were performed on comparatively large groups of patients. Unfortunately, these were not controlled studies, and no conclusive clinical trials have yet been undertaken [58, 74, 75]. In order to examine whether vitamin B_6 has neuroprotective activity we decided to check whether it influences psychotic symptoms in patients suffering from refractory schizophrenia and schizoaffective disorder, using a double blind, crossover, add-on design study [76].

Our sample contained the most severe chronic patients in our mental health center. Vitamin B_6 or placebo were supplemented to each patient's antipsychotic regular treatment on a double blind, crossover study spanning 9 weeks. All patients had stable psychopathology for at least 1 month before entry into the study and were maintained on treatment with their prestudy psychoactive medications throughout the study. All patients were assessed using the Positive and Negative Syndrome Scale (PANSS) for schizophrenia on a weekly basis. Patients randomly received placebo or vitamin B_6, starting at 100 mg/day in the first week and increasing to 400 mg/day in the fourth week by 100-mg increments each week. Five from 15 patients (30%) showed in various degrees of improvement. However, these results were not statistically considered as positive, because of only two of the five patients demonstrated significant clinical improvement. It must be bolded that the doses of pyridoxine were relatively low (maximum 400 mg/day), while the patients were severely chronic and treatment resistant. For these reasons, it can not be concluded that vitamin B_6 has no neuroprotective effect at all on schizophrenic symptoms. Further studies with larger populations and shorter duration of illness are needed to clarify the question of the possible efficacy of vitamin B_6 in treatment of psychotic symptoms in schizophrenia.

The possible assumption that higher doses of vitamin B_6 supplementation should be more effective concerning its influence and neuroprotection is supported by the followed two studies.

In these studies used high doses (1200 mg/day) of vitamin B_6 as a treatment of acute akathisia, we examined again its influence on positive symptoms of schizophrenia [77, 78]. These studies were double-blind randomized control.

The enrolled patients suffered from acute schizophrenic exacerbation and were not chronic or treatment resistant. The results clearly demonstrated a significant reduction of positive signs in BPRS compared to those patients who received placebo. The fact that the subjects were acutely schizophrenic patients may be an indication for vitamin B_6 activity as a neuroprotective agent.

Neuroprotective effect on cognitive function. Various vitamin deficiencies could influence memory function and might contribute to age-associated cognitive impairment and dementia. Results of experimental animal models studies suggest that serious cognitive deficit may occur also in vitamin B_6-deficient animals [79, 80]. These authors suggest that vitamin B_6 deficiency during gestation and lactation alters the function of N-methyl-D-aspartate receptors, which play an important role in learning and memory. Low levels of vitamin B_6 due to poor dietary intake are common in the elderly and have been related to cognitive decline and memory dysfunction. However, the underlying mechanism is not known. Studies have suggested the effect of vitamin B_6 on cognition may be mediated by vascular white matter lesions, given evidence that low vitamin B_6 levels may result in premature vascular disease, possibly due to high levels of homocysteine. Recently Mulder and colleagues found a linear relationship between white matter lesions and hippocampal atrophy in patients with probable Alzheimer's disease, suggesting that vascular pathology and typical Alzheimer's disease pathology are related [81, 82].

The neuroprotective effect of vitamin B_6 on cognitive performance is still not clear enough. Numerous studies have suggested that pregnant and lactating women may have dietary intake of vitamin B_6 that are well below the recommended dietary allowance, which may affect the vitamin B_6 status of their offspring [80].

A few recent studies have demonstrated an association between declines in cognitive function or Alzheimer's disease in the elderly and inadequate nutritional status of folic acid, vitamin B_{12}, and vitamin B_6 and thus, elevated levels of homocysteine [83]. One observational study found higher plasma vitamin B_6 levels to be associated with better performance on two measures of memory, but unrelated to performance on 18 other cognitive tests [84]. It is presently unclear whether marginal B vitamin deficiencies, which are relatively common in the elderly, contribute to age-associated declines in cognitive function or whether both result from processes associated with aging and/or disease. Malouf et al. mentioned in their review that there are no evidence found for short-term benefit from vitamin B_6 in improving mood (depression, fatigue and tension symptoms) or cognitive functions [82]. They concluded that more randomized controlled trials are needed to explore possible benefits from vitamin B_6 supplementation for healthy older people and those with cognitively impairment or dementia.

Neuroprotective effect on psychotropic-induced movement disorders. Abnormalities of serotonin (5-HT) metabolism have been associated with various neurological conditions including Parkinson's disease, tardive dyskinesia, akathisia, dystonia, Huntington's disease, familial tremor, restless leg syndrome, myoclonus, Gilles de la Tourette syndrome, multiple sclerosis, sleep disorders and dementia. The natural precursor of serotonin, L-tryptophan is hydroxylated by tryptophan hydroxylase to L-5-hydroxytryptophan (L-5HTP) and then it is decarboxylated to serotonin

by pyridoxal phosphate-dependent enzyme – vitamin B_6 and aromatic amino acid decarboxylases [85].

Although there are descriptions of many movement disorders, which can be connected to vitamin B_6 deficiency, we assume that it may have a neuroprotective action in movement disturbances induced by psychotropic drugs. This group of motor disorders includes acute akathisia, lithium-induced tremor and tardive dyskinesia (TD).

Vitamin B_6 in tardive dyskinesia. TD is a common adverse effect generally caused by chronic use of classis neuroleptics. The long-term delayed neurological side effects of these drugs are determined as tardive movement disorders. This term contains some variable types: classical TD, and other tardive extrapyramidal subsyndromes [86–94]. The prevalence of TD among subjects treated with classic neuroleptics over more than one year ranges from 3% to as high as 70% (depending on the diagnostic criteria and/or methodology) [90, 95, 96]. The annual incidence in younger adults is 4–5% [96, 97]. Many cases are persistent, often irreversible, and may result in social and physiological impairment.

Although second generation agents (SGAs) have a significant reduction potential for causing acute and tardive neuroleptic-induced extrapyramidal symptoms including TD [98–100], however, each of them may also induce tardive movement disturbances [101–110]. Furthermore, despite the extensive use of SGAs in the majority of western countries, most patients in countries of the third world and up to half in Europe are still treated with classic neuroleptics.

A large number of different medications have been studied in the treatment of TD patients, but its management remains a significant problem for patients and a therapeutic riddle for physicians [111].

Some researchers reported about motor disturbances which have been observed in vitamin B_6-deficient animals [112, 113]. Preliminary evidence in human been, demonstrate efficacy of vitamin B_6 in treatment of TD and other involuntary drug-induced movement disorders published as several case reports [60–63, 73, 114], open-label trials [115, 116], and randomized, double-blind, placebo controlled trials [78, 117–119].

We evaluated the efficacy of vitamin B_6 in treatment of TD in different doses from 400 to 1200 mg/day and both were found effective. Our clinical experience with this compound showed that the positive effect of vitamin B_6on TD connected to its dose and continued for a long period after stopping the high dose of the medication (1200 mg/day) [73, 115, 117]. The results of two double-blinded controlled studies are demonstrated in the following Figs. 17.6, 17.7, 17.8, 17.9, and 17.10.

Vitamin B_6 in acute akathisia. In the process of researching the efficacy of vitamin B_6 in treatment of patients with psychotropic-induced movement disorder, we performed two double-blind studies on 20 and 60 patients suffered from acute neuroleptic-induced akathisia (NIA) [77, 78]. In the first study, we compared vitamin B_6 with placebo, and in the second one with placebo and mianserin. The severity of akathisia was rated by the Barnes Akathisia Rating Scale (BARS) [120] on baseline and every day during the study. In both studies, the patients were treated with 1200 mg/day of vitamin B_6 as addition to their regular treatment.

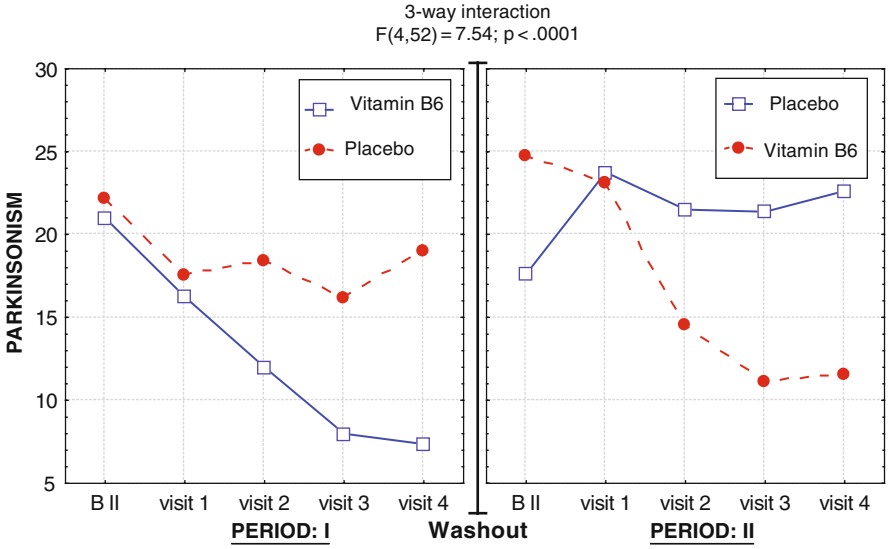

Fig.17.6 Parkinsonism Subscale Ratings of 15 Patients before and during Treatment with Vitamin B_6 and Placebo [119]

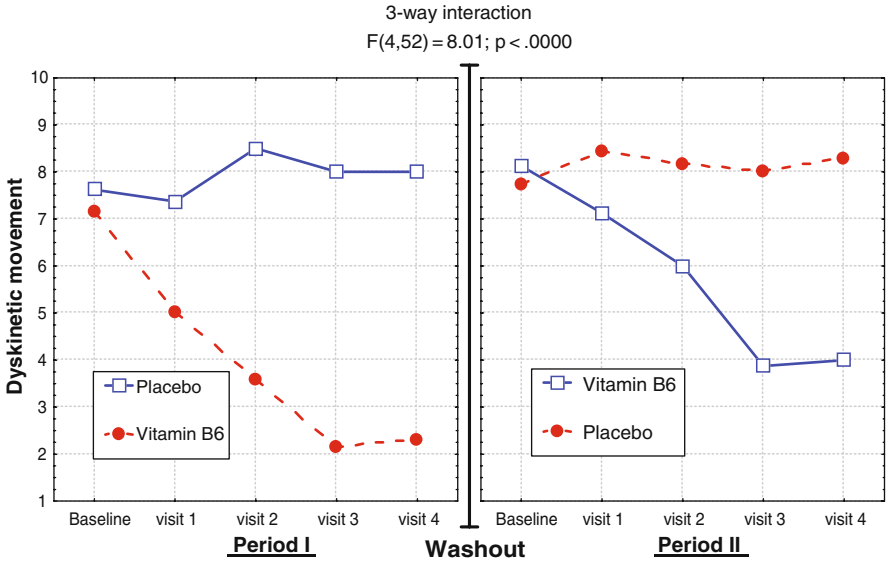

Fig. 17.7 Dyskinetic Movements Subscale Ratings of 15 Patients before and during Treatment with Vitamin B_6 and Placebo [119]

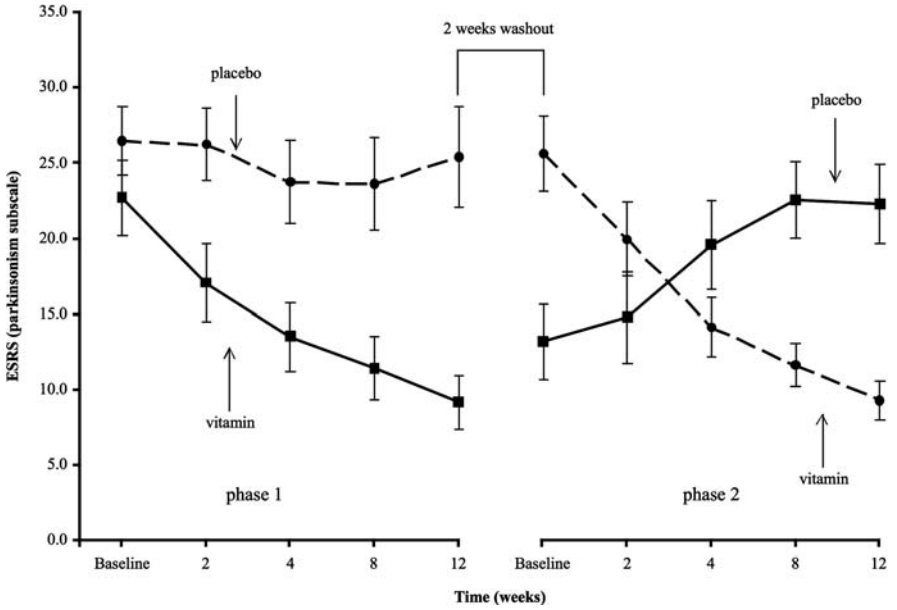

Fig. 17.8 Changes in ESRS Tardive Parkinsonism Subscale during the Crossover Design Treatment with Vitamin B_6 (1200 mg/day) (Mean±SEM; $N = 36$) [120]. Treatment-time interaction $F = 25.0$, $df = 3,102$, $p < 0.00001$; LSD post Hoc test for the 4th week, $p < 0.004$

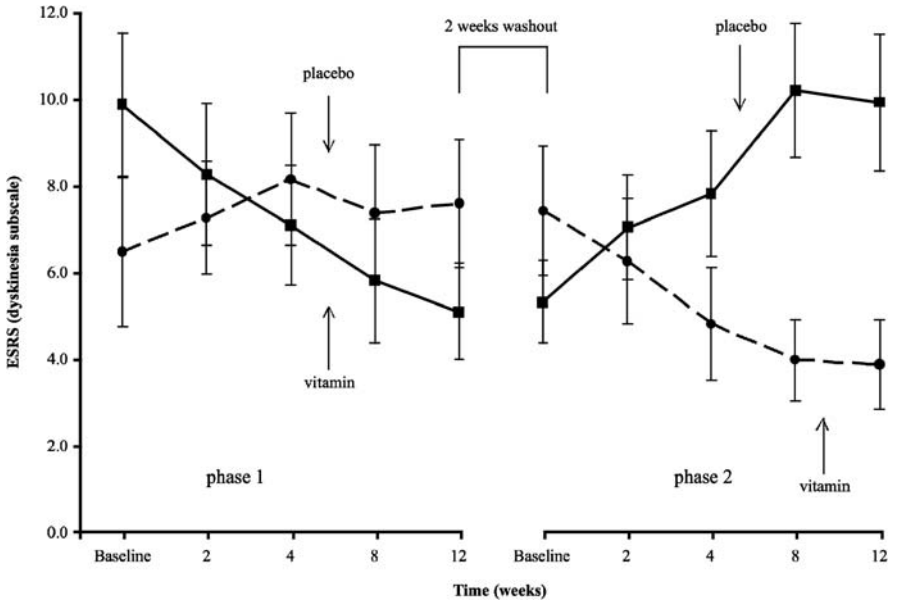

Fig. 17.9 Changes in ESRS Tardive Dyskinesia Subscale during the Crossover Design Treatment (Mean ± SEM; $N = 36$) [120]

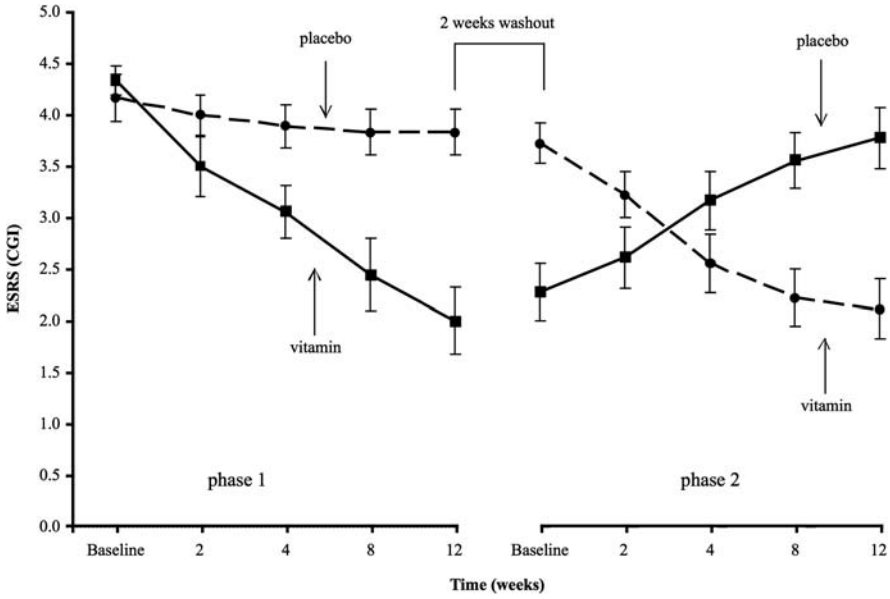

Fig. 17.10 CGI Subscale during the Crossover Design Treatment (Mean±SEM; $N = 36$) [120]. Treatment-time interaction $F = 26.5$, $df = 3,102$, $p < 0.00001$; LSD post Hoc test for 4th week, $p < 0.004$

All patients completed the trial. None of them suffered from any side effect. The authors found that vitamin B_6 produced a greater improvement in NIA than in patients from the placebo group and it was equal to mianserin [78]. The BARS subjective subscale (awareness of restlessness and distress related to restlessness) and global clinical assessment subscales, showed a significant two-way interaction of treatment and time. This beneficial effect of vitamin B_6 was come out from the third day of treatment. The same effect was obtained in the global subscale on the second day.

The efficacy of vitamin B_6 suggests that the pathophysiology of acute NIA is heterogeneous with various subtypes of acute NIA, which possibly respond to different pharmacologic approaches. The neuroprotective effect, which is demonstrated via these results suggest that vitamin B_6 also may be an option in the treatment of acute akathisia.

Vitamin B_6 in lithium-induced tremor. Tremor is one of the most bothering side effects of psychotropic medications. Tremor is a general term, which defines a regular continuous and rhythmical involuntary oscillation of a body part [121]. The neuroprotective action of vitamin B_6 on extrapyramidal symptoms in our previous studies encouraged us to investigate its possible effect on movement disorder induced by medications other than neuroleptics. Lithium-induced tremor (LT) is a very common adverse effect.

We performed open study on outpatients who were treated with lithium alone and suffering from LT. In addition to their medication regimen, they received oral vitamin B_6 (900–1200 mg per day). According to the Simpson-Angus Scale (SAS) scores four patients showed an impressive improvement until total disappearance of the tremor. Only one patient did not have any improvement. The subjective scale, in which the patients' scored their impression of clinical improvement, showed similar results. One patient reported total recovery, another three patients reported marked improvement and only one patient did not feel any change. None of the patients suffered from any side effects attributable to vitamin B_6. At the end of the study, three patients discontinued the vitamin B_6 treatment with recurrence of the tremor to previous levels. In these patients, we recommenced treatment with vitamin B_6 and the same beneficial effect was achieved. A follow-up of ten months did not reveal any side effects and the therapeutic effect was maintained [116].

The mechanism of tremor caused by lithium is still unclear and so is the mechanism of its amelioration by vitamin B_6 treatment. Anyway, there is a trial of explanation by some authors. Kane et al. [122] reported that 38 patients treated only with lithium had developed parkinsonian symptoms. Tyrer et al. explained the appearance of extrapyramidal side effects after treatment with lithium by selective blockade of dopamine receptors [123, 124]. These reports can be a clue suggesting the connection between lithium or neuroleptic induced tremor and vitamin B_6. Our preliminary results evaluating the use of vitamin B_6 in lithium-induced tremor suggest that this vitamin probably has neuroprotective activity which causes it to be an effective therapy, and repair the damage produced by lithium.

17.3.3 Folic Acid (Vitamin B_9)

History and structure. Folic acid, or folate is a water-soluble B vitamin that occurs naturally in food. Folic acid is the synthetic form of folate that is found in supplements and added to fortified foods [5, 41].

Folate helps produce and maintain new cells [125]. This is especially important during periods of rapid building of body organs or tissues, a process, which needs cell division and growth such as infancy and pregnancy. Folate is needed the synthesis of DNA and RNA, the basic building units of cells. It also helps in preventing changes of DNA that may lead to cancer [126]. All ages of mammalian need folate for production of normal red blood cells and prevent anemia.

Folic acid influences the rate of synthesis of the neurotransmitters dopamine, norepinephrine, and serotonin, and acts as a cofactor in the hydroxylation of phenylalanine and tryptophan [127–129]. Disturbance of biogenic amine metabolism may lead to various psychiatric disorders. Folate is also essential for the metabolism of homocysteine, and helps maintain normal levels of this amino acid [130, 131].

Folate gets its name from the Latin word "folium" for leaf. In 1931, an English researcher Dr. Lucy Wills (1888–1964) found folic acid as a needed nutrient to prevent anemia during pregnancy. She demonstrated that anemia could be reversed

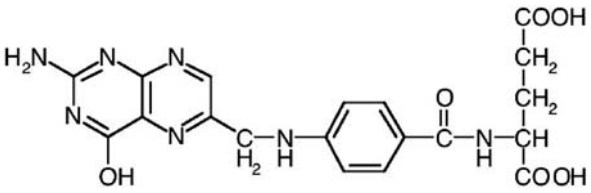

Fig. 17.11 The structure of folic acid

with brewer's yeast. Folate was identified as the corrective substance in brewer's yeast in the late 1930s. Mitchell and others in 1941 first isolated it in spinach leaves and was shown to be a growth factor for Streptococcus lactis [2, 132, 133]. E.L. Robert Stokstad (1913–1995) isolated the pure crystalline form of folate in 1943, and was able to determine its chemical structure [132]. It led to the subsequent synthesis of folic acid in 1945, by Dr. Yellapragada Subbarao (1895–1948) and many others (Fig. 17.11) [132].

Sources of folic acid. Leafy vegetables such as spinach, asparagus, turnip greens, lettuces, dried/fresh beans and peas, fortified cereal products, sunflower seeds and certain other fruits and vegetables are rich sources of folate. Liver and liver products also contain high amounts of folate, as does baker's yeast. Some breakfast cereals (ready-to-eat and others) are fortified with 25–100% of the recommended dietary allowance (RDA) for folic acid. A table of selected food sources of folate and folic acid can be found at the USDA National Nutrient Database for Standard Reference [65]. Folic acid is added to grain products in many countries, and in these countries fortified products make up a significant source of folate [134]. Since the difference in bioavailability between supplemented folic acid and the different forms of folate found in food, the dietary folate equivalent (DFE) system was established. 1 DFE is defined as 1 μg of dietary folate, or 0.6 μg of folic acid supplement. This is reduced to 0.5 μg of folic acid if the supplement is taken on an empty stomach (Table 17.4) [135].

Neuroprotective effect. Some evidence links a shortage of folate with depression [136]. There is some limited evidence that using folic acid in addition to antidepressants, specifically SSRIs, may have benefits [137]. A link between depression and low levels of folate was found in two meta-analyses [138, 139]. One of them was based on 11 relevant studies and included 15,315 subjects [139]. Dr. Reynolds has produced a comprehensive review of the association between folic acid and neuropsychiatric disorders such as depression and dementia. He showed that a folate deficiency is quite common, especially in older people. Severe folate deficiency is associated with megaloblastic anemia and it is estimated that some two thirds of patients with megaloblastic anemia also have a neuropsychiatric disorder. In elderly people a close association has been noted between a folate deficiency and apathy, depression, dementia, withdrawal, and a lack of motivation. In a study of 164 Alzheimer's patients, cognitive decline was significantly associated with raised plasma homocysteine levels and lowered folic acid and vitamin B_{12} levels. Significant improvements were noted in 24 folate-deficient, depressed persons who

Table 17.4 High folic acid foods [65]

Food	Folic acid μg/Serving Dietary Folate Equivalents*(DFE)
Ready-to-eat breakfast cereal	100–400/serving; read labels
Enriched wheat tortilla	98/one 8″ tortilla
Whole wheat tortilla	24/one 8″ tortilla
Enriched white bread	39/slice
Enriched pasta, cooked	92/half cup
Whole wheat bread	14/slice
Whole wheat pasta	23/half cup
Lentils, cooked	180/cup
Black-eyed peas, dried, cooked	105/half cup
Pinto beans or chickpeas, cooked	140–145/half cup
Sunflower seeds, dry-roasted	152/half cup
Okra, cooked	37/half cup
Orange juice	60–100/cup
Spinach, raw	58/cup
Asparagus	110/5 spears
Collards, frozen	88/half cup
Grapefruit/pineapple juice	23/cup

*Dietary Folate Equivalents account for the folic acid content, which is better absorbed than naturally occurring folate

were given 15 mg/day of folic acid for a four-month period. Other studies have shown that supplementation with as little as 0.5 mg/day of folic acid increases the effectiveness of fluoxetine. Dr. Reynolds pointed out that folic acid could excite the nervous system. For this reason, it should be used with caution in patients suffering from epilepsy. Another cautious should be taken in account when vitamin B_{12} deficiency is suspected (because there is a masking effect) [140].

Neuroprotective effect on cognitive function. Especially among the elderly population, memory and mental agility is a cardinal issue and many researches waste a lot of time in trying to find a neuroprotective way to overcome this problem. Low folate and raised homocysteine concentrations in blood are associated with poor cognitive performance. The study performed on 818 participants who received 800 mg daily oral folic acid or placebo for 3 years showed that folic acid plays an important role in prevention and treatment of disturbances short-term memory, and verbal fluency [141].

Although a few studies suggest that folic acid supplementation may provide neuroprotection among persons who are folate deficient, there is also data, which indicate that supplementation in persons without folate deficiency may pose a risk to neurological function. Folic acid supplementation has drawn much attention in recent years for the prevention of Alzheimer's disease and cognitive decline. A study performed by Tettamanti and colleagues suggest that subclinical folate deficiency may represent a risk factor for the cognitive disturbances associated with aging that could contribute to Alzheimer's disease as well as other dementia development

[142]. However, there is another opinion that the beneficial effect of folic acid on Alzheimer's disease is not yet clear-cut [143].

Folic acid protects motor neurons against increased level of homocysteine [144]. These findings also suggest that relative folate deficiency may precede Alzheimer's disease and vascular dementia onset. Hyperhomocysteinemia might also be an early risk factor for cognitive decline in the elderly, but its role in dementia development must be addressed in future longitudinal studies [145]. Further studies are needed to determine the possible risks and benefits of folic acid supplementation in older persons.

17.3.4 Vitamin B_{12} (Cyanocobalamin)

History and structure. Vitamin B_{12} is the name for a class of chemically-related compounds. It is a water-soluble vitamin, which plays an essential role in the normal functioning of the brain and nervous system, and in hemopoesis. It is normally involved in the metabolism of every cell of the body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production and has a neuroprotective activity.

Vitamin B_{12} deficiency resulting in various neuropsychiatric and thrombotic manifestations, such as neuropathy, myelopathy, myeloneuropathy, dementia, cerebellar ataxia, optic atrophy, psychosis and mood disturbances, portal vein thrombosis, myocardial infarction are well known [146–148].

Vitamin B_{12} has the most complex structure of all vitamins ($C_{63}H_{90}CoN_{14}O_{14}P$). This is a unique vitamin since it is the only one, which contains a metal ion. It is structurally similar to the position of iron in hemoglobin. Cobalamin is the generic name of the vitamin because of the presence of cobalt in its structure. Biosynthesis of the basic structure of the vitamin can only be accomplished by bacteria, but conversion between different forms of the vitamin can be accomplished in the human body. A common synthetic form of the vitamin, cyanocobalamin, does not occur in nature, but is used in many pharmaceuticals, and as food supplements, due to its stability and lower cost. In the body, vitamin B_{12} is converted to the physiological forms, methylcobalamin and adenosylcobalamin, leaving behind the cyanide, albeit in minimal concentration [5, 41].

Historically, vitamin B_{12} was discovered from its relationship to the disease pernicious anemia, which was first described in 1821, and was invariably fatal. In 1926, two American researchers George R. Minot (1885–1950) and William P. Murphy (1892–1936) could cure anemic dogs by feeding them raw liver. They discovered that pernicious anemia could also be treated by supplementing human diet with liver. In 1934, they were awarded the Nobel Prize for Physiology or Medicine. Edward L. Rickes and associates (USA) and Lester E. Smith with Parker LFJ (England), working separately, in 1948 isolated a crystalline red pigment, which they name vitamin B_{12}. In 1955, Britain chemist Dorothy Hodgkin (1910–1994) and coworkers established the molecular structure of cyanocobalamin and its coenzyme forms. This discovery was made by using X-ray crystallography. All that was known at this

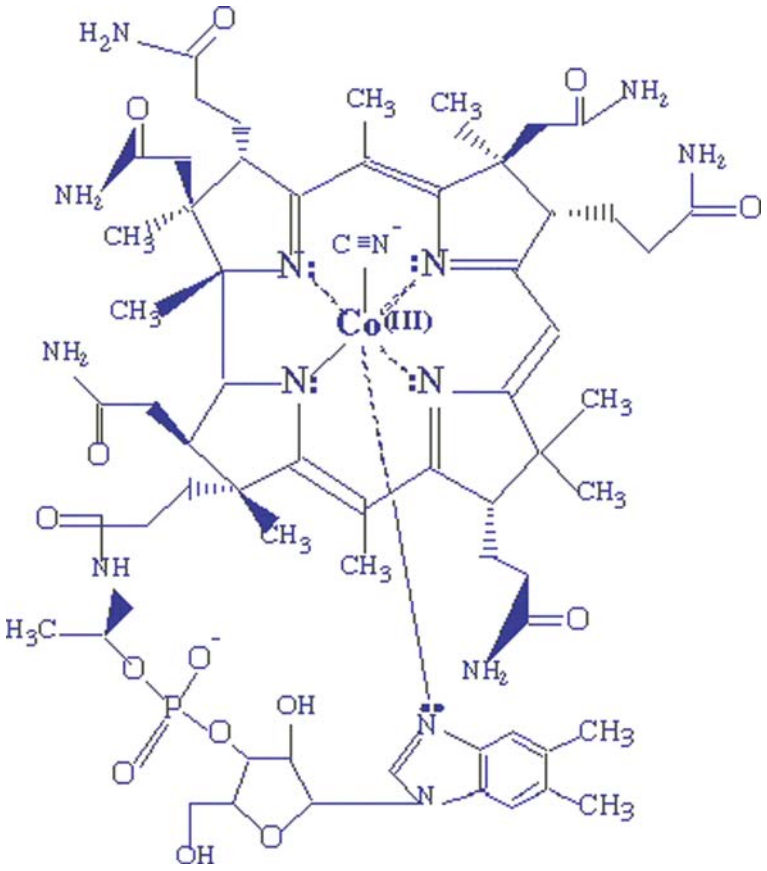

Fig. 17.12 The structure of vitamin B_{12}

stage was that the approximate empirical formula was $C_{61–64}H_{84–90}N_{14}O_{13–14}Pco$ (Fig. 17.12) [41, 149].

The human physiology of vitamin B_{12} is complex, and therefore is prone to mishaps leading to vitamin B_{12} deficiency. Unlike most nutrients, absorption of vitamin B_{12} actually begins in the mouth where small amounts of unbound crystalline B_{12} can be absorbed through the mucosa membrane [149]. Intrinsic factor is crucial for the normal absorption of vitamin B_{12}, therefore, a lack of intrinsic factor, as seen in pernicious anemia, causes a vitamin B_{12} deficiency.

The total amount of vitamin B_{12} stored in body is about 2,000–5,000 mcg in adults. Around 50% of this is stored in the liver [149]. How fast vitamin B_{12} levels change depends on the balance between how much vitamin B_{12} is obtained from the diet, how much is secreted and how much is absorbed.

Sources of vitamin B_{12}. Vitamin B_{12} is naturally found in meat (especially liver and shellfish), milk and eggs. Animals, in turn, must obtain it directly or indirectly from bacteria, and these bacteria may inhabit a section of the gut, which is posterior

Table 17.5 Selected food sources of vitamin B_{12} [65]

Food	Micrograms (μg) per serving	Percent DV*
Mollusks, clam, mixed species, cooked, 3 ounces	84.1	1,400
Liver, beef, braised, 1 slice	47.9	780
Fortified breakfast cereals, (100%) fortified), $^3/_4$ cup	6.0	100
Trout, rainbow, wild, cooked, 3 ounces	5.4	90
Salmon, sockeye, cooked, 3 ounces	4.9	80
Trout, rainbow, farmed, cooked, 3 ounces	4.2	50
Beef, top sirloin, lean, choice, broiled, 3 ounces	2.4	40
Fast Food, Cheeseburger, regular, double patty & bun, 1 sandwich	1.9	30
Fast Food, Taco, 1 large	1.6	25
Fortified breakfast cereals (25% fortified), $^3/_4$ cup	1.5	25
Yogurt, plain, skim, with 13 grams protein per cup, 1 cup	1.4	25
Haddock, cooked, 3 ounces	1.2	20
Clams, breaded & fried, $^3/_4$ cup	1.1	20
Tuna, white, canned in water, drained solids, 3 ounces	1.0	15
Milk, 1 cup	0.9	15
Pork, cured, ham, lean only, canned, roasted, 3 ounces	0.6	10
Egg, whole, hard boiled, 1	0.6	10
American pasteurized cheese food, 1 ounces	0.3	6
Chicken, breast, meat only, roasted, $^1/_2$ breast	0.3	6

*DV = Daily Value. DVs are reference numbers developed by the Food and Drug Administration (FDA) to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for vitamin B_{12} is 6.0 micrograms (μg). Most food labels do not list a food's vitamin B_{12} content. The percent DV (% DV) listed on the table indicates the percentage of the DV provided in one serving. A food providing 5% of the DV or less is a low source while a food that provides 10% to 19% of the DV is a good source. A food that provides 20% or more of the DV is high in that nutrient. It is important to remember that foods that provide lower percentages of the DV also contribute to a healthful diet. For foods not listed in this table, please refer to the US Department of Agriculture's Nutrient Database Web site: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl

to the section where vitamin B_{12} is absorbed. Thus, herbivorous animals must either obtain vitamin B_{12} from bacteria in their rumens, or (if fermenting plant material in the hindgut) by reingestion of cecotrope fæces. Eggs are often mentioned as a good vitamin B_{12} source, but they also contain a factor that blocks absorption [150]. An NIH Fact Sheet lists a variety of food sources of vitamin B_{12} (Table 17.5) [65].

An important point to be paid attention is vitamin B_{12} in vegetarians and naturists. Since the ultimate source of this essential vitamin is from animal origin, all these persons must take supplementation of vitamin B_{12}.

Neuroprotective effect. A neuroprotective effect of a vitamin B_{12} was found in animal experiment by Akaike and coworkers. They discovered that chronic exposure to methylcobalamin protects cortical neurons against NMDA receptor-mediated glutamate cytotoxicity [151].

The rational behind the idea of researching the neuroprotective efficacy of vitamin B_{12} is that its deficiency can cause an impairment of brain and nerve tissue function. The insulation around nerve cells, the myelin sheath, is misformed

and contributing to faulty nerve transmission [152]. The process of demyelinization may produce peripheral neuropathy manifested by lack of coordination, pain, numbness, and tingling in hands or feet, sensory loss and weakness. This kind of disturbances should be treated immediately with vitamin B_{12} in order to catch the reversible period of time.

Deficiency of vitamin B_{12} is known to be a pathogenesis for hematological and neural disturbances. It is less known that deficiency of this vitamin may be connected to specific mental illnesses. In a screening study performed by us, we found that about 30 percent of schizophrenic, schizoaffective and organic disorder patients had levels of vitamin B_{12} below normal, as did 17% of those with bipolar disorders. The distribution of diagnoses among those patients with low levels of cobalamin varied, with 50.8% diagnosed as having schizophrenia, almost 17% with schizoaffective and bipolar disorders, and 10% organic disorders [153]. The meaning of these data is that absence of neuroprotective umbrella of vitamin B_{12}, may be connected to the clinical picture of psychotic conditions.

In cases of dementia, some researchers found subclinical or mild deficiency in either B_{12} or folate. Moreover, they showed that replacement of these vitamins can derive a cognitive benefit [154]. Neurological disturbances accompanied by mental disorders are result from prolonged vitamin B_{12} deficiency.

Stuerenburg and coworkers investigated the correlation between plasma vitamin B_{12} levels and cognitive impairment in Alzheimer's disease. The authors conclude that vitamin B_{12} deficiency could aggravate or accelerate the course of Alzheimer disease. They assume that vitamin B_{12} possesses neuroprotective and anti-inflammatory properties [148].

Kim and colleagues found that high level of vitamin B_{12} seems to have neuroprotective effect against degenerative process, irrespective of homocysteine level, rather than its deficiency producing neural damage, because measured vitamin B_{12} levels in the patients were still within normal range [155].

It seems that a certain subset of patients with a subclinical deficiency in these nutrients derive particular benefit. Nilsson and colleagues in Sweden recently found that patients with elevated blood homocysteine levels showed a greater improvement in cognitive function on B_{12} and folate supplements than did patients with normal homocysteine levels [156].

17.4 Vitamin C (Ascorbic Acid)

History and structure. Vitamin C is a water-soluble, easily destroyed nutrient and human vitamin essential for life and for maintaining optimal health, used by the body for many purposes. It is also known by the chemical name of its principal form, L-ascorbic acid. Ascorbic acid can be broken down by ascorbic acid oxidase an enzyme which catalyses the oxidation of ascorbic acid.

Almost all animals and plants except humans, apes, guinea pigs, the red-vented bulbul, a fruit-eating bat and species of trout, synthesize their own vitamin C.

Vitamin C has many functions: it can function as a coenzyme or as a cofactor in the body. Vitamin C, as the cofactor for tryptophan-5-hydroxylase, catalyzes the hydroxylation of tryptophan to serotonin [157].

In the seventeenth century a ships surgeon to the East India Company, one Richard Woodall recommended the use of lemon juice as a preventive and cure a disease which was known from ancient time called "scurvy" in his book "Surgeon's Mate".

The first attempt to give scientific basis for the cause of scurvy was by a ships surgeon in the British Royal Navy, James Lind (1716–1794). His test was conducted at sea in May 1747 and consisted of two groups of men. One group was provided with lemon juice in addition to their regular diet, while the other group did not receive it. This test was considered to be a first example of a controlled experiment comparing results on two populations of a factor applied to one group only with all other factors the same.

The first isolation of vitamin C occurred in 1928 from various foods. In 1928, biochemist Albert Szent-Gyorgyi (1893–1986) was the first to isolate vitamin C (ascorbic acid) in Hungarian paprika. He won the 1937 Nobel Prize in Physiology and Medicine for his biological combustion discoveries.

Vitamin C is also known as, L-ascorbic acid, dehydroascorbic acid, the anti-scorbutic vitamin, L-xyloascorbic acid and L-threo-hex-2-uronic acidy-lactone. Ascorbic means "anti-scurvy." Both conventional and complementary medicine use ascorbic acid as an agent full with therapeutic properties from cancer to the common cold. Vitamin C is essential in wound healing and in the formation of collagen, a protein important in the formation of healthy skin, tendons, bones, and supportive tissues. During the 1940s and 1950s, Dr. Frederick Robert Klenner (1907–1984) firstly used large doses of vitamin C for the treatment of many viral diseases, including poliomyelitis (Fig. 17.13) [158–160].

Sources of vitamin C. Vitamin C is present in many fruits, vegetables and animal organs such as liver, kidney, and brain. Among excellent sources of vitamin C we can find oranges, green peppers, watermelon, papaya, grapefruit, cantaloupe, strawberries, kiwi and many others. Vitamin C is sensitive to light, air, and heat, therefore it is preferably to eat fruits and vegetables raw or lightly cooked [5, 162].

The recommended daily allowance (RDA) for adult men is 90 mg and for adult women it is 75 mg. According to the USDA's Dietary Guidelines in Table 17.6 cited data regarding some of vitamin C natural food sources [65].

Neuroprotective effect. The brain contains the highest level of ascorbate in the body and there are active uptake mechanisms in the choroid plexus and cell membrane to maintain intracellular levels at 16–25 times higher than blood

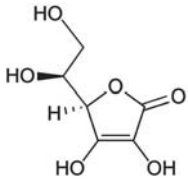

Fig. 17.13 Chemical structure of vitamin C

Table 17.6 Food Sources of vitamin C ranked by milligrams of vitamin C per standard amount; also calories in the standard amount [65]

Food, standard amount	Vitamin C (mg)	Calories
Guava, raw, ½ cup	188	56
Red sweet pepper, raw, ½cup	142	20
Red sweet pepper, cooked, ½ cup	116	19
Kiwi fruit, 1 medium	70	46
Orange, raw, 1 medium	70	62
Orange juice, ¾ cup	61–93	79–84
Green pepper, sweet, raw, ½ cup	60	15
Green pepper, sweet, cooked, ½ cup	51	19
Grapefruit juice, ¾ cup	50–70	71–86
Vegetable juice cocktail, ¾ cup	50	34
Strawberries, raw, ½ cup	49	27
Brussels sprouts, cooked, ½ cup	48	28
Cantaloupe, ¼ medium	47	51
Papaya, raw, ¼ medium	47	30
Kohlrabi, cooked, ½ cup	45	24
Broccoli, raw, ½ cup	39	15
Edible pod peas, cooked, ½ cup	38	34
Broccoli, cooked, ½ cup	37	26
Sweet potato, canned, ½ cup	34	116
Tomato juice, ¾ cup	33	31
Cauliflower, cooked, ½ cup	28	17
Pineapple, raw, ½ cup	28	37
Kale, cooked, ½ cup	27	18
Mango, ½ cup	23	54

levels. Levels of extracellular brain ascorbate vary greatly according to our activity, being lowest during sleep and highest with prolong activity and stress. These actual data direct the researches to the hypothesis that brain needs vitamin C as natural neuroprotective agent.

Since the main damage to living tissue is produced by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g. smoking), vitamin C has an important role, as it is a highly effective antioxidant. Even in small amounts, it can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage. Vitamin C may also be able to regenerate other antioxidants such as vitamin E [161].

Neuroprotective effect on stroke. In modern western life, cerebrovascular accidents take an important place in the list of causes of death among adults. Japanese researchers found that the risk of stroke in persons with the highest serum levels of vitamin C was 29% lower than in those with the lowest serum levels of vitamin C [163]. Additionally, the risk of stroke was significantly lower among people who

consumed vegetables 6–7 days of the week in contrast to those who consumed vegetables 0–2 days of the week. In this population, serum levels of vitamin C were highly correlated with fruit and vegetable intake. A recent 10-year prospective study in 20,649 adults found that people with high level of plasma vitamin C concentrations experienced a 42% lower risk of stroke compared to those in the lowest quartile [164].

Animal experiments examined the neuroprotective effects of vitamin C in adult rats after pilocarpine-induced seizures demonstrated that vitamin C is an exogenous antioxidant that can be used in treatment of seizures. The authors suggest that neuroprotective effects of vitamin C in adult rats can be the result of reduced lipid peroxidation levels and increase of catalase activity after seizures and status epilepticus induced by pilocarpine [165].

Neuroprotective effect on schizophrenia. In the first report by Horwitt in 1942 that schizophrenic patients receiving the usual dietary amounts of ascorbic acid had lower concentrations of ascorbic acid in the blood than people in good health [166].

Results of an animal study suggest that vitamin C may block the behavioral response to dopamine and enhance the effects of neuroleptic drugs [167]. Singh and colleagues found that there is an inverse correlation between ascorbic acid intake and the risk of schizophrenia [168]. Even when the dietary vitamin C intake is adequate for non-schizophrenic patients, schizophrenic patients may have depressed plasma levels and may demonstrate a greatly reduced urinary excretion of ascorbic acid after an ascorbic acid load, suggesting that the utilization of vitamin C in schizophrenic patients may be enhanced [169, 170]. The same group of researchers, in evaluation schizophrenic patients on the same hospital diet as a control group, found that the schizophrenic patients had significantly lower levels of fasting plasma vitamin C and 6-hour urinary vitamin C excretion after an ascorbic acid load test. After giving 70 mg of vitamin C daily for 4 weeks, there were no differences in plasma vitamin C levels between the schizophrenic and control subjects, but urinary vitamin C excretion after the vitamin C load test remained significantly lower in schizophrenic patients. This study supports the hypothesis that schizophrenic patients require higher levels of vitamin C than healthy control subjects for optimum vitamin C status [170].

One of the first significant controlled double-blind trials of ascorbic acid in chronic psychiatric patients was reported in 1963 by Milner. He concluded that "statistically significant improvement in the depressive, manic, and paranoid symptoms-complexes, together with an improvement in overall personality functioning, was obtained following saturation with ascorbic acid" [171].

A beneficial effect of vitamin C as addition to antipsychotic treatment in schizophrenic patients was described in a few case reports [172–174]. In recent study performed on 48 schizophrenic patients and 40 healthy subjects by Dakhale and coworkers it was found that supplementation of vitamin C to atypical antipsychotics, improves the outcome of schizophrenia [175].

Another group of researchers reported that supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia [176].

Neuroprotective effect on Alzheimer's disease. Many researchers assume that Alzheimer's disease (AD) is a result of destructive influence of free radicals oxidative process. Since vitamin C has a strong antioxidative activity, its neuroprotective abilities have received much attention. Some researchers found that vitamin C can be helpful in AD [53]. Riviere and colleagues found that plasma vitamin C is lower in AD in proportion to the degree of cognitive impairment and is not explained by lower vitamin C intake. These results support the hypothesis that oxygen-free radicals may cause damage [177].

Most studies were performed by using a combination of vitamin C with other vitamins. Gray et al. [178] and Zandi et al. [179] reported that treating with a combination of vitamin E and vitamin C was associated with reduced prevalence and incidence of AD. Further studies should be taken in order to detect whether antioxidant supplements may be effective as agents for primary prevention of AD.

In another study that lasted for 10 years took part 5395 non-hospitalized participants. At baseline, they were aged at least 55 years, and without symptoms of dementia. The researchers have assessed the subjects' diet. Participants were reexamined twice after five and ten years. Researchers found that incidence of AD is associated with dietary intake of beta-carotene, flavonoids, vitamin C, and vitamin E. They concluded that high dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer's disease [180].

Neuroprotective effect on affective disorders. Vitamin C may be valuable for patients with depression associated with low levels of serotonin. In one study, 40 chronic psychiatric inpatients received 1 g/day of ascorbic acid or placebo for three weeks, in double-blind fashion. In the vitamin C group, significant improvements were seen in depressive, manic and paranoid symptom complexes, as well as in overall functioning [171]. According to other study, vitamin C supplementation may be therefore helpful for depression as it may decrease levels of the trace mineral vanadium in the body. Raised levels of vanadium have been found in the blood plasma and hair of individuals with depression [181].

Supplementation with vitamin C associated not only with mood elevation as well as increases intercourse frequency. In one randomized double-blind study was found that vitamin C supplementation decreases stress reactivity, approach anxiety and prolactin release, improves vascular function, and increases oxytocin release [182].

Neuroprotective effect on other psychiatric disorders. Conditions such as autism [49–52, 183], attention deficit hyperactivity disorder (ADHD) [184], Down's syndrome [185] and anxiety disorder are some of those which have been reported to be treated by vitamin C supplementation.

There are few intervention studies with vitamin C. According to our knowledge, there are only two studies in which it was specifically evaluated as an intervention in autism. The first study reports that vitamin C in dosages range from 1 to 3 grams per day was beneficial in patients with autism [186]. In the second, 30-week's, double-blind, placebo-controlled trial, scientists have studied the effectiveness of ascorbic acid as a supplemental pharmacological treatment for autistic children in residential treatment. In this study the dose range was significantly higher – 8 g/70 kg/day. The investigators reported that the ascorbic acid treatment led to

a significant reduction in symptoms severity in total scores and also sensory motor scores [183].

Vitamin C and other antioxidants and nutrition may be relevant in Down's syndrome. Although unfortunately, there is no study in which the efficacy of vitamin C solitarily was examined, there are clues for it. Individuals with Down's syndrome have signs of possible brain damage prior to birth. Some of the cognitive impairments are resemble to post-natal hydrogen peroxide-mediated oxidative stress.

There are some anecdotal reports regarding improvement of the outcome of ADHD after vitamin C supplementation [184]. Although the results of this study are encouraging in improvement of few components of the disorder such as impulsivity, restlessness, inattention and self-control, it is still not convenience enough. It should be mentioned that this study has some limitations. First of all it is an open study, and the second the researchers used two substances, and we can not be sure whether the improvement was due to each of them.

There is a study in which the authors tried to measure possible brain and central-nervous-system stimulants and sedative effect of multivitamins. One of the components was vitamin C (3 g). Their conclusion was that it has potent sedative and antianxiety properties in large doses. This conclusion should be taken cautiously, because this study was performed with a polyvitamin formula, especially as other vitamins were in high doses [187].

17.5 Vitamin D

History and structure. The term "vitamin D" refers to a group of several chemically related compounds that has antirachitic activity. Two forms from this family are important in humans: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_2 is synthesized by plants. Vitamin D_3 is synthesized by humans in the skin when it is exposed to ultraviolet-B (UVB) rays from sunlight. The major biologic function of vitamin D is to maintain normal blood levels of calcium and phosphorus. Vitamin D aids in the absorption of calcium, helping to form and maintain strong bones [5, 188]. Vitamin D may provide protection from osteoporosis.

Recently, it was found that hypertension (high blood pressure), cancer, several autoimmune diseases are also influenced by Vitamin D. Concerning our subject of the neuroprotective effect, it has an ability in cognitive impairment, depression and schizophrenia [146, 188–194].

In one of the oldest surviving Egyptian medical texts Ebers Papyrus, there is recommendations of exposure to the sun [195], and some of the most famous figures in Greek, Roman and Islamic medicine used sunlight to prevent and cure different diseases. For example, an ancient Greek physician Hippocrates (460–377 BC) recognized the importance of sunlight for human health. He believed that the south face of a hill, which receives the most part of day sunlight in the northern

hemisphere, was the healthiest place to live. Despite the fact that the relationship between disturbances caused by lack of sunlight exposure, rickets continuing to be a widespread illness. Only thousands years later, the first scientific description of a vitamin D-deficiency, namely rickets, was provided in the 17th century by two British physicians Dr. Daniel Whistler (1618/19–1684) in 1645, and Professor Francis Glisson (1599?–1677) in 1650 [188]. The French physician Cauvain in 1815 had recommended sunlight as a treatment for rickets [196]. Almost in that time, Polish physician, professor of chemistry at the University of Vilnius, Jedrzei Sniadecki (1763–1838) wrote in 1822, that children living in rural areas did not develop rickets ("English Disease" as it had become known on the Continent), in contrast to children living in the city, who had high incidence of the disease [197]. He hypothesized that increased exposure to sunlight in country children prevented them from developing rickets. Understanding the mechanism of vitamin D activation, especially by influence of sunlight, was developed step by step as an interesting process like many other scientific discoveries. In 1937, investigators were able to isolate and identify a form of cholesterol as the precursor of vitamin D_3. They isolated 7-dehydrocholesterol (7-DHC) from animal skin and induced formation of vitamin D_3 by irradiating 7-DHC with ultraviolet radiation [198, 199].

The chemical structures of the vitamins D were determined in the 1930s by German chemist Professor Adolf Windaus (1876–1959).

Molecular formula of vitamin D is C28H44O. Chemical name is 3-[2-[1-(5, 6-dimethylhept-3-en-2-yl)-7a-methyl-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene] ethylidene]-4-methylidene-cyclohexan-1-ol [188].

Although sunlight play essential role in activation of provitamin D it should be remembered that exposure to the sun must be carefully as it has potential hazards (Fig. 17.14).

Sources of vitamin D. Vitamin D is found in a small number of foods. The richest sources are oily fish, particularly those of the halibut and the cod, and eggs. Other sources include fortified foods such as margarine, butter, cheese cream, yogurt, milk and sunlight. It is soluble in fats, milk, butter and eggs.

Most people should be able to get all the vitamin D they need from their diet and by getting a little sun. However, pregnant or breastfeeding women should take 10 micrograms (0.01 mg) of vitamin D each day. Older people should also consider taking 10 micrograms (0.01 mg) of vitamin D each day.

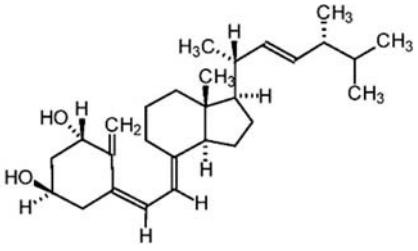

Fig. 17.14 The chemical structure of vitamin D

Adequate intake is defined as 200 IU/day for ages infant to 50, 400/day for 51–70, and 600/day for >70. The 100% Daily Value used for product labels is 400 IU. The safe upper limit is set at 2000 IU. According to the USDA's Dietary Guidelines in Table 17.7 cited data regarding vitamin D natural food sources [200].

Neuroprotective effect. Vitamin D as a neuroactive compound, a prohormone, is highly active in regulating cell differentiation, proliferation, and peroxidation in a variety of structures, including the brain. Since the brain contains big amount of vitamin D, many researchers were interested in its part in mental disorders. There is a theory concerning vitamin D as a component in pathogenesis of schizophrenia and its deficiency play a role in other mental disorders [192, 194, 201, 202]. Yan and colleagues in their study on schizophrenic patients described three novel structural variants of the vitamin D receptor [194].

Since vitamin D is a fat-soluble vitamin and a steroid hormone, it can also inhibit the synthesis of inducible nitric oxide synthase and increase glutathione levels, suggesting a role for the hormone in brain detoxification pathways [203]. Neuroprotective and immunomodulatory effects of this hormone have been described in several experimental models, indicating the potential value of vitamin D pharmacological analogs in neurodegenerative and neuroimmune diseases [203].

During the last period the interest in neuroprotective effect of vitamin D became spread among many researchers in this area [190, 204].

Table 17.7 Selected food sources of vitamin D [65]

Food	International Units (IU) per serving	Percent DV
Pure Cod liver oil, 1 Tablespoon (Note: most refined cod liver oils today have the vitamin D removed! Check your label to be certain.)	1,360	340
Salmon, cooked, 3½ ounces	360	90
Mackerel, cooked, 3½ ounces	345	90
Tuna fish, canned in oil, 3 ounces	200	50
Sardines, canned in oil, drained, 1¾ ounces	250	70
Milk, nonfat, reduced fat, and whole, vitamin D fortified, 1 cup	98	25
Margarine, fortified, 1 Tablespoon	60	15
Pudding, prepared from mix and made with vitamin D fortified milk, ½ cup	50	10
Ready-to-eat cereals fortified with 10% of the DV for vitamin D, ¾ cup to 1 cup servings (servings vary according to the brand)	40	10
Egg, 1 whole (vitamin D is found in egg yolk)	20	6
Liver, beef, cooked, 3½ ounces	15	4
Cheese, Swiss, 1 ounce	12	4

DV = Daily Value. DVs are reference numbers developed by the Food and Drug Administration to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for vitamin D is 400 IU for adults

Llewellyn and coworkers published the study in which they found that low serum 25-hydroxyvitamin D is associated with increased cognitive impairment [205].

The old age is associated with many bone fractures and pathologies, and on the other hand with mood disorders age related. The connection between bone metabolism is quite established, but is there a link between vitamin D and depression, several studies have investigated this question. Wilkins et al. [206] examined a group of elderly subjects and found mean vitamin D levels of 18.6 nM/L, with 58% of subjects being frankly deficient, defined as a level below 20 nM/L. Low vitamin D was robustly associated with the presence of mood disorder (odds ratio 11.7, 95% CI 2.0–66.9). Vitamin D deficiency has also been associated with depression and anxiety in a cohort of individuals with fibromyalgia [207].

Berk and coworkers found that a substantially higher proportion of depressed individuals are vitamin D deficient, which supports other published data in this regard [146]. This deficiency is particularly exacerbated for those aged 70 years. Vitamin D supplementation has been shown to have a positive effect on mood and wellbeing, however previous studies have been limited by small numbers, short treatment duration, or a lack of a placebo control. A therapeutic role for vitamin D supplementation in the treatment of mood disorders could provide a safe, low cost therapy with additional advantages to general and bone health [146].

Grant and colleagues assume that vitamin D is neuroprotective agent and suggest the hypothesis that it can reduce the risk of developing dementia, presenting the evidence from observational and laboratory studies. In continuation to their theory, the evidence includes observational studies supporting a beneficial role of vitamin D in reducing the risk of diseases linked to dementia such as vascular and metabolic diseases, as well as an understanding of the role of vitamin D in reducing the risk of several mechanisms that lead to dementia [208].

17.6 Vitamin E (Tocopherol)

History and structure. Vitamin E is an essential, fat-soluble nutrient as are vitamins A, D, and K. It is functions as an antioxidant in the human body. The chemical name for vitamin E is tocopherol, which is derived from the Greek tokos (childbirth) and pherin (to bear). The ending ol is the chemical suffix to denote an alcohol. The name tocopherol was bestowed on this vitamin in 1938.

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties [209, 210]. Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form [211].

It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [209, 212]. This removes the free radical intermediates and prevents the propagation reaction from continuing. However, the roles and importance of the various forms of vitamin E are presently

unclear [212, 213], and it has even been suggested that the most important function of α-tocopherol is as a signaling molecule, with this molecule having no significant role in antioxidant metabolism [213, 214].

From the discovery of vitamin E until now, the awareness to it passed a long and crooked way. At beginning, it was massively rejected as a treatment agent. It took about 40 years to come into conclusion that this vitamin has healing agent characteristics.

Vitamin E was first discovered in 1922 by American anatomist and physiologist Herbert McLean Evans (1882–1971) and his assistant Katherine Scott Bishop (1889–1976). The researchers found that laboratory rats failed to reproduce when lard was their only source of food fat. They assumed that this nutritional component has an influence on the reproductive ability. They called it the "anti-sterility factor." In 1925, Evans decided that the component should be renamed vitamin E.

Evans and his coworker biochemist Gladys A. Emerson (1903–1984) isolated vitamin E from wheat germ oil, corn oil, and cottonseed oil in 1936. In 1938 it was synthesized by Paul Karrer (1889–1971) and his coworkers. The investigators decided that the vitamin's biochemical function was primarily a protective one. The chemical formula of vitamin E is C29H5002 (Fig. 17.15).

In 1933, two Canadian physicians named brothers Evan (1905–1978) and Wilfred Shute (1907–1972) were the first doctors to use large doses of vitamin E to treat heart disease. This therapeutic attitude was not conventional, since physicians were not aware to the concepts of oxidation and its applications.

Today's growing appreciation of the role of d-alpha tocopherol in preventing and reversing cardiovascular disease is primarily due to the Shute brothers.

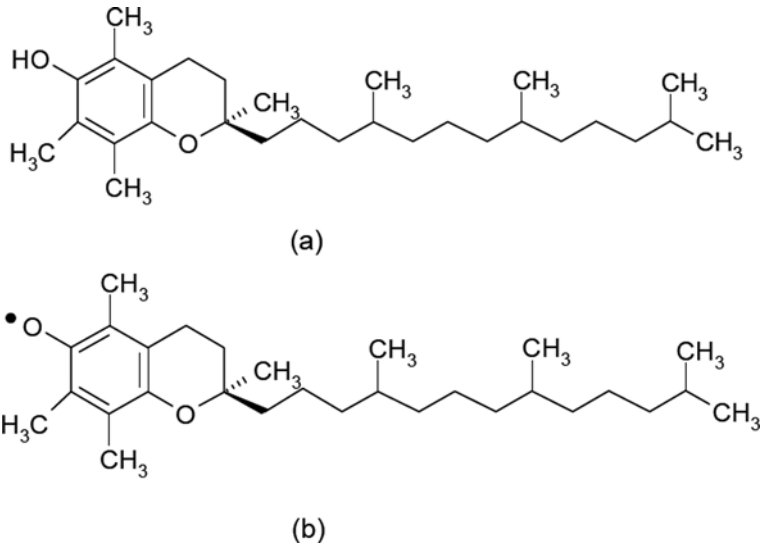

Fig. 17.15 Chemical structure of vitamin E

Sources of vitamin E. Wheat germ oil is the most abundant source. Other good sources of vitamin E are almonds, bread, eggs, broccoli, peaches, margarine, salad dressings, vegetable oils (soybean, corn, safflower, and cottonseed) and whole grain cereals. It is estimated that 2/3 of our intake of vitamin E comes from salad oils, margarine, and shortening. The rest is from vegetables, and grains. The US RDA for vitamin E is 10 milligrams per day. The normal US diet only supplies about 85% of the US RDA (recommended dietary allowance, not daily allowance) (Table 17.8) [65].

Neuroprotective effect. Since most neural damages are associated with oxidation process, until recently, it appeared that antioxidants were almost a panacea for continued good health and keeping neural system in good condition. A new area of research, led by the study of the human genome, suggests that the interplay of human genetics and diet may play a role in the development of chronic

Table 17.8 Food sources of vitamin E [65]

Food, standard amount	AT (mg)	Calories
Fortified ready-to-eat cereals, ~1 oz	1.6–12.8	90–107
Sunflower seeds, dry roasted, 1 oz	7.4	165
Almonds, 1 oz	7.3	164
Sunflower oil, high linoleic, 1 Tbsp	5.6	120
Cottonseed oil, 1 Tbsp	4.8	120
Safflower oil, high oleic, 1 Tbsp	4.6	120
Hazelnuts (filberts), 1 oz	4.3	178
Mixed nuts, dry roasted, 1 oz	3.1	168
Turnip greens, frozen, cooked, ½ cup	2.9	24
Tomato paste, ¼ cup	2.8	54
Pine nuts, 1 oz	2.6	191
Peanut butter, 2 Tbsp	2.5	192
Tomato puree, ½ cup	2.5	48
Tomato sauce, ½ cup	2.5	39
Canola oil, 1 Tbsp	2.4	124
Wheat germ, toasted, plain, 2 Tbsp	2.3	54
Peanuts, 1 oz	2.2	166
Avocado, raw, ½ avocado	2.1	161
Carrot juice, canned, ¾ cup	2.1	71
Peanut oil, 1 Tbsp	2.1	119
Corn oil, 1 Tbsp	1.9	120
Olive oil, 1 Tbsp	1.9	119
Spinach, cooked, ½ cup	1.9	21
Dandelion greens, cooked, ½ cup	1.8	18
Sardine, Atlantic, in oil, drained, 3 oz	1.7	177
Blue crab, cooked/canned, 3 oz	1.6	84
Brazil nuts, 1 oz	1.6	186
Herring, Atlantic, pickled, 3 oz	1.5	222

Food Sources of Vitamin E ranked by milligrams of vitamin E per standard amount; also calories in the standard amount. (All provide > –10% of RDA for vitamin E for adults, which is 15 mg a-tocopherol [AT]/day.)

diseases. This science, while still in its infancy, seeks to provide an understanding of how common dietary nutrients such as antioxidants can affect health through gene-nutrient interactions [215].

D'Souza and D'Souza report about the study performed on schizophrenic patients and healthy relatives control subjects. The main finding was that schizophrenic patients were more susceptible than control subjects to oxidative damage. The other finding in this study was that antioxidant levels are depleted in schizophrenic patients when compared to normal subjects as evident from decreased levels of vitamins E and C in the plasma. They came into conclusion that using at the initial stages of illness may prevent further oxidative injury and deterioration of associated neurological deficits in schizophrenia [216].

The issue of the therapeutic effect of vitamin E on tardive dyskinesia was examined in a lot of investigations. The results of these studies remained this issue still controversial [217–228]. In recent review performed by Soares and McGrath, the reviewers came to the conclusions that vitamin E has a value of preventive protection against deterioration of tardive dyskinesia, but there is no evidence that this vitamin improves its symptoms [229].

Studies performed on animals suggest that alpha-tocotrienol can exert anti-apoptotic neuroprotective action independently of its antioxidant property. Among the vitamin E analogs examined, alpha-tocotrienol exhibited the most potent neuroprotective actions in rat striatal cultures [230].

In another study performed by these researchers, the authors suggest that alpha-tocopherol protects striatal neurons by the reduction of oxidative stress, presumably by decreasing intracellular O(2)(-) levels, and at least partly by the inhibition of apoptosis [231].

Neuroprotective activity studied also in other movement disturbances such as Parkinson's disease (PD). Roghani and Behzadi performed a study on rats in order to investigate the neuroprotective effect of vitamin E in the early model of PD. According to their results, administration of vitamin E produces a rapid protective effect on the nigrostriatal dopaminergic neurons in the early unilateral model of PD [232].

Post and colleagues in another experiment on animal studied mechanisms underlying the protective potential of α-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. The authors found that vitamin E led to substantial reduce the haloperidol-induced impairment of locomotor activity in rats. They comment that the data indicate the usefulness of vitamin E as an adjunct to haloperidol treatment and provide initial clues about the underlying molecular mechanisms involved in these effects [233].

17.7 Conclusions and Future Directions

The process underlying the onset of mental disorders is not well understood, at least not in all mental disturbances. There is an ocean of papers and investigations concerning the question whether there is any biological marker or clear-cut connection

between pathogenesis of mental illnesses or disorders, and nutrition or other supplements. The modern medicine is limited in full understanding of this conundrum. In this gap between scientific knowledge and great hope and will of helping suffering people, the relatively new phrase "neuroprotective activity" enters.

In this chapter, we try to illuminate the issue of neuroprotective activity of some vitamins. We try to bring the history and the update information about this topic from an objective point of view and to bring our own personal experience by citing our studies regard vitamin B_6 and B_{12}. We are trying to confirm and better understand the changes observed in the emerging phase of mental disorders caused by addition of vitamins.

It is difficult to summarize the whole material but it is quite clear that there is something more than guts feeling about the efficacy of all these supplements. Even those, which are not clear-cut benevolent, we can have the impression that bigger samples and studies are needed to strengthen the objective data.

There is a growing body of evidence to suggest that dietary supplementation of antioxidants may improve/ assist recovery in schizophrenia or other psychiatric disorders Antioxidants such as vitamins A, B_6, folic acid, B_{12}, C, D and E have demonstrated neuroprotective effects. This may be particularly relevant in first-episode schizophrenia, where antioxidant deficiencies have been observed and the resultant oxidative stress/damage may contribute to the pathophysiology of onset of the disorder.

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Chapter 18
Smoking and Mental Disorders: Focus on Neuroprotection

Tsafrir Loebl

Abstract Smoking is the number one preventable risk factor for mortality in general, especially through its actions on the respiratory and cardiovascular systems. As such, it can be considered as a highly offensive factor for the organism nervous system, mostly through its actions on its vasculature. On the other hand, smoking can be regarded as a protective factor against one neurodegenerative disease (Parkinson Disease), while its major psychoactive ingredient, nicotine, and perhaps other substances, can act in the central nervous system to alleviate many symptoms of various cognitive and psychiatric disorders – Alzheimer Disease, Schizophrenia, depression and more. Therefore, smoking cessation, that can reverse cerebrovascular disease, could be thought of as a neuroprotective treatment, but with some risk potential regarding the above mentioned benefical role of smoking and nicotine on the central nervous system disorders. The literature on smoking cessation in psychiatric disorders is very recent and relatively sparse, focusing mostly on schizophrenia and depression.

This chapter will focus on the association between smoking and neuroprotection – from one hand, smoking as a risk factor for one of the major offenses on the central nervous system – cerebrovascular disease. From the other hand, smoking putative beneficial role on neuropsychiatric disorders: degenerative disorders – Alzheimer disease and Parkinson disease, schizophrenia and depressive disorder.

Hence smoking cessation can be looked upon as an intervention with neuroprotective features there would be a brief summary of the existing experience of smoking cessation intervention in schizophrenia, depression as well as in the general population.

Abbreviations

ADHD	Attention Deficit/Hyperactivity Disorder
CBT	Cognitive Behavioral Therapy

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_18,

CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CVD	Cerebro Vascular Disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECA	Epidemiological Catchment Area
ERP	Event Related Potential
GNHIES	German National Health Interview and Examination Survey
IHD	Ischemic Heart Disease
MAO	Mono Amine Oxidase
MDD	Major Depressive Disorder
MRI	Magnetic Resonance Imaging
NCS	National Catchment Survey
NESARC	National Epidemiologic Survey on Alcohol and Related Conditions
NRT	Nicotine Replacement Therapy
OR	Odds Ratio
PD	Parkinson Disease
PET	Positron Emission Tomography
RR	Relative Risk
RT	Reaction Time
SNICAS	Smoking and Nicotine Dependence Awareness and Screening
TACOS	Transitions in Alcohol Consumption and Smoking
VTA	Ventral Tegmental Area
WML	White Matter Lesions

18.1 Introduction

Humanity has been using tobacco as early as 5000 BC, first used as part of shamanistic rituals that began in the Peruvian and Ecuadorian Andes and spread all over the Americas. Following the discovery of the "New World" by Columbus in 1492, smoking and cultivation of Tobacco has spread, first to Spain and Portugal, and from there, by the assistance of Jean Nicot (On which called the active ingredient, nicotine), to France in the 16th century. During that century Tobacco use quickly spread all over the globe. Its use for smoking by means of rolled paper cigarettes became especially popular during the 19th century, following the Crimean war.

Cigarette smoke is composed of volatile and particulate phases. The gaseous portion comprises about 95% of cigarette smoke by weight. Some 500 gaseous compounds, including nitrogen, carbon monoxide, carbon dioxide, ammonia, hydrogen cyanide and benzene, have been identified in the volatile phase. The other 5% of cigarette smoke is composed of particulates. There are about 3,500 different compounds in the particulate phase, of which the major one is the alkaloid nicotine. Other alkaloids include nornicotine, anatabine and anabasine. The particulate matter minus its alkaloid and water content is called tar.

18.2 Smoking and Nicotine Dependence

According to the World Health Organization, in 2008 there were more than one billion smokers in the world; more than 80% of them live in low- and middle-income countries. Two-thirds of the world's smokers live in 10 countries (with prevalence of current tobacco smoking, males and females, respectively): China (66.0%, 3.1%), India (57.0%, 3.1%), Indonesia (63.2%, 4.5%), Russia (60.4%, 15.5%), USA (27.5%, 19.0%), Japan (43.3%, 12.0%), Brazil (20.3%, 12.8%), Bangladesh (48.6%, 25.4%), Germany (33.2%, 22.4%) and Turkey (52.0%, 17.3%) [1].

Nicotine, the most potent psychoactive substance in cigarette smoke is highly addictive, with a substantial proportion of its users eventually developing nicotine dependence. Nicotine dependence, defined by the DSM-IV TR as having 3 out of 7 criteria (see Table 18.1) [2].

Like other drugs of abuse (i.e. cocaine, morphine, ethanol), nicotine activates the mesolimbic dopamine system within the VTA system to initiate processes which are critical to the reinforcing properties of the drug as seen in nicotine self-administration animal models, which serve as a model for addictive disorders [3].

Specifically, nicotine has been shown to increase burst activity in dopamine neurons of the VTA, a mode of firing pattern in these cells which is physiologically associated with basic motivational processes. Continuous infusion of nicotine into the VTA produces a long-lasting increase in accumbal dopamine release, which is known to be associated with feelings of euphoria as well as part of other substances of abuse self-administration animal models [4].

Nicotine dependence is highly prevalent in the general population – In the US, the National Catchment Survey (NCS) and Epidemiological Catchment Area (ECA) found 24 and 37%, respectively, of the sample to meet criteria for lifetime dependence. The National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) found 13% of the sample to meet criteria for current dependence. In

Table 18.1 DSM-IV TR criteria for nicotine dependence [2]

Criteria
Tolerance
Withdrawal
The substance is often taken in larger amounts or over a longer period than was intended
Persistent desire or unsuccessful effort to stop
A great deal of time is spent to obtain, to use, or to recover from the drug
Important activities are given up or reduced because of substance use
Use continues despite knowledge of problem caused by substance

Germany the prevalence of current dependence were 14, 11 and 9%, according to the SNICAS, TACOS and GNHIES surveys, respectively. Surveys in Asia found lifetime prevalence of 13% in Hong-Kong, 20% in Seoul and 12% in Takayama, Japan. Surveys in young adults found similar rates of lifetime dependence – 20% (Detroit) and 19% (Germany) [5].

18.3 Smoking, Mortality and Cerebrovascular Disease

Tobacco use is a risk factor for six of the eight leading causes of deaths in the world: Ischemic heart disease (IHD), cerebrovascular disease (CVD), Lower respiratory infections, chronic obstructive pulmonary disease (COPD), Tuberculosis and Trachea, bronchus and lung cancers (The other 2 leading causes of death are HIV/AIDS and diarrheal diseases). About 100 million deaths were caused by tobacco in the 20th century. Tobacco use kills 5.4 million people a year – an average of one person every six seconds - and accounts for one in 10 adult deaths worldwide. It kills up to half of all its users [1].

Due to the nature of this book, this chapter will focus on the relationship between smoking and central nervous system disorders. The relationship between smoking and CVD was one of the first topics already addressed by the general surgeon's report in the 1960s, based on studies done as early as the 1950s. The main pathophysiologic process underlying CVD is the development of atherosclerosis.

Briefly, smoking shown to have casual relationship with the development of atherosclerosis in several pathophysiological mechanisms: endothelial injury, increased thrombosis/reduced fibrinolysis, inflammation, changes in lipid metabolism, increased blood rheology by increased oxygen demand and decreased oxygen supply.

The process of atherosclerosis in the brain's blood vessels can be manifested sub-clinically as white matter lesions (WML), seen by magnetic resonance imaging (MRI) in asymptomatic patients [6–8].

Studies conducted in the 1950s described the increased mortality from strokes in smokers compared with non-smokers. Smoking has been shown to be associated with an increase in incidence and mortality from both main subtypes of stroke, ischemic stroke and subarachnoid hemorrhage [9].

The overall relative risk of stroke associated with cigarette smoking was 1.5 (95% confidence interval 1.4–1.6). Considerable differences were seen in relative risks among the subtypes: cerebral infarction 1.9, cerebral hemorrhage 0.7, and subarachnoid hemorrhage 2.9. An effect of age on the relative risk was also noted; <55 years: 2.9, 55–74 years: 1.8, and 75+ years: 1.1. A dose response between the number of cigarettes smoked and relative risk was noted, and there was a small increased risk in women compared with men [10].

The risk of stroke in smokers is also mediated by hypertension - Pharmacologic treatment in mildly hypertensive smokers is much less effective in reducing the incidence of stroke than in mildly hypertensive non-smokers. The relative risk of stroke

among hypertensive smokers is five times that among normotensive smokers, but 20 times that of normotensive non-smokers [9].

In a prospective study for over 12 years, looking on more than 7,000 middle aged men, smoking cessation benefits on the reduction in the risk of stroke was seen shortly after cessation and reached its full effect within 5 years post cessation, Light smokers of less than 20 cigarettes per day did not have a higher risk than never smokers while ex-heavy smokers retained considerable risk of more than 2-fold, but less than current smokers (RR of 3.7 than never smokers). The benefit was seen both in hypertensive and normotensive men , more remarkably so in hypertensive men [11].

18.4 Smoking and Neurodegenerative Disorders

In contrast to the robust evidence for smoking being a major risk factor for cerebrovascular disorders, smoking plays a different role when we look on its effect on non-vascular neurodegenerative disorders, especially Parkinson disease, and to a lesser extent, in Alzheimer's disease – the prevalence of these 2 diseases in non-smokers is about twice compared to its prevalence among smokers [12].

Many studies repeatedly detected a protective effect of smoking on the risk of developing an idiopathic Parkinson's disease, including Morens et al. 29-year follow-up study that found RR of 0.4 fpr PD among smokers [13]. Already in 1959 Dorn detected decreased risk for PD among smokers (RR 0.36) [14]. The highest protection against PD among smokers was detected by Kahn (RR 0.23) [15], but most of the results were aroung RR of 0.4 [16, 17]. The issue of survival bias, i.e. that smokers are underrepresented due to earlier mortality from cardiovascular or other diseases was addressed in several studies and the protective effect of smoking was confirmed – Subjects with and without PD showed similar increased mortality in smokers than in nonsmokers. A dose-response relationship between smoking, smoking cessation and PD was shown in a retrospective, population-based case-control study (Table 18.2) [18].

Possible mechanism in which smoking exert neuroprotective action against development of PD can be attributed to the actions of nicotine as seen in animal models – (1) protection of nigral dopaminergic neurons from cell loss induced by partial mesodiencephalic hemitransection [19] and (2) reduction ofage-related

Table 18.2 Dose-response relationship – smoking, cessation and PD [18]

Smoking history adjusted	OR (95% CI)
Never smoked	1 (reference)
Current light smokers	0.6 (0.2–1.5)
Current heavy smokers	0.1 (0.01–0.6)
Former heavy smokers – stopped >20 years ago	0.9 (0.4–1.8)
Former heavy smokers – stopped 1–20 years ago	0.4 (0.2–0.7)

nigrostriatal neuronal loss by chronic nicotine treatment [20]. Another hypothesis suggests the common mechanism by upregulation of nicotinic cholinergic receptors and the resulting increased activity of the ubiquitin-proteasome system, which is believed to prevent neurodegeneration caused by the accumulation of misfolded or damaged proteins [21]. Inhibition of brain monoamine oxidase-B (MAO-B) activity by cigarette smoke has been associated with protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in mice [22] and smoking has been found to reduce human brain MAO-B activity substantially, as assessed by Positron Emission Tomography (PET) [23].

18.4.1 Smoking and Alzheimer's Disease

The relationship between smoking and AD has not been consistent as it is with PD. While 5 case-control studies and 2 meta-analysis found smoking to be a protective factor, 3 case-control and 2 follow-up studies found the opposite, 2 follow-up studies found no association and 14 case-control studies had reported inconsistent results [12]. A survival bias might explain the results in those studies showing smoking to be a protective factor. Hence, it is likely that smokers who succumb to dementia might be eliminated early from the population.

18.5 Smoking and Mood Disorders

18.5.1 Epidemiology

With the decline in the prevalence of smoking among the general population, the association between smoking and depression transferred from being small and non-significant to being more prominent and a matter of clinical significance. In a series of clinical interviews and following cohorts in the years 1952–1970 and 1970–1992 Murphy et al. [24] are showing that increase, when in 1992, for the first time compared to the previous interviews, the odds that a smoker would be depressed were three times the odds that a nonsmoker would be depressed. The subjects in the cohorts who became depressed were more likely to start or continue smoking and less likely to quit than those who never had a depression.

With the emergence of that association, the first epidemiological studies demonstrating it were done in the early 1980s.

Hughes et al. compared the prevalence of smoking among 277 psychiatric outpatients to that among either local ($N = 1{,}440$) or national ($N = 17{,}000$) population-based samples. Of the depressed outpatients, 49% smoked, compared to around 30% among the general population. The significant difference was not associated with age, sex, marital status, socioeconomic status, alcohol or coffee use [25].

Using a population-based data ($n = 3{,}213$) collected in the early 1980s in the St Louis Epidemiologic Catchment Area Survey of the National Institute of Mental

Health, Glassman et al. [26] observed that among people with lifetime history of major depressive disorder a history of regular smoking was observed more frequently than among those with no psychiatric diagnosis. Smokers with history of major depression were also less successful at their attempts to quit. In addition, among the depressed group, the gender differences in prevalence of smoking were not evident.

An analysis of a national scale data set – the first National Health and Nutrition Examination Survey (done in the early 1970s, with more than 20,000 participants) and its 9-years follow-up study was performed by Anda et al. [27]. The cross-sectional analysis showed a positive correlation between depression severity score and the prevalence of current smoking as well negative correlation with quit ratio among the cohort of smokers in the Follow-up Study, the estimated incidence of quitting after 9 years was 9.9 vs. 17.7%, for depressed smokers and nondepressed smokers, respectively.

Breslau et al. [28, 29] have published several other epidemiological studies, specifically looking into prevalence of smoking and mental illness among younger adults in the Detroit area.

In a 5-year longitudinal study of a sample of 1,007 young adults [28], a history of major depression at baseline increased significantly the risk for progression to daily smoking (OR, 3.0; 95% confidence interval, 1.1–8.2), but did not decrease their rate of quitting (OR, 0.8; 95% confidence interval, 0.4–1.6). On the other way around, a history of daily smoking at baseline increased the risk for major depression (OR, 1.9; 95% confidence interval, 1.1–3.4). A weaker association was present when a history of early conduct problems was controlled for. When specifically looking into the diagnosis of nicotine dependence [29], the lifetime prevalence of in the whole sample was 20%, and was associated with higher rates of major depression, that varied by level of severity of nicotine dependence. On the contrary, the nondependent smokers did not had higher rates of major depression.

Analysis of the 2000–2001 NESARC sample was able to find increased rates of 12-month prevalence of nicotine dependence among people who met DSM-IV criteria for mood disorders: 29.2% for any mood disorder, 30.0% for major depressive disorder, 32.0% for dysthymia, 35.3% for mania and 33.4% for hypomania [30].

On the other hand, the 12-month prevalence for these disorders was also increased among people with nicotine dependence: 21.1% for any mood disorder, 16.6, 4.6, 4.6 and 3.0% for major depressive disorder, dysthymia, mania and hypomania, respectively [30].

Breslau et al. [31] showed in subsequent survey ($N = 4{,}414$, ages 15–54) that active psychiatric disorders (including mood, anxiety and substance use disorders) predicted onset of daily smoking, progression to nicotine dependence, and the persistence of smoking. Persons with four or more active disorders were at higher risk for daily smoking (2.1 vs. 1.4) and for nicotine dependence (2.9 vs. 1.4) than were persons with one active disorder. Preexisting psychiatric disorders did not influence smokers' potential for quitting.

The association between smoking and depression was replicated in non-american samples as well. John et al. [32] performed a cross-sectional analysis and a 3-years follow-up of a population-based random sample in Germany ($N = 4{,}075$, ages

18–64). 2,458 were daily smokers, of whom 320 (13.0%) had a lifetime diagnosis of depression. Among females, presence vs. absence of nicotine dependence was associated with increased rate of depressive disorder 31.6% vs. 13.7%, respectively ($\chi^2 = 49.9$, df = 2, $p < 0.001$); a significant difference was present in males as well: 13.4 vs. 5.6% ($\chi^2 = 20.2$, df = 2, $p < 0.001$). Lifetime history of depressive disorder did not affect significantly the rates of smoking cessation in this cohort.

Twins – Specifically looking into relationship between smoking and depression in female twins register [33], Kendler et al. found a relative risk for ever smoking given a lifetime history of MD of 1.48 and 1.18 or 0.98, respectively, in dizygotic and monozygotic twin pairs discordant for a history of MD. The reverse relationship was a relative risk for a history of MD given ever smoking of 1.60 and 1.29 or 0.96 in dizygotic and monozygotic twins discordant for smoking, respectively. The conclusion suggested that the association between smoking and MD in women is not a causal one but arises largely from familial factors, which are probably genetic, that predispose to both smoking and MD.

A study that looked in male twins [34] from the Vietnam Era Twin Registry extended Kendler's findings by demonstrating in men that shared genetic factors predispose not only to major depression and daily smoking but also to major depression and nicotine dependence.

18.6 Mechanisms

The biological mechanisms that correlate smoking to depression are attributed to the cigarette smoke major psychoactive substance, nicotine, and to some degree to its Monoamine oxidase (MAO) inhibitor properties.

18.6.1 Nicotine

18.6.1.1 Animal Models

The standard animal models for the human depression are the forced swim test and the learned helplessness models. A specific rat line, the Flinders Sensitive Line rats have been proposed as an animal model of depression. These rats show an exaggerated immobility in the forced swim test. Tizabi et al. found that either acute or chronic (14 days) administration of subcutaneous nicotine significantly improved their performance on the forced swim test, dissociable from its effects on locomotor activity [35]. He also found in this rats line higher binding of the alpha4beta2 nicotinic receptor subtype in the frontal cortex, striatum, midbrain and colliculi. Nicotine also showed to have an effect on rats that were conditioned thru the learned helplessness model – rats receiving nicotine for 14 days had a better success rate in an escape test compared to non treatment or to blockade with the nicotine antagonist, mecamylamine [36].

18.6.1.2 Emotional Aspects of Depression

Nicotine acts on the brain reward circuitry systems all other drugs of abuse acts – by activating dopaminergic projections in the ventral tegmental area (VTA) to the shell of the nucleus accumbens. This activation tends to cause repeated administration as well as feeling of reward that experienced as "euphoria". Henningfield et al. conducted a study in which 8 drug users were administered several doses of nicotine by intravenous route and by inhalation. Subjects reported more euphoria and relaxation and mis-identified the drug as cocaine [37].

Cognition – The depressive disorder affects areas of cognition such as attention, short-term memory, cognitive retardation and more. The cholinergic system that modulates cognitive processes can be activated by nicotine agonist effect on nicotinic acetylcholine receptors. There is a great body of evidence on the positive effect of nicotine in these areas:

Working memory – Pineda et al. tested smoking status on event-related potentials (ERPs) and reaction times (RTs) and performance accuracy. They were recorded from smokers and nonsmokers during a recognition memory task – smokers exhibited fast RTs relative to nonsmokers. They exhibited even faster RTs when tested for the first or last item in the memory set, suggesting for enhanced primacy and recency effects of smoking, perhaps related to the motor output aspects of working memory [38].

Attention – Nicotine has been shown to improve attentiveness in normal smokers, attenuate attentional deficits in Alzheimer's disease, schizophrenia, attention-deficit/hyperactivity disorder (ADHD) and also in non-smokers without attentional deficits [39].

Information Processing – In a series of studies, using nicotine, cigarettes and scopolamine and measurements on information processing, Wesnes, Warburton and Revell demonstrated smoking effects on enhanced information processing, mediated thu central cholinergic pathways [40–43].

MonoAmine Oxidase (MAO) Inhibitor – MAO B is involved in the breakdown of dopamine, and as such it is implicated in reinforcing and motivating behaviors. In a PET binding study, Fowler et al. reported a 40% decrease in the level of monoamine oxidase B in the brains of living smokers relative to non-smokers or former smokers. He suggested that the reduction of MAO B activity may synergize with nicotine to produce the effects that are associated both with depression and smoking [23].

18.6.2 Post-Smoking Cessation

Studies focusing on associations between smoking cessation and emergence of depressive symptoms and or episodes began in the 1990s: Covey et al. described that in the first week post cessation, the smokers with a history of depression experienced more frequent and intense psychological withdrawal symptoms, especially depressive mood – that were eventually associated with the outcome of the treatment [44]. Kinnunen et al. who followed 269 smokers for 3 months describes earlier

relapse among those with depression history, again with more frequent and intense emotional withdrawal symptoms and more frequent smoking as a response to negative affect. He strengthens the evidence for the effectiveness of NRT among the depressed as well, with successful quit rate of 29.5 vs. 12.5% for those treated with nicotine gum vs. placebo, respectively [45].

Particularly looking on the emergence of a new depressive episode, Covey et al. found a 3-month incidence, post cessation of 30% among people with lifetime history of recurrent depressive episodes, 17% among those with history of a single depressive episodes and 2% among those with no history of depression [46].

Expanding the post cessation observation to 12 months, Tsoh et al. found an incidence of 14% at a 12-month follow-up after treatments for smoking cessation [47], predicted by history of depression, baseline depression, education, and age at smoking initiation. Reviewing this study as well as 6 others, Hughes et al. [48] found similar evidence, of past history of MDD as a predictor, but could not reach further conclusions. Similar results were reported by Borrelli et al. in a descriptive study where those who developed MDD after treatment scored significantly higher on measures of depressed mood at baseline [49].

18.7 Smoking and Schizophrenia

18.7.1 Epidemiology

Smoking is highly prevalent among people with schizophrenia, a finding that was replicated in many studies with prevalence reported to be around three-quarters of the patient population, regardless of setting (inpatient, outpatient or community samples) [25, 50–55], higher than the general population and even higher than people with affective disorders [54].

In a worldwide meta-analysis of 42 studies by De-Leon and Diaz [56], that finding was consistently repeated: OR of current smoking in schizophrenia was 5.9 – more in males (7.2 in 32 male studies), but also among females (3.3 in 25 female studies).

The result was not dependent on illness severity (OR of 1.9 compared to non-schizophrenia severely ill). Accordingly, heavy smoking, high nicotine dependence and low rates of smoking cessation were more prevalent among schizophrenia patients.

The greater prevalence of smoking among men, younger age, poor education and lower socioeconomical status is seen among schizophrenia patients as well as in the general population [25, 53, 55]. Schizophrenia patients who smoke tend to be heavier smokers, assessed by number of cigarettes per day and nicotine metabolites level [55, 57].

Smoking was also found to be a predictor of future hospitalization for schizophrenia, as seen in a cohort of more than 14,000 Israeli recruits [58] – adjusted RR was 1.94 in a dose-dependent fashion: smoking of more than 10 cigarettes/day

had a RR of 2.24. This finding is contradictory to an earlier observation among moe than 50,000 Swedish conscripts [59], where smoking found to be a protective factor (OR of 0.8).

18.7.2 Clinical Correlates

Smoking in schizophrenia is frequently associated with younger age, earlier age at onset of schizophrenia, more hospitalizations, and higher doses of neuroleptic medication, possibly because of enhanced metabolism [53]. Former smokers suggested to have higher levels of functioning and fewer negative symptoms than current smokers [60]. Gray matter differences were noted with greater volumes in lateral prefrontal and superior temporal gyri among smokers [61].

Smoking is a risk factor for dyskinesias independent of medication exposure [62]. On the contrary, smokers were reported to have less medication-induced parkinsonism [53, 63, 64].

Smokers reported on more positive symptoms than non-smokers [51, 53]. Heavier smoking (>25 cigarettes per day) seems to report more positive symptoms and less negative symptoms than light or non-smokers [60].

Of smoking – smokers with schizophrenia report similar reasons for smoking as in the general population (relaxation, enjoyment etc.) and similar withdrawal symptoms. Some cases of exacerbation psychiatric symptoms during smoking cessation or reduction were noted [50].

Post-mortem studies of nicotine binding showed that schizophrenic smokers had reduced nicotinic receptor levels in hippocampus, cortex, and caudate compared to control smokers. This effect was suggested not to be dependent on chronic neuroleptic treatment, as seen in a rat model [65].

Normally, repeated auditory stimuli should produce a diminished evoked response compared to the first stimuli, a phenomena called sensory gating. A typical finding in patients with schizophrenia as well as in half of their first degree non-affected relatives is a diminished gating of the P50 auditory evoked response. This gating deficit is shown to have a genetic linkage, with the gene for one type of nicotinic receptor expressed in the human hippocampus [66]. Adler et al. found that nicotine would transiently reverse the sensory gating deficit in nonsmoking relatives of individuals with schizophrenia [67] as well as the patients themselves [68].

Another physiological deficit often found among patients with schizophrenia, the impaired smooth pursuit eye movement, was partially improved by smoking [69].

Cognitive performance – In a series of cognitive tasks in first-episode psychosis patients, Zabala et al. found that the smokers demonstrated better cognitive functioning in the areas of attention and working memory than non-smokers [70].

Nicotine reversed cognitive deficits related to haloperidol treatment, including memory performance and reaction time to a complex spatial task and improved attentiveness during a continuous performance task, independent of haloperidol dosage.

Smoking also had an specific effect on visuo-spatial working memory, with impairment after overnight abstinence or nicotine blockade by mecamylamine, as shown by Sacco et al. [71].

18.7.3 Antipsychotic Medications

Smoking results in increased metabolism of neuroleptics [72–74]. The opposite effect, of neuroleptics on smoking behavior is dependent on medication type – Haloperidol administration results in increased smoking in both schizophrenia patients [75] as well as in normal smokers [76]. Clozapine holds 2 interesting effects that might be correlated – clozapine decrease smoking [77] and improves gating of the P50 auditory evoked response [78].

Models of schizophrenia proposed dysfunction in both dopaminergic and glutamatergic modulation of brain activity in nigro-striatal and mesocorticolimbic systems – both systems been demonstrated to contain a variety of nicotinic receptors that have a modulatory effects mediated by nicotinic receptors, which can be manifested both by an effect on psychotic positive symptoms, postulated to be the result of dissociation of cortical-subcortical dopaminergic activity and on negative symptoms that proposed to be the result of cortical hypoactivity [50].

18.8 Smoking Cessation in the General Population

The common practice of smoking cessation is either initiated by the smoker him/herself or by a professional – a primary care provider, a hospital nurse, a therapist etc. It could be either any of the listed behavioral interventions (Table 18.3 summarize the Cochrane database reviews of various interventions), a pharmacotherapy – nicotine replacement therapy (NRT), antidepressants (such as bupropion or nortryptiline), the newer nicotine receptor partial agonist, varenicline, other miscellaneous drugs (Table 18.4) or a combination of both.

18.8.1 Nicotine Replacement Therapy (NRT)

An agonist therapy is an attractive option for treatment of dependence, similar to methadone treatment for opioid dependence. Nicotine is available in many over the counter preparations since the early 1980s: trans-dermal skin patches, gum, nasal and oral spray and sub-lingual lozenges.

Stead et al. [79] reviewed 132 trials – 111 of them with over 40,000 smokers. They found that the RR of abstinence for any form of NRT relative to control was 1.58 (nicotine gum 1.43, nicotine patch 1.66, nicotine inhaler 1.90, nicotine lozenges

Table 18.3 Behavioral interventions

Intervention	Trials	Results
Exercise [96]	13	3 studies showed an effect at 3-months follow-up
Brief advice [97]	41	rate of quitting RR 1.66, intensive intervention RR 1.84
Hospitalized patients [98]	33	With 1 month follow-up OR 1.65, no effect for adding NRT
Telephone counseling [99]	48	Multiple sessions OR 1.41
Self-help interventions [100]	60	OR 1.24
Individual behavioral counseling [101]	21	OR 1.56
Group behavior therapy [102]	55	Compared to self-help OR 2.04, compared to no intervention OR 2.17
Hypnotherapy [103]	9	No evidence
Acupuncture and related interventions [104]	24	No consistent evidence, OR 1.36
Relapse prevention [105]	40	No benefit
Aversive smoking [106]	25	Insufficient evidence for an effect

Table 18.4 Other interventions

Intervention	Trials	Results
Clonidine [107]	6	OR 1.89, prominent side-effects
Anxiolytics [108]	6	No effect
Mecamylamine (Nicotine receptor antagonist) [109]	2	Possible effect
Nicobrevin [110]	0	No evidence
Opioid antagonists [111]	4	No evidence
Cannabinoid type 1 receptor antagonists (rimonabant) [112]	3	*N*>3000, Rimonabant 20 mg OR 1.6, depression/suicidal ideation concern
Silver acetate [113]	2	OR 1.05
Topiramate [114]	1 RCT	Gender specific for males (OR 3.8)

2.00 and nicotine nasal spray 2.02. The effect was mostly independent on duration, setting or additional support provided. NRTs increase the rate of quitting by 50–70%.

18.8.2 Nicotine Partial Agonists

The most recent pharmacotherapy approved for smoking cessation is Varenicline, a partial agonist at the alpha4beta2 nicotinic acetylcholine receptor. Two large multicenter randomized, double-blind, placebo-controlled trials conducted in the mid 2000s, one in UK [80] and one in US [81] with a 12-week treatment period and a one year follow-up. During the last month of treatment 44% of the smokers treated with

varenicline continuously abstinent compared with 18% in the placebo group (OR 3.85) and 30% in the bupropion SR group (OR 1.90). In the one year follow-up 23% of the smokers treated with varenicline were continuously abstinent compared with 10% in the placebo group (OR 2.66) and 15% in the bupropion SR group (OR 1.77).

People taking Varenicline especially mentioned reduced withdrawal symptoms, cravings and since the treatment begins before the quit date, reduced euphoria from smoking cigarettes. The most common side effect were nausea (around 30%) and insomnia. Also, possible links were reported with dysphoric mood [82].

18.8.3 Antidepressants

Due to the nature of the nicotine withdrawal syndrome that contains, among others, mood symptoms of irritability and depression and because of some shared biological pathology, the theory that anti-depressants should assist in smoking cessation was tested several times with different classes of anti-depressants. Hughes et al. reviewed a total number of 53 studies [83], of which were 40 trials of bupropion and eight trials of nortriptyline, the most tested medications from this class. Both medications doubled the odds of a successful smoking cessation outcome: bupropion OR 1.94, nortriptyline OR 2.34. There is no evidence for an added effect of either long-term therapy with bupropion or to a supplemental therapy with NRT. Three trials found bupropion to be of smaller efficacy than varenicline (OR 0.60). Only eight trials of other anti-depressants were found of fluoxetine, sertraline, paroxetine, moclobemide and venlafaxine. No significant benefit was found. Evidence suggests that the mode of action of bupropion and nortriptyline is independent of their antidepressant effect.

In another meta-analyis, looking at bupropion and nortryptiline [84], also found higher abstinence with nortryptiline than placebo (RR 2.4) and smaller, but not statistically significant than wit bupropion (RR=1.7).

18.8.4 Smoking Cessation in Depression

CBT – Hall et al. provided CBT with mood management for smokers who had a history of MDD that improved their chances to become abstinent compare to NRT alone. That effect was specific only to those with positive history for MDD, but did not affect mood after quitting [85].

Nortryptiline – Trying to tailoring treatment for depressed smokers, Hall et also conducted a 2 × 2 study (Nortriptyline × CBT) [86] where he showed that nortryptiline produced higher abstinence rates than placebo, regardless of depression history, where CBT was more effective for participants with a history of depression. Nortriptyline did alleviate a negative affect occurring after smoking cessation. Hall also noted a sex-by-depression history interaction; MDD history-positive women were less likely to be abstinent than MDD history-negative women, but depression history did not predict abstinence for men.

Bupropion –In a RCT that measured if adding bupropion to NRT and CBT in 200 smokers with current or past depression, Evins et al. [87] reported that bupropion

seemed to provide an advantage to NRT and CBT only to those who did not dropped out of the trial. Abstinence was associated with increased depressive symptoms, regardless of bupropion treatment.

Sertraline – Sertraline did not add to the efficacy of an intensive individual counseling program for depressed smokers in a study held by Covey et al. [88].

18.8.5 Smoking Cessation in Schizophrenia

The prospect of smoking cessation treatment for schizophrenia patients was affected by the discouraging success rate and by stigmatizing views such as that smoking in among the few pleasurable activities left to this patient population.

The odds ratios of smoking cessation among schizophrenia patients compared to the general population were varied between 0.04 and 0.72 [56].

In a delay of about 20 years, some research on smoking cessation treatments for this specific population began to emerge – Addington et al. performed an open trial of 7-weeks group treatment for smoking cessation with promising results [89]: 42% quit rate at endpoint and 16 and 12% abstinence rates at the 3 and 6 months follow-up, respectively, without any observable deterioration in their mental health.

George et al. published a study on NRT for smoking cessation in schizophrenia in 2000 [90], where it shown to have a modest effect – the interesting results were the interaction with the patients medication class – those treated with atypical agents (especially risperidone and olanzapine) had significantly better outcome of 55.6% abstinence rates compared to only 22.2% among those treated with typical antipsychotics. This finding was replicated in future study [91].

Introduction of bupropion as treatment for smoking cessation led to further studies in the 2000s on its efficacy and safety in schizophrenia, especially regarding the concern about possible psychotic exacerbation due to its dopaminergic activity.

Several studies published by George et al. and Evins et al. further established bupropion as an effective and safe treatment for smoking cessation in schizophrenia with abstinence rates at endpoint of 50 vs. 12.5% [91] or 16 vs. 0% [92]. When added to NRT, in one study bupropion found to enhance reduction in smoking compared to NRT alone – 60 vs. 31% after 12 weeks, but not to have a significant effect on total abstinence [93]. In another study of 10 weeks treatment, bupropion did found to have an effect on total abstinence, even when added to NRT (27.6 vs. 3.4%) [94].

Odds of achieving abstinence were higher as the age of initiation of smoking was older as well as with less attentional impairment at baseline [95].

18.9 Conclusions and Future Directions

Smoking tobacco carries a substantial risk for harming the central nervous system by accelerated atherosclerosis and ischemic or hemorrhagic stroke. By simply eliminating this risk factor, the risk drops significantly within a short period of time.

After considering the existing evidence for possible neuroprotection against neurodegenerative disorders, the benefit of smoking prevention outweighs the negligible benefits, which are especially evident in protection against PD.

Still, a lesson must be learned from the beneficial effect of smoking as a protective factor against the development of PD and as a symptom mediator in other neuropsychiatric disorders – cognitive enhancer, specifically regarding attention, in schizophrenia and depression, mood alleviator in depression and for the negative symptoms of schizophrenia and possibly as a sensory modulator for the positive symptoms of schizophrenia.

Most of these effects are attributed to the tobacco major psychoactive ingredient, nicotine – its role as an adjuvant therapeutic tool are still need for more substantial evidence than the existing one. One of the challenges is the mode of delivery, since no current delivery mode (nicotine patch, gum etc.) really resemble the delivery mode as a volatile gas in the cigarette smoke.

Further research should target the diversity of the nicotinic receptors subtypes and areas of distribution; with possible more specific targeting than the general affinity that nicotine itself carries. Current promising agent is the nicotinic partial agonist, varenicline, which carries an affinity for its alpha4beta2 sub unit.

But the major challenge would be still tailoring smoking cessation treatments for the majority of smokers which are, after the existing treatments available, still continue smoking or relapsing back into smoking after a short period of abstinence. The proportion of that population is even higher among people with psychiatric disorders – schizophrenia and depression as described in this chapter. People with anxiety disorders, eating disorders and personality disorders also seems to suffer from poorer prognosis regarding smoking cessation and further research should target these populations as well.

Future treatments expand beyond the agonist approach of nicotine and varenicline. Recent research has targeted the glutamate receptors by a partial antagonist as a possible target for relapse prevention with probably negative results. What thought to be a promising agent for smoking reduction by blocking the endocannabinoid system – the CB1 receptor antagonist, rimonabant, showed initial promising results, while later been taken of clinical research due to possible increased risk of depression and suicidality. The most promising target for relapse prevention is by blocking the corticothalamic-pituitary-cortisol pathway which is considered to mediate the relapse mechanism in response to stress, a major risk for smoking relapse.

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Chapter 19
Inhibition of Glycine Transporter-1 Improves the Functional Outcome of Schizophrenia

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Abstract Although current antipsychotic medications are able to reduce psychotic symptoms by blocking dopamine 2 (D_2) receptors, they are limited in their ability to treat certain aspects of schizophrenia, such as flat affect, social withdrawal, and cognitive impairments, all which influence an individual's ability to function in society. For over fifteen years, increasing emphasis has been placed on hypofunction at the N-methyl-D-aspartate receptor (NMDAR) as a potential mechanism underlying the pathophysiology of schizophrenia. Altering NMDAR-mediated neurotransmission by targeting components of the glutamatergic synapse offers a new paradigm by which to generate new drugs to treat schizophrenia. The potential for improving cognitive symptoms, with the ultimate goal of improving functional outcome, is especially alluring given NMDAR's critical role in learning and memory. Several clinical trials have been conducted with agents that enhance NMDAR function by increasing activity at the glycine co-agonist site (GCS). The GCS can be directly activated by amino acids, such as glycine or D-serine, or indirectly, by blocking glycine transporter-1 (GlyT-1) reuptake of glycine from the synaptic cleft. While there have been limited clinical trials of GlyT-1 inhibitors in humans, translational research in animal models has demonstrated the antipsychotic effects and cognitive enhancement of these agents. Thus, the 21st century holds promise as the era in which an entirely new type of medication for schizophrenia may be created, one with more success at treating the debilitating negative and cognitive symptoms of this disease.

Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isozadole-4-proprionate
BDNF	brain derived neurotrophic factor
CATIE	Clinical antipsychotic trials of intervention effectiveness

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_19,

CREB	cAMP response element binding protein
DAAO	D-amino acid oxidase
DAT	dopamine transporters
EPS	extrapyramidal symptoms
GABA	gamma-aminobutyric acid
EAAT	glutamate transporters
GCS	glycine co-agonist site
GlyT-1	glycine transporter-1
LTD	long term depression
LTP	long term ptoentiation
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
MK-801	diclozipine
NFPS	N[3-(4′-flurophenyl)-3-(4′phenyl-phenylphenoxy)propyl]sarcosine
NMDA	N-methyl-D-aspartate
PANSS	Positive and Negative Syndrome Scale
PCP	phencyclidine
PET	positron emission tomography
PICK1	protein interacting C-kinase-1
PSD	postsynaptic density
PPI	prepulse inhibition
RCT	randomized control trials
SR	serine racemase
SNPs	single nucleotide polymorphisms
vGluT1	vesicular glutamate transporter-1

19.1 Introduction

Schizophrenia is a debilitating mental disorder characterized by a set of distinguishing clinical features. The symptoms of schizophrenia can be divided into three clusters. Positive symptoms refer to the psychotic elements of the disease, such as illogical thought processes, hallucinations, and delusions. Negative symptoms reflect the loss of social relatedness, demonstrated by flat affect, withdrawal, and ambivalence. Finally, cognitive symptoms allude to impaired information processing and executive dysfunction often exhibited by patients with schizophrenia [1]. Inherent in the diagnosis of schizophrenia is that these symptoms "impair one or more major areas of function, including work, interpersonal relationships, or self care" [2]. Consequently, patients with schizophrenia are often unemployed, homeless, and stigmatized by society. They frequent inpatient psychiatric hospitals and have repeated encounters with the forensic system. First described nearly a century ago, schizophrenia continues to affect 0.5–0.8% of the population worldwide and to present a pharmacological challenge to treat adequately today [3].

The advent of typical, or first generation, neuroleptic medications in the 1950s, and their success in controlling psychotic symptoms via D_2 receptor blockade,

gave credence to the concept of dysregulated dopaminergic neurotransmission as the underlying pathophysiology of schizophrenia [4]. The dopaminergic hypothesis has subsequently guided most of schizophrenia drug research and development [5]. Atypical, or second generation, antipsychotic medications were first introduced in the 1970s and later proliferated the market in the 1990s. The atypicals were created with the intent having less side effects, such as extrapyramidal symptoms (EPS) and tardive dyskinesia that characterized the high potency first generation neuroleptic medications. In addition, the second generation medications were purported to improve negative symptoms [6]. While their mechanism of action was founded primarily upon D_2 receptor antagonism, they also targeted other receptors as well. For example, clozapine, which is used for treatment resistant schizophrenia, has high affinity for D_4 receptors and also affects serotoninergic, muscarinic, and histaminergic receptors [7]. While atypical antipsychotic medications have become the mainstay of treatment, their efficacy is comparable to those of typical neuroleptics. The CATIE trial also demonstrated that, as with the typical neuroleptic, perphenazine, the atypical antipsychotic medications, namely, olanzapine, was discontinued by patients secondary to side effects. While perphenanize treated patients complained of EPS, olanzapine was associated with weight gain, diabetes, and hyperlipidemia [8].

Although current antipsychotic medications alleviate positive symptoms, their efficacy in terms of negative symptoms and cognitive impairments is less clear. In Hagan and Jones' [9] brief review, they found data that support atypical medications, and to a lesser degree, typical neuroleptics, to improve a variety of cognitive domains, such as verbal fluency, memory, information processing, and executive functioning. Keefe et al. [10] examined the effect of risperidone, olanzapine, and quetiapine on neurocognition in 400 patients with psychotic symptoms less than 5 years duration. There was significant improvement in neurocognition at week 12 for all three treatments, and no significant difference in efficacy among the three medications. There was also significant improvement in functioning as measured by Heinrich-Carpenter Quality of Life scale at weeks 12 and 52. The variance in functional outcome, however, was also predicted by the change in overall symptoms and baseline cognitive functioning. Thus, it is not evident whether risperidone, olanzapine, and quetiapine exerted their effects via improvement in cognition, or via alleviation of general symptoms alleviation. What has remained clear is functional outcome (i.e. tasks of daily living, occupation, interpersonal relationships) for patients with schizophrenia remains dismal [11].

In 1996, Green et al. [12] attempted to identify "rate limiting factors," or specific cognitive deficits of schizophrenia that correspond to specific social deficits that prevent patients from functioning in society. He reviewed 17 studies, which examined the relationship between various cognitive impairments to patients' community outcome, social problem solving, and social skill acquisition. Green et al. found that secondary verbal memory, which requires the ability to retain information, was associated with all types of functional outcome. Deficits in vigilance, which refers to a person's ability to discriminate between what is important or irrelevant, hindered problem solving in social situations and in learning social skills. Succeeding at the

Wisconsin Card Sorting Test, which assesses executive functioning and adapting to situations, is related to whether patients are successful in the community (e.g. social interactions, occupation). Negative symptoms were associated with social interactions, and no association was found between psychotic symptoms and functioning. The studies were limited by the small sample sizes, lack of standardized outcome measures, and differing definitions of neurocognitive deficits. The idea that specific cognitive deficits hinder patients with schizophrenia from being able to learn, retain, and implement skills necessary for real world functioning, and the potential of developing specific interventions to target the deficit has spurred efforts to further characterize the "rate limiting factors" and systemize outcome measures. The National Institute of Mental Health (NIMH) sponsored the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative, which has identified the following cognitive deficiencies in schizophrenia: processing speed, attention/vigilance, working memory, verbal learning, visual learning, reasoning/problems solving, and social cognition. MATRICS emphasized creating cognitive batteries to assess these parameters and the development of appropriate animal models to exhibit these cognitive deficits for translational research [13]. NIMH also sponsored Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) which identified five different social-emotional domains, such as mentalization, based on underlying biological mechanisms identified by functional neuroanatomy, which could be potentially targeted by therapeutic intervention [14].

Over the past decade, research regarding schizophrenia and cognition has increased, and much remains to be elucidated between identifying a specific cognitive impairment and how it ultimately affects an individual's functioning in society. That relationship likely involves many different steps. For example, Fisher et al. [15] recently published a study involving a computer based training program based on the premise that targeting auditory perceptual processes would increase speed and quality of information processing, which would subsequently improve verbal encoding and retrieval. Verbal memory, as mentioned, is implicated in different tasks of daily function. Understanding all the connections that exist has implications for therapeutic intervention, whether it be by pharmacology or other programs, such as cognitive remediation or vocational rehabilitation. Recently, Green [16] described a paradigm for cognitive remediation, which requires developing techniques that patients will use; that will induce lasting improvements in cognitive performance, improve symptoms, and ultimately improve day-to-day functioning.

Despite the emphasis on cognitive symptoms, there are still studies examining the influence of positive and negative symptoms on both cognition and functional outcome. Perlick et al. [17], studied a population of 309 veterans and found that the Positive and Negative Syndrome Scale (PANSS), which measures positive and negative symptoms accounts for 16% of variance in quality of life scores. In a meta-analysis of 73 studies, Venture et al. [18] found that negative symptoms, but not positive symptoms significantly related to functional outcome. They also contended that negative symptoms mediated the relationship between neurocognition and functional outcome. Finally, Mohammed et al. [19] found that positive

symptoms accounted for 6% of variance and neurocognition 5% of variance of quality of life total scores.

In this context of current antipsychotic medications, limited efficacy, their side effect profile, and the continued morbidity secondary to poor functioning, drug development for schizophrenia treatment is primed for the creation of a novel drug that operates by a mechanism other than $D_2/5HT_2$ antagonism and that intervenes in the relationship between initial cognitive deficit and subsequent impairments in societal functioning.

Over the past fifteen years, there has been increasing focus on the role of NMDAR mediated glutamatergic neurotransmission in the pathophysiology of schizophrenia. This chapter will discuss in detail the putative role of NMDAR hypofunction in producing symptoms of schizophrenia and the relevant components of the glutamatergic synapse. Consequently, the new paradigm for drug development has been focused on enhancing activity at the NMDAR. Of particular interest is the glycine co-agonist site (GCS), which can be activated directly, by glycine or D-serine, or indirectly by inhibiting glycine transporter-1 (GlyT-1) reuptake of glycine from the synaptic cleft. While clinical trials of GlyT-1 inhibitors have been limited, animal studies show potential of GlyT-1 inhibitors as having antipsychotic characteristics and ability to enhance cognition via the ability to correct a hypofunctioning NMDA state. As cognition, and in some studies, general psychopathology, predict how patients manage activities of daily life, GlyT-1 inhibitors offer the potential to improve functional outcome in schizophrenia.

19.2 N-methyl-D-aspartate Receptor (NMDAR) Hypofunction and Schizophrenia

The hypothesis that the pathophysiology of schizophrenia could be explained by NMDAR hypofunction was first proposed based on the observation that dissociative anesthetics, such as ketamine and phencyclidine (PCP), could replicate the full spectrum of symptoms consistent with schizophrenia in healthy individuals. This was in contrast to drug model of schizophrenia induced by amphetamine, which through increased dopamine release, only mimicked psychosis, represented by animal models as hyperactivity and stereotyped behaviors [20]. In addition to creating, or in patients with schizophrenia, exacerbating psychotic symptoms, PCP caused withdrawal, catatonia, and flattened affect. In rodents treated with PCP, these negative symptoms are assessed by disruption in social behavior and immobility. Finally, individuals treated with PCP exhibited cognitive impairments, including disrupted attention and memory [21]. Animal models are analogously tested for sensorimotor gating, working memory, spatial memory, and associative learning [20]. Dissociative anesthetics also produce physiologic findings seen in patients with schizophrenia. Ketamine, for example, impairs prepulse inhibition, evokes abnormal cortical event-related potentials, and causes abnormal eye saccade movements [4, 22–27].

PCP and ketamine are both use dependent, noncompetitive antagonists of the NMDAR, binding to a site within the ion channel. Thus, as PCP and ketamine exerts their effect by blocking NMDAR, it follows that an endogenous reduction of NMDAR mediated neurotransmission could be the underlying cause of schizophrenia [28, 29].

19.3 N-methyl-D-aspartate Receptor (NMDAR): Structure and Function

NMDAR is an ionotropic glutamate receptor. It is named after NMDA, which has a high affinity to the receptor. There are 3 types of subunits, NR1, NR2, and NR3, which combine in sets of four subunits, to form the NMDAR. NR2 is encoded by four different genes, A, B, C, and D. NR3 is encoded by two different genes, A and B. The subunit structure determines characteristics of the ion channel, such as which ions—Na^+, K^+, or Ca^{2+} the channel is preferably permeable to [30]. All NMDARs must have a NR1 subunit to function. NR2 units influence channel permeability, duration of channel opening, and sensitivity to Mg^{2+} blockade. NMDARs at adult CNS synapses are usually composed of two NR1 subunits paired with two NR2A subunits. The type of subunit expressed changes during development and depends on the location of the NMDAR [31]. NR2B, for example, is more prominent in early development and has a higher proportion in CA1 pyramidal neurons. NMDAR subunits also determines the affinity of NMDAR for glutamate. Kalbaugh et al. [32] studied NMDAR mediated excitatory postsynaptic currents (EPSCs) depolarization in rat retinal ganglion cells, and found that synaptic response to light stimulus, in the "ON" pathway was significantly reduced in the presence of Ro- 25–6981, a NR2B antagonist. EPSCs in the retinal ganglion are also reduced when release of glycine or D-serine are blocked. NR3 is allegedly involved with decreasing NMDAR activity, given that it is less permeable to Ca^{2+}.

NMDAR mediated activity is both ligand and voltage dependent. NMDAR requires two agonists to be activated: L-glutamate, the primary excitatory neurotransmitter of the mammalian brain binds to NR2, and glycine or D-serine, an amino acid, binds to NR1 at the GCS. Furthermore, the closed channel is physiologically blocked by Mg^{2+}, which must be removed prior to NMDAR activation [33]. A second type of ionotropic glutamate receptor, alpha-amino-3-hydroxy-5-methyl-4-isozadole-4-proprionate (AMPAR), is co-localized with NMDARs and creates the initial depolarization that removes the Mg^{2+} block [34]. When the channel is open, it is subject to the aforementioned, noncompetitive antagonists, such as PCP, ketamine, and MK-801 (diclozipine), which bind to residues within the channel wall [29]. In addition, NMDARs can also be modulated by zinc, polyamines, and protein kinases [30].

NMDARs are found both on pre-synaptic and post-synaptic neurons. The postsynaptic membrane is where the NMDAR of interest resides. Once open, the NMDAR ion channel is permeable to Ca^{2+}[31]. The NMDAR, associated via the NR2

subunit, is part of the postsynaptic density (PSD) complex in excitatory synapses. The PSD is a physically thickened area composed of a network of scaffolding proteins that convert the glutamate signals into secondary messengers that lead to persistent changes (i.e. gene transcription) and provide signals for trafficking receptors, such as recruiting additional AMPARs [30, 31, 35]. Activation of postsynaptic NMDARs can also upregulate transcription factors such a cAMP response element binding protein (CREB), which promote trophic factors, such as brain derived neurotrophic factor (BDNF) [36].

NMDAR mediated glutamate plutamatergic transmission plays a critical role in neurodevelopment and synaptic plasticity, and thus, is further implicated in the pathology of schizophrenia. After a period of neurogenesis, subsequent brain development is guided by cell differentiation, proliferation, and migration. During this time, NMDARs are exquisitely responsive to fluctuations in glutamate concentration. Activation by glutamate can cause outgrowth and development of synaptic connections. Hyperactivation of NMDAR results in excitotoxicity. Understimulation of NMDAR can lead to apoptosis, which eliminates redundant synapses [37]. Perinatal exposure to NMDAR antagonists, such as PCP, results in cell death, impaired cognition, sensorimotor gating, and abnormal D_2 receptor expression.

Du Bois et al. [38] studied patterns of NMDAR and γ-aminobutyric acid receptor (GABAR) binding in the hippocampus, prefrontal cortex, and anterior cingulated cortex of rats treated with PCP on postnatal day 7, 9, and 11. NMDAR and GABAR similarly showed increased binding in the thalamus and hippocampus throughout life, while there was increased receptor binding in the prefrontal cortex and anterior cingulate only in adolescence. PCP induced cell death may result in upregulation of the receptors. Either hyperstimulation or NMDAR blockade can lead to the consequences of disrupted circuits, such as a loss of NMDAR neurons, which may re-emerge later, during adolescence, when there is a high rate of synaptic pruning i.e. pervasive changes in receptor densities. The sensitivity of neurons to NMDAR blockade also depends on the age of development. Rats are particularly sensitive to NMDAR toxicity during postnatal days 7–14. This could be consistent with the neurodevelopmental model of schizophrenia, whose premises that an insult early in development, subsequently remerges at a later age, under inauspicious environmental influences [39]. Antagonism at NMDAR, resulting in neuronal loss, can have severe long term detrimental effects such as reduced functional connections producing a hypofunctional NMDAR state [40].

The ability of NMDAR mediated glutamatergic neurotransmission to change neuronal connectivity and adapt to environmental stimuli is related to concept of "synaptic plasticity," including long term ptoentiation (LTP) and long term depression (LTD) [31]. LTP describes how, via NMDAR excitatory potentials, repeated external stimulation of the same synapse causes an enhanced response over a prolonged period of time. The neurons fire more efficiently, or, the synaptic "strength" is increased. Synaptic strength is affected by frequency of glutamate release and by the number and characteristics of the postsynaptic NMDARs [30]. LTP, mediated by NMDAR in the hippocampus, is the neuronal model for learning and memory. Neurotransmission at NMDAR can be increased or decreased, and dysregulation of

NMDA receptors can lead to pathology. The subunits that form the NMDARs and the number of NMDARs at synapse change during development and in response to neuronal activity [41].

The cognitive deficits observed in schizophrenia are sometimes described as "deficit symptoms," which are consistent with frontal lobe dysfunction. These symptoms include apathy, difficulty planning, and inability for abstract reasoning [42]. These tasks rely heavily on the ability of an individual to use stored information, to associate present situations with past experiences in order to guide behavior. These tasks, which involve memory formation and learning, are localized to the prefrontal cortex and are associated with the bidirectional modulation that occurs between D_1 receptors and NMDARs [43]. Patients with schizophrenia demonstrate decreased function in the prefrontal cortex. A recent meta-analysis looked at 36 studies through February 2008 that measured activity via functional MRI (fMRI) and positron emission tomography (PET) in healthy individuals and patients with schizophrenia. During the formation of episodic memory, which involved encoding, consolidation, and retrieval of pictures or word lists, patients with schizophrenia had less activity in the prefrontal cortex [44]. NMDAR function thus plays a critical role in maintaining cognitive processes that are disrupted in patients with schizophrenia who often present with negative symptoms refractory to treatment.

On the cellular level, NMDAR hypofunction manifests as complex interactions between multiple neurotransmitter systems, such as glutamate, dopamine, acetylcholine, and GABA. The role of these different neurotransmitters may be related to the different types of symptoms seen in schizophrenia. NMDAR blockade can lead to excessive release of glutamate through GABA interneurons [28]. The glutamatergic dysregulation which accounts for symptoms of schizophrenia does not exclude the role of dopamine dysfunction in the neurochemical basis of schizophrenia. In fact, NMDAR hypofunction may lead to dopaminergic dysfunction, depending on the anatomic location of NMDAR, and possibly, the time course of NMDAR antagonism. When given to healthy volunteers, ketamine produces an increase of metabolic activity in the prefrontal cortex when viewed with PET, that corresponds to an acute psychotic state. Research has shown that there is a resulting increased subcortical dopamine release [45, 46]. Study of nonhuman primates seems to indicate a difference in DA levels depending on acute versus chronic administration of the NMDA noncompetitive antagonist, MK-801. Acutely, PET shows increased in extracellular levels of glutamate and dopamine. With daily administration of MK-801, however, glutamate and DA levels are lowered. There is also a change in affinity of dopamine receptor in the prefrontal cortex. Acute treatment of MK-801 resulted in decreased binding at D_2 receptors and chronic MK-801 administration shows increased binding at D_1 receptors. In both cases, the subjects displayed impaired working memory performance [47].

Dysregulated glutamatergic neurotransmission may also affect activity of GABA neurons. Consistent with the other dissociative anesthetics, PCP affects the mesolimbic dopamine pathway preferentially [48]. Schiffer et al. [49] performed a PET study in nonhuman primates that suggested PCP exerted its effects on DA via GABA neurons and blocking dopamine transporters (DAT). Their study showed

that effects of PCP could be attenuated by GABA agonists and competitive inhibition of DAT. This suggests that antagonism of NMDA receptors on GABA neurons results in decreased glutamatergic neurotransmission at GABA neurons. GABA is a major inhibitory neurotransmitter in the brain. Decreased GABA activity subsequently results in decreased inhibition, or net excitement, of other neurons. GABA neurons modulate other excitatory neuronal circuits, such as cholinergic pathways in the basal forebrain and glutamatergic pathways in thalamus. In addition, some noradrenergic and serotoninergic neurons also function in an inhibitory fashion, when their NMDARs are activated. The net result of decreased NMDAR activity mainly on GABA neurons, and possibly, noradrenergic neurons and serotoninergic neurons, is increased activity at cholinergic and glutamatergic neurons. This ultimately causes increased glutamatergic neurotransmission in the cortex, resulting in a hyperexcitable state, which produces psychotic symptoms [50]. The relationship between dopamine, GABA, and glutamate is demonstrated in the prefrontal cortex. Prefrontal cortex hypofunction, is likely secondary to a reduction in glutamatergic output to GABA neurons in the ventral tegmentum. Furthermore, GABA regulates dopamine activity, and in the absence of GABA inhibition, it can cause an exaggerated response of dopaminergic neurotransmission in the mesoaccumbens causing psychotic symptoms [43].

The theory that dysregulated glutamatergic neurotransmission secondary to hypofunction of NMDARs causes the symptoms of schizophrenia offers a new paradigm for drug research and development. As discussed, there are multiple neurotransmitters systems involved, but the most straightforward goal would be to restore NMDAR functioning to baseline. As with the case of the dopamine blockading agents, if drugs were developed that alleviated the symptoms of schizophrenia via increasing NMDAR signal transmission, this would provide further credence to the NMDAR theory of schizophrenia. The involvement of NMDAR mediated glutamatergic neurotransmission in memory formation is alluring as, perhaps, pharmacotherapy can be effective in treating the cognitive symptoms of schizophrenia, and ultimately, improve functional outcome. Over the past two decades, academic researchers and pharmaceutical companies have begun testing potential agents that enhance NMDAR signal transmission in humans and in animal models [51]. Even in considering the approach "of systemic stimulation of the NMDA receptor," the possibilities of therapeutic intervention remain vast [52].

19.4 The Glutamatergic Synapse: Components and Relationship to Schizophrenia

The glutamatergic synapse is composed of the presynaptic terminal, the postsynaptic dendrite, and the encapsulating astrocyte. Some of the receptors and ligands, with their associated enzymes and transporters, located at the synapse, have genetic linkage association with schizophrenia and may be potential targets for the development of drug therapy (Fig. 19.1).

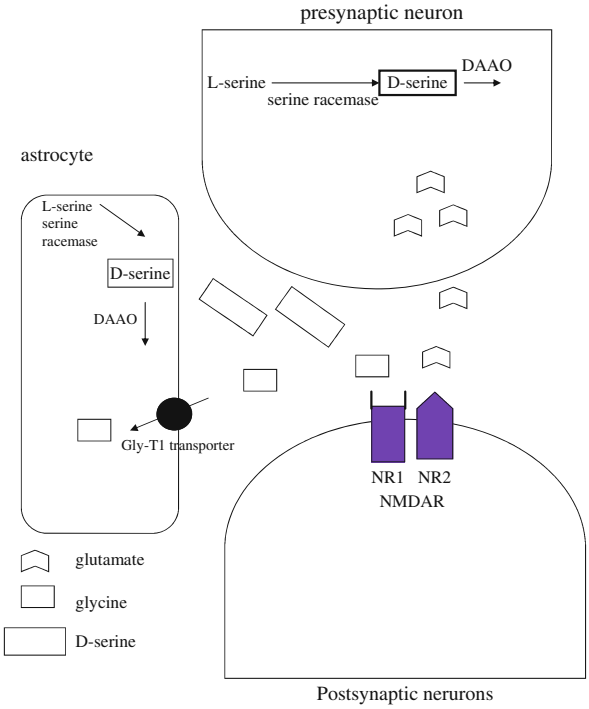

Fig. 19.1 The glutamatergic synapse. The three main components of the glutamatergic synapse are the presynaptic neuron, the astrocyte, and the postsynaptic neuron. The NMDAR is shown at the postsynaptic membrane, composed of two subunits, NR1 and NR2. It is activated by glutamate and glycine or D-serine. The glycine transporter 1 D-serine is located in the astrocyte, maintaining glycine concentration at subsaturating levels at the synapse. The astrocyte also contains the enzymes, serine racemase and D-amino-acid oxidase (DAAO), that regulate levels of D-serine. DAAO and serine racemase are also found in neurons

Glutamate Receptors. The characteristics of NMDARs have already been described. As discussed, they are located both on the presynaptic and postsynaptic neuron. NMDAR subunit expression are regulated by the gene, neuregulin-1 (NRG1), that encodes the protein, neuregulin-1, which acts on erbB4, a tyrosine receptor [37]. Stefansson et al. [53] first demonstrated an association between NRG1 and schizophrenia in an Icelandic population. The results have been confirmed in several follow-up studies of Scottish, Northern European, Han Chinese, and Portuguese populations [54–58]. Neuregulin-1 and erbB4 have been shown to play a crucial role in glutamatergic synapse maturation and plasticity [59], and NRG1 suppression of NMDA receptor activation was greater in schizophrenics than in controls as measured in post-mortem tissue samples [60]. NRG1 decreases NMDAR mediated neurotransmission in the prefrontal cortex and hippocampus [37]. NRG1 has also been show to reverse LTP in the hippocampus. This effect can be prevented by erB receptor inhibitors [61].

Presynaptic NMDA receptors, (preNMDARs) have a role in enhancing neurotransmitter release [62]. Dysbindin (6p22.3) is located in the preNMDAR complex and modulates release of glutamate. Straub et al. [63] reported a strong association between single nucleotide polymorphisms (SNPs) in the 140-kb gene and schizophrenia in their sample of 270 Irish pedigrees. Schwab et al. [64] replicated the above findings in a sib-pair sample and sample of triads comprising 203 families.

Post-mortem comparison between schizophrenic patients and matched controls showed reductions in presynaptic dysbindin-1 in intrinsic glutamatergic terminal within the hippocampus, thus implicating dysbindin dysfunction with glutamatergic dysfunction [65]. Additionally, dysbindin has been associated with both the severity of cognitive decline and the presence of negative symptoms in schizophrenic subjects [66, 67].

Metabotropic glutamate receptors (mGLURs) are the second type of glutamate receptors that are also found at glutamatergic synapses. Unlike the ionotropic glutamater receptors, mGLURs are guanine nucleotide binding [G] protein coupled receptors [31]. There are 8 subtypes of mGluRs, classified into 3 groups based on signaling pathways, pharmacology properties, and genetic homology (Table 19.1). mGluRs have a role in promoting NMDAR-mediated neurotransmission. Of particular interest are Group 1 receptor, mGluR5, and Group 2 receptors, mGluR2 and mGluR3. mGluR5 is found near synaptic dendritic spines in the cortex, and hippocampal. mGluR2 and mGluR3 are located both pre and postsynaptically on glutamatergic and GABAergic neurons. mGluR3 is also found in glia [68]. Group 1 mGluRs increase presynaptic glutamate release, while Group 2 mGluRs decrease presynaptic glutamate release. mGluR3 has been shown an association with schizophrenia in at least three independent studies [69]. N-acetylaspartylglutamate (NAAG) is a potent and specific activator of mGluR3, which downregulates glutamate release and may be implicated in NMDA receptor hypofunction [70, 71].

The first study linking mGluR3 to schizophrenia showed a significant association in a German sample of 265 schizophrenic patients when compared to 227 controls, but this finding could not be replicated in a second sample of schizophrenic patients, controls, and schizophrenic trios [72]. However, Fujii et al. [73] genotyped 100 Japanese schizophrenic patients and 100 controls for 6 SNPs and found a significant association with a SNP and with three haplotypes containing that SNP. Egan et al. [74] found a significant association between schizophrenia and mGluR3 haplotype in a sample of 335 American families. The allele was associated with poorer performance on cognitive tests of prefrontal and hippocampal function, even in normal

Table 19.1 Two classes of glutamate receptors[a]

Ionotropic	Metabotropic	
NMDA (NR1, NR2A-D, NR3A,B)	Group 1	mGluR1, **mGluR5**
AMPA (GLUR1–4)	Group 2	**mGluR2, mGluR3**
Kainate (GLUR5–7)	Group 3	mGluR4, mGluR6, mGluR7, mGluR8

[a]There are two main types of glutamate receptors, ionotropic and metabotropic. The three classes of the ionotropic receptors are named after their high affinity agonists: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isozadole-4-proprionate (AMPA), and kainate. There are three groups of metabotropic glutamate receptors, in which the 8 receptor subtypes are classified depending on their characteristics. Group 1 uses both phospholipase A&C and adenylyl cyclase secondary messenger systems, while Group 2 and 3 depend on adenylyl cyclase [30]. The receptors of interest for targeting in treating schizophrenia have been bolded.

subjects, and homozygotes showed lower levels of prefrontal N-acetylaspartate. A more recent study confirmed that schizophrenic patients homozygous for the allele, initially identified by Egan et al., showed decreased NAA levels in dorsolateral prefrontal regions when compared to controls [75]. These studies provide evidence of mGluR3 as a susceptibility gene in schizophrenia and suggest that the mechanism involves prefrontal and hippocampal glutamatergic neurotransmission.

Ligands and their Transporters. Glutamine is stored in the surrounding astrocyte for synthesis of glutamate by glutaminase. Glutamate is packaged into presynaptic secretory vesicles by a group of vesicular glutamate transporters (vGluT1-vGluT3). Glutamate is stored in these vesicles until the presynaptic neuron depolarizes, and releases glutamate into the synaptic cleft [76]. In some locations, such as the CA1 hippocampal slices, a neuropeptide, NAAG is also released and functions as an antagonist by decreasing NMDAR mediated currents [77]. In addition, glutamate transporters EAAT (EAAT 1 and 2) regulate the availability of glutamate in the synaptic cleft, thereby protecting against excitotoxicty by ending the glutamatergic signal [78].

The endogenous amino acids of glycine, D-serine, and D-alanine are obligatory co-agonists of the NMDAR at the GCS. They regulate NMDAR mediated transmission, as they promote NMDAR's affinity for glutamate and lower the threshold for depolarization of the postsynaptic neuron [79]. In addition, their presence decreases the strength of Mg^{2+} blockade and desensitization of NMDARs.

Glycine is an endogenous amino acid that functions as a neurotransmitter in humans. Glycine is synthesized from the conversion of L-serine by the enzyme, serine hydroxymethyltransferase, and the glycine cleavage system [80]. As part of the glutamatergic synapse, glycine acts on strychinine insensitive receptors (glycine B binding site) and participates in excitatory neurotransmission [81]. In more caudal areas of the CNS, glycine primarily functions in inhibitory processes, via strychinine sensitive receptors (glycine A binding site), increasing Cl^- conductance, thereby hyperpolarizing neurons [77]. Through this mechanism, glycine coordinates reflex responses in the brain stem and spinal cord. During development, glycine also participates in signaling between neurons and oligodendrocyte progenitor cells [80, 82].

Glycine transporters are 12 transmembrane Na^+/Cl^- coupled transporters that are encoded by two genes, GlyT-1/SLC6A9 and GlyT-2/ SLC6A5. GlyT-2 are thought to be predominantly located near the inhibitory synapses. Unlike GlyT-1, GlyT-2 is not inhibited by sarcosine [83].

GlyT-1 has five variants, a-e, distinguished by different combinations of their N and C terminal exons. GlyT-1 is found mostly in astrocytes, located in the hippocampus, cortex, and cerebellum. GlyT-1 mRNA has been localized in the neurons of cerebellum, cortex, and hippocampus [83, 80]. Regulation of glycine transporters are likely dependent on protein kinase phosphorylation, involving protein kinase C, protein kinase A, and calmodulin dependent protein kinase. GlyT-1 is co-localized with NMDARs, associated by the scaffolding protein, PSD95 [84, 81]. The distribution of GlyT-1 is uneven along the cell membranes, to optimize glycine clearance from the synaptic cleft and to maintain subsaturating concentrations of glycine at the

glutamatergic synapse [83]. At inactive synapses, extracellular glycine concentration is 0.1–0.2 uM. Intracellular glycine concentrations can be up to 2 mM. Glycine transporters are characterized by their function in substrate translocation and as an ion channel [80]. GlyT-1 exchanges 1 glycine for 3 Na^+ and 1 Cl^-. Because GlyT-1 relies on concentration gradients, it can also work in reverse mode, releasing glycine into the synaptic cleft [83, 80]. This can occur when there is an increase in intracellular Na^+, allowing glycine to be transported backdown the concentration gradient, if there is an addition of external glycine, or if there is local membrane depolarization [80].

Although several knockout rodent models of GlyT-1 have been created, there is no established genetic defect of GlyT-1 and schizophrenia in humans [82]. Tsai et al. [85] examined four GlyT-1 SNPs in 249 patients with schizophrenia and 210 controls in a Chinese population. They found no significant difference in the frequency of these polymorphisms and no association to schizophrenia. In a Japanese population, Deng et al. [86] examined the association of 21 SNPS in 100 case-control pairs and found a nominally significant SNP associated with schizophrenia near SLC6A5. Finally, four SNPs associated with GlyT-2, which tend to be localized to the nonglutamatergic synapses, were examined in 328 patients with schizophrenia and 307 controls in a German population. Again, there was no common genetic variant associated with schizophrenia [87].

D-serine is an endogenous amino acid that has been characterized as a glial neurotransmitter [79]. It is co-localized with NMDARs, found in the forebrain and pyramidal cells of the cortex and hippocampus. D-serine is made from L-serine by serine racemase (SR), which was initially found in astrocytes, though SR mRNA is also found in neurons [88]. D-serine is released from astrocytes after NMDAR activation. At the glutamatergic synapse, it displays three times higher affinity for the GCS than glycine [79, 88]. Consistent with its role as a coagonist at NMDA receptor, D-serine mediates LTP and functions in granule cell migration. It has also been proposed that a dysfunction in SR would cause a reduction in D-serine, resulting in NMDA receptor hypofunction, indicating an association with schizophrenia. A genetic variant in the promoter region of the gene encoding SR lowers the expression of the gene is associated with schizophrenia, especially the paranoid type [89]. SR has been implicated as a risk gene for schizophrenia through its interaction with protein interacting C-kinase (PICK1). PICK1 is located on chromosome 22q13, a region frequently linked with schizophrenia, and in a study of schizophrenic subjects the PICK1 gene was associated with schizophrenia [90].

D-serine is metabolized by D-amino-acid oxidase (DAAO) [91]. DAAO oxidizes D-amino acids, including D-serine and D-alanine, and is preferentially found in the liver and kidneys, as well as the brain [92]. Given that there are reduced levels of D-serine in patients with schizophrenia, there is thought that this is caused by increased metabolism of D-serine [93–95]. DAAO has been linked to risk for schizophrenia in several studies [96]. Studies have shown a correlation between DAAO levels in the hippocampus of schizophrenic individuals and the duration of their illness [97], as well as increased levels of DAAO activity in patients with schizophrenia when compared with controls [98]. Activity of DAAO correlates

inversely with levels of D-serine both regionally and developmentally [93, 94], and low levels of D-serine result in NMDAR hypofunction [99]. G72 codes for a protein that is a regulator of DAAO. G72 are of recent evolutionary appearance [92], and were discovered by Chumakov et al. [100] in a chromosomal region previously associated with vulnerability to schizophrenia. Genetic variations of G72 and DAAO had been reported to be associated with schizophrenia and bipolar disorder.

19.5 Human Trials of Agonists for Glycine Coagonist Site

The components of the glutamatergic synapse offer several areas of potential intervention in order to increase NMDAR-mediated neurotransmission. The most straightforward way to increase activity at the NMDAR would be through increasing the available glutamate. However, excessive glutamatergic activity leads to cellular excitotoxicty, which causes neuronal injury and death [101]. Clinically, excessive NMDAR function is linked to acute ischemic stroke, traumatic brain injury, and neuronal loss in neurodegenerative diseases such as Alzheimer's and Parkinson's Disease [36]. Excess glutamate can cause damage via accumulated intracellular calcium and inhibition of the cystine-glutamate exchanger. The cystine-gluatmate exchanger is located in astrocytes and functions in glutamate reuptake. Increased levels of extracelluar glutamate prevents cellular uptake of cystine. When cystine is unavailable, it cannot be incorporated into the production glutathione, an antioxidant. The combination of extracellular glutamate and oxidative stress disrupts cell membrane lipids, gene expression, and protein signaling [52].

As increasing the amount of glutamate to activate NMDAR is not a viable option, the focus has been on intervening at NMDAR's GCS. GCS is located on the NR1 subunit of the NMDAR and can be activated by full agonists—glycine, D-serine, D-alanine—or the partial agonist, D-cycloserine. Even if NMDAR dysfunction in schizophrenia is not due to dysfunctional GCS per se, increasing the concentration of agonists at this site should increase NMDAR activity [51]. Low GCS function is evident in the mouse model, $Grin1^{D481N}$ which has a fivefold decrease in glycine affinity at the GCS and manifests symptoms analogous to those seen in schizophrenia, including: decreased LTP, increased startle, decreased anxiety, and impairments in learning and memory. A recently published study of these mice demonstrated the mice also had difficulty with latent inhibition, social interactions, and spatial recognition. Supporting the GCS approach, these impairments were reversed by treatment with GCS full agonist, D-serine [102].

While the focus in schizophrenia treatment is promoting GCS activity, glycine has also been observed to cause NMDAR mediated excitotoxicity. As such, there is interest in reducing glycine activation in order to treat ischemic or hypoxic brain damage. Adding glycine to NMDA in primary rat cortical cultures results in at least 20% greater cell death than for neurons exposed to NMDA alone. Glycine is thought to exert its toxicity first by allowing activation of NMDAR and second, via actions on the strychinine sensitive receptors, by increasing Cl^- load, which disrupts

intracellular pH balance and causes neuronal swelling [103]. In another study, however, Schleper et al. [104] found D-serine played a role in NMDAR mediated cell neurotoxicity. In rat organotypic hippocampal slices pretreated with D-serine deaminase, which removed D-serine, and subsequently exposed to NMDA, there was no resultant cell death. Excitotoxicity did not occur in the presence of glycine, until GlyT-1 was inhibited, thereby increasing glycine concentration, suggesting that the reuptake transporter prevents glycine's role in excessive NMDAR activity causing cell death. These regulatory mechanisms of glycien and D-serine can prevent their toxicity. However, the idea of excessive glycine and D-serine mediating cell death may need to be considered when increasing NMDAR activity to treat schizophrenia.

Since the 1990s, there have been more than twenty published randomized control trials (RCT) conducted in humans that involve enhancing NMDA neurotransmission by directly activating GCS using glycine, D-serine, D-alanine, or D-cycloserine. Nearly all the trials were short term, lasting less than 12 weeks. The number of patients ranged from 7 to about 50 per treatment group. The majority of participating patients had been stable on an antipsychotic regimen, but still had refractory symptoms. GCS agonists were added for patients on typical and atypical neuroleptics. The majority of trials were double-blind and placebo controlled. There were some cross-over study designs. Most trials used the following scales to assess efficacy of the GCS agonist on symptoms: BPRS, PANSS which includes a cognitive subscale that measures symptom reduction related to neurocognition, and SANS. There was focus on gauging EPS, most commonly assessed by the Simpson Angus Scale (SAS) and Abnormal Involuntary Movement Scale (AIMS). These trials have yielded predominantly positive findings, as well as negative and equivocal results. The positive findings tend to show that when GCS agonists are added to an existing regimen of antipsychotic medications for stable chronic schizophrenic patients, there has been improvement in symptoms. Early studies showed improvement in negative symptoms, specifically. Later studies showed an effect on positive, cognitive, and depressive symptoms. In general, GCS agonists were well-tolerated, and no consistent systemic side effects were noted in the trials. Although further studies are required, the initial evidence gives hope that this approach may indeed yield the development of an efficacious treatment for schizophrenia [51].

In 2005, Tuominen et al. [105, 106] published a meta-analysis of 18 short-term RCTs of NMDA agonists used as adjunctive treatment, with a total of $N = 358$ participants. Studies were obtained through searching the Cochrane Schizophrenia Group's registry between May 2002 and October 2003. Selected trials had been completed between 1992 and 2002, and each study lasted at least 2 weeks. Patients were predominantly male (>74%) with average age 39.8. They tended to be inpatients, treatment refractory, and stable on an antipsychotic regimen. There were seven studies of glycine, seven studies of D-cycloserine, and two studies of D-serine. Glycine doses ranged from 15 g to 60 g/day, D-cycloserine doses from 10 mg to 50 mg/day, and D-serine was dosed at 30 mg/kg/day. The pooled data showed that glycine and D-serine had moderate effect on reducing negative symptoms of schizophrenia (N = 132). This correlated to a four point decrease for the PANSS-negative subscale. Tuominen et al. noted a trend, though not statistically

significant, of glycine and D-serine's effect on improving cognitive symptoms. D-cycloserine was less effective in reducing negative symptoms (N=119). There was no consistent significant occurrence of systemic side effects reported across trial. Again, this data is limited, given sample size and length of trial.

More recently, Tsai and Lin [107] performed another meta-analysis of agents used to increase activity at the GCS, termed "NMDA-enhancing agents." They covered a longer time period than Tuominen et al, ranging between 1996 and July 2008 and included 26 double-blind, placebo-controlled trials, with a total of 802 participants. They included studies of glycine, D-cycloserine, D-alanine, D-serine, and sarcosine (N-methylglycine). Sarcosine is an inhibitor of GlyT-1, which prevents glycine reuptake, thereby increasing the amount of glycine available for the GCS. Sarcosine is the lead compound for the development of GlyT-1 inhibitor.

Tsai and Lin's goal was to analyze overall efficacy of NMDA enhancing agents, compare the efficacy among the different agents, determine whether the antipsychotic regimens affected efficacy, and note side effects. Efficacy was calculated by determining the effect size, which described the degree to which improved symptoms were secondary to NMDA enhancing agents versus placebo. They examined five symptom domains in addition to overall psychopathology: negative symptoms, positive symptoms, cognitive symptoms, depressive symptoms and general psychopathology. Negative symptoms were rated by SANS or PANSS-negative subscale. Positive symptoms were rated by BPRS- or PANSS-positive subscale. Cognitive symptoms were measured by PANSS-cognitive subscale. Depressive symptoms were rated either by PANSS-depressive subscale or the Hamilton Depression Rating Scale (HDRS). General psychopathology was assessed by the PANSS-general subscale. Total psychopathology was assessed by PANSS or BPRS. Side effects were measured by AIMS and SAS. The effect size of the pooled data was small to medium range, similar to atypical antipsychotic medications. For total psychopathology, effect size was 0.4. As per symptom domain, in decreasing order of effect size: depressive symptoms, 0.4; negative symptoms, 0.38; cognitive symptoms, 0.28; positive symptoms, 0.26; general psychopathology, 0.26. Finally, EPS symptoms were unchanged by the NMDA-enhancing agents.

It is important to mention the relationship of NMDA-enhancing agents and clozapine. To date, all trials in which NMDA-enhancing agents were added to patients on clozapine have yielded negative results. Diaz et al. [108] published 28 week crossover trial in which high dose glycine (60 g/day) or placebo was added to clozapine (mean dose = 575 mg) in 12 inpatients. There was no benefit or exacerbation noted in patients treated with clozapine. D-serine was also used adjunctively with clozapine in a 6 week trial. There were no significant improvements or exacerbations found when D-serine was used to augment clozapine [109]. Finally, sarcosine at 2 g/day did not produce any additional improvement when added to clozapine [110].

The ineffectiveness of NMDA enhancing agents when used in combination with clozapine has been theorized to be due to clozapine's inherent effect on glutamatergic neurotransmission. Animal studies have shown that clozapine increases glutamate release, potentiates NMDA transmission, and can reverse effects of

NMDA antagonists [111]. Recent animal studies have tried to elucidate the mechanism by which clozapine affects glutamate transmission. For example, clozapine increases glutamatergic neurotransmission through calcium/calmodulin kinase II, and clozapine down regulates glutamate transporter 1, thereby increasing extracellular glutamate [112, 113]. Clozapine also demonstrates the ability to block MK-801 induced increases in both glutamate and serotonin mediate neurotransmission [114]. Nevertheless, it is premature to consider that a ceiling effect prevents GCS agonists to elicit further efficacy because clozapine is already altering NMDARs. It is likely the characteristics of clozapine treated patients render the treatment resistance to NMDA agents. Clozapine treated patients may have severe NMDA deficits that they will respond to the treatment once NMDA activation can be achieved.

19.6 Glycine and D-Serine

Given the moderate effect size of trials of NMDA enhancing agents in humans, these studies lend support to the concept of targeting the GCS for developing drugs to treat schizophrenia (Fig. 19.2). Two of these direct GCS agonists, glycine and D-serine, will be discussed in detail. Glycine was the first GCS agonist to be studied. D-serine has been noted to most approximate a marketable drug [115].

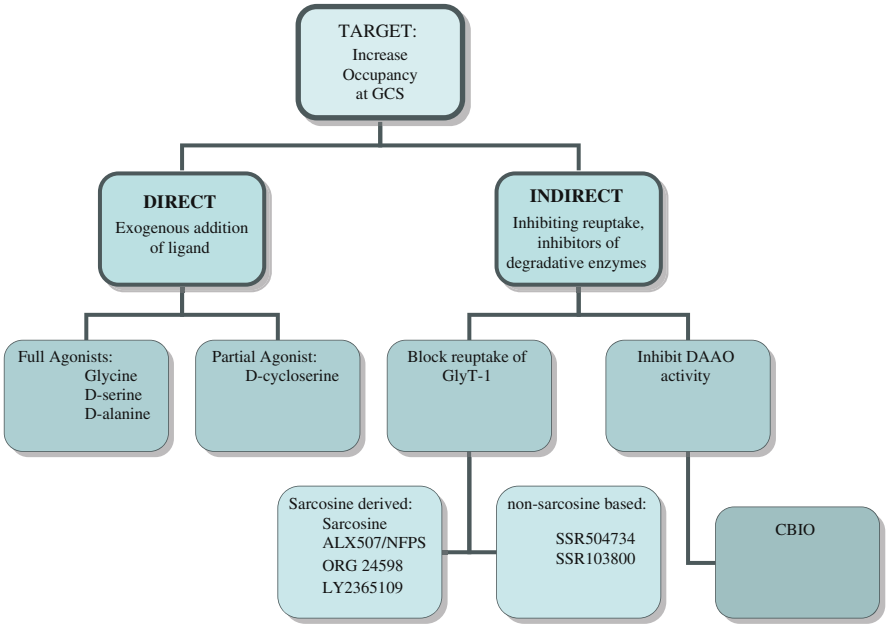

Fig. 19.2 Strategies and molecules that increasing activity at the glycine co-agonist site

Glycine is an amino acid. The average daily diet contains about 2 grams of glycine. At this amount, glycine has minimal effect on the CNS. At higher doses, as those targeted in drug trials, sufficient quantities of glycine crosses the blood brain barrier to raise glycine levels in the CNS [116]. In 1988, Waziri [117] reported, in an open label study, the use of daily doses of glycine 5–25 g, to treat 11 patients with chronic schizophrenia. Patients were maintained on glycine alone. The improvements noted in psychosis and psychosocial functioning were based on clinical observations [111]. Rosse et al. [118] also published a small study in 1989 of glycine 10.8 g daily, administered adjunctively to 6 patients, duration of treatment ranging from 4 days to 9 weeks. Findings were equivocal – some patient exhibited improved behavior, and others had no change.

Beginning in the early 1990s, RCTSs of glycine, with doses ranging from 15 to 60 g daily, were conducted. Glycine has been used in conjunction to typical and atypical antipsychotic medications. High dose glycine, i.e. 0.8 g/kg body weight, equivalent to 60 g daily, was first used by Heresco-Levy et al. [116] in a 6 week crossover trial of 22 patients diagnosed with treatment resistant schizophrenia. They found the treatment led to significant increases in both glycine and serine serum levels. They also observed a 30% reduction in negative symptoms, as measured by the PANSS-negative subscale, and a significant reduction in BPRS. Javitt et al. [119] also found high dose glycine to be effective in 21 inpatients of a state hospital, with a 34% reduction in negative symptoms. In 2004, Heresco-Levy [120] studied high dose glycine added specifically either to olanzapine and risperidone in a similar patient population as before, albeit one with a lower mean pretreatment PANSS-negative symptom score. There were 17 patients in a double blind 6 week crossover treatment. Glycine treatment resulted in a significant 23% reduction in negative symptoms as per PANSS-negative subscale. It also showed reduction in PANSS-cognitive subscale scores, and in PANSS-positive subscale scores, thereby extending the scope of symptoms glycine affected. Tsai and Lin's [107] meta-analysis pooled ten trials of glycine. Glycine showed significant improvements in three areas: total psychopathology, effect size = 0.71, depressive symptoms = 0.59, and positive symptoms, effect size = 0.42.

Given the preliminary positive findings of glycine, the limits of glycine requiring high oral doses to affect the CNS, and the negligible therapeutic benefits of the partial agonist, D-cycloserine, researchers continued to look for other agents that could strengthen activity at GCS and further prove the concept of NMDAR hypofunction theory of schizophrenia. D-serine is another full agonist, more potent than glycine and only acts at the GCS, unlike glycine which also acts on strychnine-sensitive site. D-serine is found in corticolimbic regions that parallel the location of NMDARs. Its regulatory enzymes of SR and DAAO are of interest as potentially being altered to regulate levels of D-serine. Animal models have further characterized the effect of altered SR and DAAO activity. A mouse model with an exon deletion of SR, with homozygotes resulting in 10% of cortical D-serine levels found in wild-type animals, demonstrates slower decay kinetics of NMDAR generated EPSCs, hyperactivity, and impaired spatial memory [121]. There is also a mouse line that lacks DAAO activity secondary to a point mutation at G181R ($Dao1^{G181R}$).

Almond et al. [122] showed that these mice had no changes in mRNA expression of DAAO, nor were there any changes in SR, ASC-1, NMDAR, or GlyT-1 expression, contending that the lack of DAAO activity, with resulting increase in D-serine levels, accounted for the behaviors observed. In their study, the mutant mice were resistant to PCP-induced hyperlocomotor activity. They also found that mutant mice were able to overcome GCS antagonism because of increased GCS occupancy by D-serine and reported a significant increases in cyclic GMP expression, purportedly secondary to increased NMDAR activity. Hashimoto et al. [123], reported that this animal model had been resistant to effects of MK-801-induced behaviors, and in their study, found that the mutant mice showed a significant decline of methamphetamine-induced stereotyped behaviors compared with wild type. Finally, $Dao1^{G181R}$ had been reported to have enhanced LTP and improved spatial memory. LaBrie et al. [124], also confirmed significantly elevated levels of D-serine in the entire brain & cortex, hippocampus, and cerebellum, and through Morris Water Maze testing, showed the animal model had improved cognitive flexibility and was able to adapt to changing circumstances. These studies lend further credence to the idea that decreased D-serine can mimic symptoms seen in schizophrenia, while increasing D-serine levels, can be a potential therapeutic intervention for schizophrenia.

Tsai et al. [125] conducted the first human trial of D-serine. It was a 6 week placebo controlled trial involving 31 patients with schizophrenia that met the criteria for primary deficit syndrome. D-serine was dosed at 30 mg/kg/day to augment both typical and atypical neuroleptics. Unlike prior GCS agonists, D-serine resulted in improvement in all three main symptom domains. There was a 17% reduction in positive symptoms, 21% reduction in negative symptoms, and 12% improvement in cognitive symptoms, and improved performance on the Wisconsin Card Sorting Test.

A follow up study performed in 2005 in a different ethnic population, Heresco-Levy et al. [126] focused on adding of D-serine to either risperidone or olanzapine in a 6 week double blind, placebo controlled crossover trial. Again there were significant reductions in negative, positive, and depressive symptoms. There was significant order effect in patients who received D-serine initially, and they did worse when switched to placebo.

Tsai and Lin's [107] meta-analysis pooled five trials of D-serine was found effective in total psychopathology effect size = 0.40; negative symptoms, 0.48; and cognitive symptoms, 0.42.

The advantages of D-serine are the lower oral dosing required than glycine, and D-serine should not have a curvilinear dose response as D-cycloserine as it is a full agonist. Animal studies indicated that at doses of 800 mg/kg/day, D-serine causes renal toxicity. The current dosing at 30 mg/kg/day is much lower and has not been linked to significant systemic side effects at this time [125, 126]. In addition, D-serine has demonstrated the potential to alleviate cognitive symptoms, thereby influencing functional outcome, as seen in improved performance in Wisconsin Card Sorting Test, and in animal models, the ability to retain and relearn information to adapt to the changing environment.

19.7 GlyT-1 Inhibitors

Given the aforementioned characteristics, D-serine is likely the closest drug of direct agonists of GCS that could be marketed. In general, however, agents that directly activate the GCS tend to require high doses in order to exert CNS effects and are subject to the endogenous regulatory systems that limit their activity. In the mid-1980s, glycyldodecylamide (GDA), a glycine derivative, was observed to prevent PCP-induced hyperactivity. In 1997, its mechanism of action was identified as a GlyT-1 inhibitor [127]. Since the late 1990s, then, focus expanded to increase activity at the GCS by inhibiting GlyT-1 in order to prevent reuptake of glycine. This concept is analogous to that of selective serotonin reuptake inhibitors used to treat depression [115].

There have been two mouse models created to further characterize the role of GlyT-1 and its potential in improving cognitive symptoms that are seen in schizophrenia. In 2004, Tsai et al. [128] studied a GlyT-1 knockout mouse model. Homozygous mice were not viable after birth, due to respiratory depression and motor impairments, likely caused by glycine effect on strychnine-sensitive receptors in the brain stem. Heterozygous mice, however, exhibited normal growth and physical characteristics. They had a 50% decreased mRNA transcription for GlyT-1, which corresponded to a 50% decreased reuptake of glycine when compared to wildtype mice. These mutant mice were less sensitive to the effects of both amphetamine and MK-801 and exhibited better memory retention than their wildtype littermates. Furthermore, in heterozygous mice, the addition of glycine or D-serine did not show enhanced NMDAR-mediated currents in pyramidal cells, which suggests that because of decreased reuptake of glycine, glycine concentration was increased and fully occupied the GCS. This confirms that GlyT-1 routinely maintains glycine at subsaturating concentrations at the NMDAR synapse and lends a way by which to enhance NMDAR-mediated transmission. If GlyT-1 activity was blocked, there would be increased amount of glycine to activate the GCS. In 2006, another transgenic murine model (CamKIIalphaCre; GlyT1tm1.2 fl/fl) was created that exhibited a selective deletion of GlyT-1 in forebrain neurons. GlyT-1 protein levels were reduced by 30% and resulted in a 35% decrease in glycine reuptake. When compared to wildtype mice, the mutant mice had 2.5 times the number of NMDAR generated EPSCs. The transgenic mice were less affected by PCP and amphetamine, consistent with antipsychotic properties. In regards to cognitive symptoms, these mice exhibited enhance latent inhibition and were able to react significantly faster to noise signals to avoid foot shock compared to controls [129]. A follow up study of these mice in 2007 showed that transgenic mice had improved object recognition memory, able to recall which object was novel 2 hours later compared to controls. In summary, increased glycine by blocking GlyT-1, and subsequent NMDAR activity could potentially enhance memory and associative learning [130].

Randomized control trials of GlyT-1 inhibitors have been conducted in humans. Sarcosine, or N-methylglycine, is an endogenous amino acid and a GlyT-1 antagonist. When demethlyated by sarcosine dehydrogenase, sarcosine becomes glycine.

Because of the similarity in structure to glycine, Zhang et al. [131] proposed that sarcosine may also function as a direct agonist at the GCS site. In their study of embryonic mouse hippocampal neurons, sarcosine was able to increase the number NMDAR mediated action potentials, even when GlyT-1 transporter was antagonized by another agent. In regards to toxicity from elevated sarcosine, patients with sarcosinemia, which is a rare autosomal metabolic disease in which sarcosine dehydrogenase is defective,have highly elevated levels of sarcosine in their blood and urine. There are no known clinical sequelae to this disorder, suggesting that higher level of sarcosine is benign.

The first trial of sarcosine in humans involved 38 participants in a 6 week double-blind placebo controlled. Sarcosine 2 g/day or placebo was added to patients' antipsychotic regimen. These patients had been stable on their regimen over 3 months. Twenty of the patients were being treated with risperidone, The others were taking typical neuroleptics. At the end of 6 weeks, sarcosine patients had a 17% reduction in positive symptoms, 14% reduction of negative symptoms, and 13% improvement in PANSS-cognitive subscale. When looking at risperidone treated patients alone, there were similar findings: 14% reduction in negative symptoms, 16% improvement in cognitive symptoms and 15% improvement in total PANSS scores. No significant systemic side effects reported [132].

Working from the premise that animal studies had shown that sarcosine modulation of NMDAR-mediated transmission could be influenced by environmental stress, Lane et al. [133] tested sarcosine as the sole agent to treat acute exacerbation of schizophrenia. This study involved 20 patients, 11 whom had never been treated with an antipsychotic medication before. They were randomized to either sarcosine 1 or 2 g for 6 weeks. The data did show that antipsychotic naïve patients treated with 2 g did show better improvement in outcome (i.e. 20% reduction of PANS total score) compared to patients who had been treated before. Tsai and Lin's [107] meta-analysis, which pooled five trials of sarcosine, found sarcosine effective in total psychopathology 0.43 (effect size), negative symptoms 0.33, and general psychopathology 0.49.

Because of these preliminary findings, several research groups associated with pharmaceutical companies have attempted to create GlyT-1 inhibitors. There have been over 100 compounds created, with 30 patents published between 2003 and 2005 [80, 134]. The compounds can be divided into sarcosine derived compounds and non-sarcosine derived compounds. There have yet to be clinical trials completed with these compounds, likely because of safety and pharmacokinetic limitations. The compounds continue to be modified in hopes of developing a drug to be used in clinical trials. For example, in 2006, Merck reported a number of compounds generated as potential potent, reversible, and specific GlyT-1 inhibitors, based on a series of [4-phenyl 1 (propylsulfonyl)piperidin-4-yl]methyl benzamides. There was a proof-of-concept trial with compound 13-h that did show increased glycine levels in the prefrontal cortex by 340%, of freely moving rats and the ability to enhance prepulse inhibition in DBA/J mice [135]. In 2009, there was a follow up report indicating that Merck had to adjust moieties of these compounds in hopes of increasing oral bioavailability of the compounds [136]. In 2007, Lilly reported their work in

trying to create GlyT-1 inhibitors, which are sarcosine-based, linked to a substituted biphenyl system via an ethanolamine linker [137].

While the search continues for synthetic GlyT-1 inhibitors continue, there have been several compounds developed and studied in animal models. Two sarcosine based inhibitors, ALX 5407, racemic form NFPS, and ORG24598, racemic form ORG24461, and two non-sarcosine based inhibitors, SSR504737 and SSR103800 will be reviewed here. Mezler et al. [138] conducted a study of these two classes of GlyT-1inhibitors in Xenopus laevis oocytes in order to clarify the mechanism by which the inhibitors functioned. The sarcosine derivatives, NFPS and ORG 24589, demonstrated irreversible, noncompetitive inhibition of GlyT-1. On the other hand, SSR504737 demonstrated competitive reversible inhibition. When SSR504737 was labeled with N-methyl, it was competitively displaced by both SSR504737 and glycine, but not be the sarcosine derived compounds.

In general, when evaluating the efficacy of these novel molecules in animal models, these studies have sought to assess their antipsychotic properties. This is done by comparing the GCS agonists activity to that of existing antipsychotic medication. For example, the ability to decrease psychotic symptoms can be assessed by the capability of the potential drug to reduce amphetamine induced hyperactivity. These studies also assess the ability of the potential drug to attenuate the effect of NMDA antagonists, such as PCP, ketamine, or MK-801. Given the effect of NMDA antagonism on memory, it is of interest whether these drugs can promote LTP, improve novel object recognition, and spatial memory. These are all skills required for social functioning [139]. Finally, patients with schizophrenia are notorious for their deficits in attention. Clinically, patients with schizophrenia are unable to determine which external stimuli are relevant to guide behavior, and which are inconsequential. Furthermore, there is a lack of ability to adapt, i.e. cognitive inflexibility or perseveration. These deficits in attention are assessed by PPI and latent inhibition. Both are impaired in patients with schizophrenia. PPI is a task of sensorimotor gating. PPI consists of a prepulse stimulus, a pulse stimulus, and a resulting startle reflex. The exposure to a prepulse stimulus should attenuate the startle response to the pulse stimulus, demonstrating learning from prior exposure. Patients with schizophrenia have decreased PPI, and the extent to which PPI is disrupted is associated with symptom severity. In animal models, impaired PPI can be induced by dopamine agonists, NMDAR antagonists, and by lesions to the ventral hippocampus during development. Latent inhibition (LI) describes the ability to disregard insignificant environmental stimuli. It also refers to capacity to change behavior in response to a previously irrelevant stimulus that becomes associated with a condition that now makes it relevant for the individual. Thus, latent inhibition requires that the individual can re-learn and remember the new association, otherwise the individual will default back to ignoring the stimuli even though the stimuli is now relevant. Patients with schizophrenia can have low levels of LI and abnormally persistent LI.

Decreased PPI can serve to model positive symptoms, consistent with, possibly auditory hallucinations. Abnormally persistent LI can model negative

and cognitive symptoms, exhibiting perseveration and an inability to interact socially with others. LI can be disrupted in rats and humans when treated with amphetamine and corresponds to acute stages of schizophrenia [140]. NMDAR antagonists cause abnormally persistent LI. In animal models, atypical antipsychotics and NMDAR agonists, such as glycine, can be distinguished by which phase of LI they effect. In mice treated with MK-801 with resulting abnormally persistent LI, atypical neuroleptics reverse this by acting in the preexposure phase, while glycine affects the conditioning phase [141].

The sarcosine-based GlyT-1 inhibitor, ALX 5407, or NFPS, N [3-(4′-flurophenyl)-3-(4′phenyl-phenylphenoxy)propyl] sarcosine), was created by Allelix Neuroscience, Inc. and described in 2001. NFPS selectively and irreversibly inhibited glycine transport at human GlyT-1 transporters expressed in a quail fibroblast cell line at an IC50 value of 3nM. NFPS, at 10 mg/kg administered by mouth, resulted in a significant increase of glycine levels in the rat prefrontal cortex by 40% at a dose that represented 50% occupancy of available GlyT-1 sites [142]. According to Harsing et al. [80] NFPS was expected to entered clinical trials, but is now used, instead, as a tool for studying glycine transporters. Perry et al. [143] found that ALX507 at higher doses (30 mg/kg) affected motor performance and caused respiratory distress in rodent models. Clinically, rodents exhibited repetitive behaviors, autonomic changes, "toe walking" ataxia, lateral recumbency, and tremors, likely due to strong blocking of GlyT-1 throughout the CNS. While GlyT-2 is often thought of near inhibitory glycine synapses, GlyT-1 is also present in the cerebellum and brain stem. In fact, the GlyT-1a isoform has the highest concentration in the brainstem, then cerebellum, followed by the hippocampus and frontal cortex. An hour after administration of ALX507, glycine levels in the CSF increased 250% versus controls, with a 150% increase in the prefrontal cortex, and a 220–250% increase in cerebellum, which was sustained for 8 hours.

NFPS has exhibited similar properties as current antipsychotic medications. Similar to clozapine, NPFS increased c-Fos immunoreactivity in the nucleus accumbens [81]. NFPS also attenuate effects of NMDAR antagonists. NFPS prevented PCP-induced hyperactivity in mice and undid PCP induced EEG changes in conscious rats [134]. Rats treated with PCP demonstrate an increased susceptibility to amphetamine challenge, evidenced by increased dopamine release in the prefrontal cortex and striatum. Javitt et al. [144] examined the effects of both glycine and NFPS to rectify dopamine dysregulation in rats that had been exposed to PCP for 3 months (chronic) or 2 weeks (subchronic). PCP exposed animals treated with NFPS for two weeks showed significantly lower levels of DA when compared to untreated PCP exposed animals. In PCP treated animals, there was a significant negative correlation between serum glycine levels and DA release. NFPS also enhanced NMDAR mediated transmission, the underlying mechanism of LTP and memory. Kinney et al. [81] showed that NFPS at 3 mg/kg significantly enhanced high frequency electrical stimulation-induced LTP, measured by EPSP slope, in the dentate gyrus of rats. In the rat medial prefrontal cortex, risperidone, clozapine, and NFPS enhanced NMDA induced transmission. Adding NFPS to risperidone augmented

the effects of risperidone on NMDA induced currents. However, NFPS did not augment the effect of clozapine, which is similar to findings from human trials in which NMDA enhancing agents do not augment the effect of clozapine [145]. For MK-801 treated animals, NFPS treated animals exhibited improved LTP and reversed reference memory deficits when compared to controls that had been treated with vehicle. The improvement could be mediated by recruitment of NMDARs and affected by level and duration of postsynaptic calcium concentration [146]. Hashimoto et al. [147] examined effect of GCS activation, via NFPS and D-serine, on mice after 10 days of PCP (10 mg/kg/day) exposure. PCP treated animals displayed decreased exploratory preference in the novel object recognition test, mirroring negative symptoms of social withdrawal in humans. These deficits were significantly improved by subsequent 2 week administration of NFPS (1 and 3 mg/kg/day), or D-serine 600 mg/kg/day. On a cellular basis, the PCP treated animals showed significantly increased levels of GlyT-1 and decreased levels of glycine in the hippocampus. MK-801 exposed rats that were treated with clozapine, NFPS, and D-serine exhibited significant improvements in cognitive deficits as tested by novel object recognition [148].

Studies also indicate that NFPS can correct disruptions in attention. In the $Grin^{1D48}$ mouse line, NFPS, and D-serine, reversed LI defects [139]. In the DBA/2 J mouse line, which has decreased basal PPI, NFPS enhanced PPI at all prepulse intensities examined [81]. Lipina et al. [140] evaluated the effects of GCS modulators by comparing clozapine to GCS modulators, D-serine (600 mg/kg) and ALX547 (1 mg/kg) in the C57Bl/6 J mouse line, treated with MK-801. MK-801 caused persistent LI which was reversed by all three agents. MK-801 reduction of PPI was reversed by clozapine and ALX547 but not D-serine. Clozapine and D-serine facilitate PPI independent of MK-801 impariment. AlX5407 did not affect PPI at the lowest dose but higher doses reduced PPI, differing from the findings by Kinney et al., and may be due to different mouse line tested.

ORG 24598, designed by Organon, Organon R-(-)-N-methyl-N-[3-[4-flurormethyl]phenoxy-3-phenyl-propylglycine, is a fluoextine analogue, sarcosine based GlyT-1 inhibitor [149]. It increased extracellular glycine concentrations in rats and reversed PPI deficits caused by lesions to the ventral hippocampus. It also counteracted PCP effects.

The non-sarcosine based GlyT-1 inhibitor, SSR504734, (2-chloro-N-[(S)-phenyl[(2S)-piperidin-2-yl]]-3-trifluromethyl benzamine, monohydrochloride), developed by Sano-Sythelabo has shown efficacy in animal models of schizophrenia [134] SSR504734 is a selective and reversible GlyT-1 inhibitor. Depoortere et al. [150] tested SSR504734 extensively in vivo and in vitro, with the purpose of assessing whether SSR504734 could increase glycine levels in the CNS in order to enhance glutamatergic neurotransmission, assess its potential antipsychotic activity, and counter the effects of hypofunctiong at NMDARs. SSR504734 inhibited GlyT-1 in rat and human cells, at a potency below that of ALX5407 and ORG24598 and above that of sarcosine. Microdialysis of rat cells showed a significant increase of glycine and dopamine in the prefrontal cortex. SSR504734 was also able to

increase NMDAR mediated eEPSCs in pyramidal neurons. Ketamine induced psychosis correlated to increased metabolic acvitiy in the frontal cortex, assessed by uptake of 2-DG. At 10 mg/kg, SSR504734 significantly reduced 2-DG uptake in the hippocampus, PFC, and cingulate cortex. At 30 mg/kg, it reversed ketamine induced activity in the cingulate cortex, dorsal hippocampus, nucleus accumbens, and anteroventral thalamic nucleus. SSR504734 antagonized MK-801 induced hyperactivity and attenuated the hypersensitive motor reaction to D-amphetamine administered to rat treated with PCP neonatally. Finally, SSR504734 significantly increased PPI in the DBA/2 J mouse line. There was also some evidence that SSR504734 was effective in the chronic stress rodent models of anxiety and depression.

Subsequent studies have assessed SSR504734 in wild type C57BL/6 mice, which showed pretreatment of mice with 30 mg/kg improved choice accuracy, at a delay of 12–16 seconds, in an automatic continuous alternation task, thereby suggesting the potential use of SSR504734 to enhance cognition [151]. In another study, the relationship between glutamate and dopamine was studied in the nucleus accumbens. In rats, DA is released in response to 20 or 40 Hz stimulation of the basolateral amygdale, mediated by glutamatergic neurotransmission. NMDAR antagonists cause decreased dopamine release in the nucleus accumbens. Thus, if SSR504734 increases extracellular glycine concentration (which reached statistical significance after 45 minutes of its administration), it should enhance glutamatergic neurotransmission and thus increase dopamine neurotransmission. Indeed, SSR504734 significantly enhanced DA release by 88% after 20 hz stimulation and by 35% at 40 hz. In the presence of a NMDAR antagonist, release of DA evoked by amygdala electrical stimulation was reduced by 69.3%. Thus, enhancing NMDAR activity will improve modulation of DA release in the nucleus accumbens [152].

The impact of SSR504734 antipsychotic profile was assessed in a study in which its effect in wild type mice exposed to amphetamine, PCP, and apomorphine, which acts at presynaptic DA autoreceptors to decrease locomotor activity. At a dose of 30 mg/kg, SSR504734 attenuated all PCP induced hyperlocomotor activity. Consistent with the findings in the nucleus accumbens, SSR504734 potentiated DA. It potentiated amphetamine hyperactivity, while also potentiating the depressive effects of apomorphine. It is unclear whether SSR504734 could thereby normalize excessive DA transmission by acting on presynaptic DA receptors and increasing negative feedback [153].

Recently, Boulay et al. [154] published an article profiling SSR103800, another non-sarcosine based GlyT-1 inhibitor manufactured by Sanofi-Aventis. SSR103800 was specific to GlyT-1 and increased levels of glycine in the rat prefrontal cortex. It also increased NMDA mediated EPSCs in rat hippocampal neurons. In regards to a hypoglutamatergic state, it decreased MK-801 and PCP induced hyperactivity. It also addressed cognitive symptoms by correcting impaired social recognition and short term visual episodic memory in rats after PCP exposure. SSR103800 also increased PPI in the DBA/1 mouse line.

19.8 Conclusion and Future Directions

Consideration of the NMDA hypofunction theory of schizophrenia comes at an important time when half a century of their use has indicated that current antipsychotic medications are limited in their ability to treat the debilitating effects of schizophrenia. The bidirectional interactions between multiple neurotransmitters, such as glutamate, dopamine, and GABA, which also depend on location in the brain, are complicated and cannot be characterized with one general statement. However, given the evidence of NMDAR antagonists ability to reproduce symptoms consistent with schizophrenia, and preliminary findings in small clinical trial, approaching drug development on the premise of enhancing NMDAR functioning is likely a solid beginning. The full implications of manipulating glutamatergic neurotransmission can only be elucidated with further studies, both in animal models and in humans.

The components of the NMDAR glutamatergic synapse offer many junctures at which potential intervention can occur. The focus of this chapter has been on the GCS, which must be activated in order for NMDAR to depolarize. Even though stimulation of GCS does not appear to be as excitotoxic as increasing glutamate concentrations, there are some in vitro studies that seem to indicate that both glycine and D-serine may play a role in excitotoxicty. Furthermore, it may be that GlyT-1 prevents excess glycine activation of GCS which can cause cell death. There are factors to consider as drug development is now headed in the direction of enhancing activity at this very site, and potentially, by manipulating some of these endogenous regulatory mechanisms, i.e. GlyT-1.

Other than sarcosine, none of the other GlyT-1 inhibitors have yet findings in human trials. This is likely due to toxic effects as demonstrated by NFPS, which exhibits noncompetitive, irreversible inhibition of GlyT-1. While these inhibitors are specific to GlyT-1, it is not specific to particular isoforms of GlyT-1, like the ones in brain stem and spinal cord. In addition, it has not been possible to localize the GlyT-1 inhibition to specific areas of the brain, such as the hippocampus or prefrontal cortex. Thus, increased glycine levels at inhibitory synapses in caudal brain areas result in respiratory and motor toxicity. In this regard, the nonsarcosine derived, SSR504734, a noncompetitive, reversible GlyT-1 inhibitor has shown promise in terms of its ability to modulate glutamatergic neurotransmission to counteract NMDA antagonist induced state, exhibit antipsychotic properties, and enhance cognition. Importantly, inhibition of GlyT-1 should not be maximized due to the potential toxicity concern. In fact, competive antagonists like sarcosine is promising due to its moderate potency. This is likely why most GhyT-1 inhibitors falten during drug development and sancosine is the only drug show safety and efficacy in human subjects.

Treatments of schizophrenia will require “polypharmacy,” the rational being that different drugs will target specific symptom domains [3]. In some ways, this is how the clinical trials for increasing NMDAR mediated glutamatergic neurotransmission have been constructed. In many cases, the focus has been on improving cognitive and negative symptoms, and the agents tested have been used as augmentation

strategies. It is highly plausible that new therapy for schizophrenia will target at more than a single neurotransmitter system. Either targeting at multiple neurotransmitters, including glutamate, dopamine, and serotonin, at the same time or tailor the molecular treatments according to the different mechanisms involved in individual person.

A search of clinicaltrials.gov, in April 2009 reveals eight ongoing trials including D-serine as monotherapy, ORG25935 (glycine uptake inhibitor), acamprosate (partial agonist of NMDAR), glycine, and D-cycloserine as agents. Recently completed trials also show interest in the use of these medications as preventive/prodromal stages [155]. These trials are necessary to continue proof-of-concept, and as more evidence is gathered, the studies will continue to include larger numbers of people increasing length of treatment and follow-up, and different context in which these drugs can be tested. In regards to DAAO inhibitor, Ferraris et al. [156] have synthesized several, the most promising one being CBIO (6-chlorobenzol[d]isoxazol-3–0 l) that when given to rats increased D-serine plasma levels. It is with great anticipation that this century awaits the development of a novel glutamatergic drugs that could target the cognitive symptoms of schizophrenia, and ultimately improve functional outcome for patients through their actions on neuroplasticity and neurocognition.

Acknowledgements GET is supported in part by Los Angeles Biomedical Research Institute and National Alliance of Research on Schizophrenia and Affective Disorder.

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Chapter 20
The Role of Oxytocin in Neuropsychiatric Disorders: Concepts and Mechanisms

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Abstract The role of oxytocin in the pathophysiology and treatment of major neuropsychiatric disorders has recently received increased attention. Although oxytocin has an established role as a circulating hormone involved in parturition and lactation, it also acts as a neurotransmitter and neuromodulator. Oxytocin receptors are found in several brain areas such as amygdala, nucleus accumbens and hippocampus, which have been heavily implicated in the pathophysiology of schizophrenia, depression and anxiety disorders. Converging lines of evidences suggest that oxytocin is a key mediator of complex emotional and social behaviors, including attachment, social recognition, and aggression. Moreover, oxytocin alleviates anxiety and impacts on fear conditioning and extinction and on social reward systems. Furthermore, recent data suggest that oxytocin has neuroprotective effects by increasing the resistance of fetal neurons to insults during delivery. Due to its influence upon a wide range of behaviors and its antistress neuroprotective properties the role of oxytocin-related dysfunctions and therapeutics are presently assessed in major neuropsychiatric disorders. In this chapter we will review and summarize some of the mechanisms and concepts relevant to the role of oxytocin in the pathophysiology and therapeutics of neuropsychiatric disorders.

Abbreviations

ACTH	adrenocorticotrophic hormone
AMPA	$\propto$-amino-3-hydroxy-5-methylisoxazole propionic acid
ASD	autism spectrum disorders
CRH	corticotrophin-releasing hormone
GABA	γ-aminobutyric acid

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_20,

GPCR	G protein-coupled receptor
HPA	hypothalamic-pitutary-adrenal
KA	kainic acid
mGluRs	metabotropic glutamatergic receptors
NMDA	N-methyl-D-aspartate
OCD	obsessive-compulsive
PCP	phencyclidine
PPI	prepulse inhibition
PTSD	posttraumatic stress disorder
SAD	social anxiety disorder

20.1 Introduction

Oxytocin is a nonapeptide, which can be found in most bony vertebrate species and exhibits in its structure, as well as in the locations of main cell groups in the brain, a great evolutionary stability [1]. This unordinary evolution stability suggests a strong selective pressure [2] and a significant role of oxytocin in the survival of species. Although oxytocin has an established role as a circulating hormone involved in parturition and lactation, it can also act as a neurotransmitter and

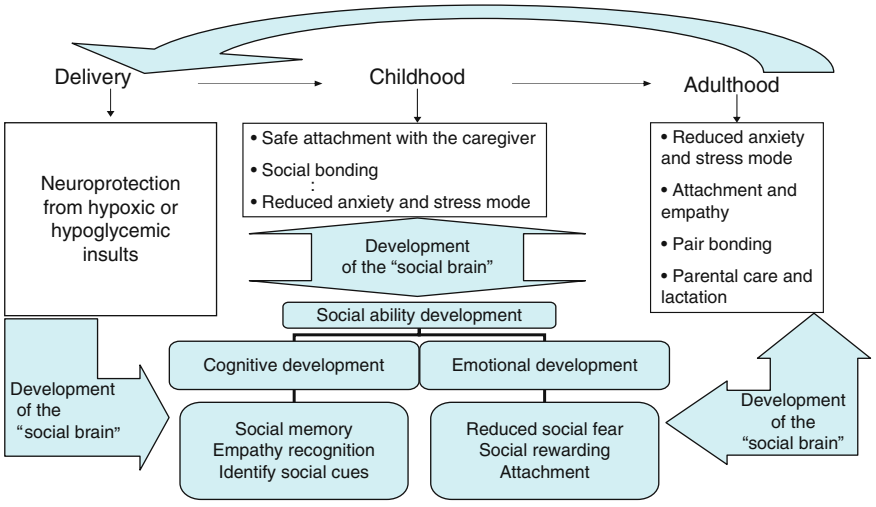

Fig. 20.1 Oxytocin influences on social behavior and development throughout life. Interruption in one or more stages may cause a neuropsychiatric disorder. e.g. Hypoxic insults during delivery may cause insufficient neurological development. This may cause deficits in social ability development. Poor and unrewarding social interactions during childhood will lead again to social abilities and neurological deficits during adulthood. Deficits in social behavior in adulthood may lead to neuropsychiatric disorders

neuromodulator by interacting with the central oxytocin receptors within the brain [3]. Oxytocin can be found in brain regions that are involved in social emotions and social reward such as amygdala and the tegmental area. Accumulating evidence indicate that oxytocin is a key mediator of complex emotional and social behaviors, including attachment, social recognition, and aggression in humans. Several studies have found associations between oxytocin and neuropsychiatric disorders such as depression and autism. Furthermore, intranasal administration of oxytocin reduces anxiety and impacts on fear conditioning and extinction [4]. It seems that oxytocin is also involved in reducing hypoxic and/or hypoglycemic stress during delivery. Thus, it is assumed that oxytocin may be involved in the neuroprotection of the fetus as well as in neuroregulation of normal and disturbed social behaviors throughout life (Fig. 20.1). In this chapter we will review and summarize some of the mechanisms and concepts relevant to the role of oxytocin in the pathophysiology and therapeutics of neuropsychiatric disorders.

20.2 Evolution and Oxytocin

All neurohypophysial hormones are nonapeptides with a disulfide bridge between cysteine residues 1 and 6. This results in a peptide constituting a six-amino acid cyclic part and a COOH-terminal a-amidated three-residue tail. These peptides are classified into vasopressin and oxytocin families based on the amino acid at position 8. The vasopressin family contains a basic amino acid and the oxytocin family contains a neutral amino acid at this position [2]. There is evidence that the oxytocin and vasopressin genes may have arisen by duplication of a common ancestral gene approximately 450 million years ago, forming a second oxytocin-like peptide—isotocin. Two evolutionary molecular lineages have been proposed: an isotocin-mesotocin-oxytocin line, associated with reproductive functions, and a vasotocin- vasopressin line involved in water homeostasis. Vasotocin has been found in the most primitive cyclostomes. Bony fishes (Osteichthyes), predecessors of land vertebrates, possess both isotocin and vasotocin. Most vertebrates possess two nonapeptide forms, including an arginine vasopressin-like form and an oxytocin-like form [5].

The evolutionary conservation in the nonapeptides structure is substantially mirrored by the conservative evolution in the locations of main cell groups in the brain. In all vertebrates, the arginine vasopressin-like and oxytocin-like peptides are produced by populations of magnocellular and parvocellular neurons located in the preoptic area and anterior hypothalamus. These cell groups project to the neurohypophysis as well as to the adenohypophysis, allowing the nonapeptides to exert a wide range of peripheral effects. Some of these effects are actions that may be integrated with the central regulation of social behaviors [5].

The exceptional structural stability of the nonapeptides and their location in the brain during evolution suggest a strong selective pressure [2] and highlight the significance of oxytocin in the survival of species.

20.3 Genes, Receptor Structure and Physiology

In all species, oxytocin and vasopressin genes are located on the same chromosomal locus but are transcribed in opposite directions. The intergenic distance between these genes ranges from 3 to 12 kb in different species [6, 7]. The human gene for oxytocin-neurophysin I encoding the oxytocin prepropeptide is mapped to chromosome 20p13 [8] and consists of three exons: the first exon encodes a translocator signal, the nonapeptide hormone, the tripeptide processing signal glycyl-lysyl-arginine and the first nine residues of neurophysin; the second exon encodes the central part of neurophysin (residues 10–76); and the third exon encodes the COOH-terminal region of neurophysin (residues 77–93/95) [2].

The oxytocin receptor gene is present in single copy in the human genome and was mapped to the gene locus 3p25–3p26.2. Human oxytocin receptor mRNAs were found to be of two sizes, 3.6 kb in breast and 4.4 kb in ovary, endometrium, and myometrium [9–11]. The gene size is 17 kb and it contains 3 introns and 4 exons. Exons 1 and 2 correspond to the 59-prime noncoding region. Exons 3 and 4 encode the amino acids of the oxytocin receptor. Intron 3, which is the largest, separates the coding region immediately after the putative transmembrane domain 6. Exon 4 contains the sequence encoding the seventh transmembrane domain, the COOH terminus, and the entire 39-noncoding region, including the polyadenylation signals. Oxytocin receptor could be susceptible to epigenetic regulation due to CpG islands, genomic regions that contain a high proportion of cytosine guanine dinucleotides that lie upstream of the oxytocin receptor gene [12]. Therefore, lifelong differences in oxytocin receptor function could potentially be influenced by environmental circumstances (e.g. inappropriate early maternal care) [13]. The oxytocin receptor is a typical member of the rhodopsin- type class I G protein-coupled receptor (GPCR) family with seven transmembrane a-helices which are highly conserved among the GPCR family members. Mutations at the conserved tripeptide aspartic acid-arginine-cysteine motif result in an either inactive or a constitutively active oxytocin receptor [14]. The cysteine residues in the first and second extracellular loops are highly conserved within the GPCR family and are probably connected by a disulfide bridge. Besides the seven transmembrane helices there is a relatively short N-terminal extracellular domain, a moderately long C-terminal intracellular domain, which includes several sites for phosphorylation, and a double-cysteine isoprenylation anchor [15]. For the “core” oxytocin receptor, a molecular mass of 40–45 kDa can be calculated on the basis of the amino acid sequence derived from the known cDNA sequences of several species. The oxytocin receptor has two (e.g. mouse, rat) or three (e.g. human, pig, sheep, rhesus monkey, bovine) potential N-glycosylation sites (N-X-S/T consensus motif) in its extracellular NH2-terminal domain [2].

As noted earlier, oxytocin is synthesized primarily in magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus. Following its synthesis, oxytocin is transported mainly along the neuronal axons to the posterior pituitary lobe, where it is stored until its secretion in neuronal terminals. Later, oxytocin is released to regulate parturition and lactation. Oxytocin is also released in brain

structures that are relevant to emotions and social interaction [16–18]. In rodents, oxytocin receptors are found in the olfactory bulb, which is related to social cognition, in the central and lateral amygdala which are related to social stress and in the nucleus accumbens which is related to social reward [19, 20]. In humans, expression is prominent in the basal nucleus of Meynert, the nucleus of the vertical limb of the diagonal band of Broca, the ventral part of the lateral septal nucleus, the preoptic/anterior hypothalamic area, the posterior hypothalamic area, the substantia nigra pars compacta, the substantia gelatinosae of the caudal spinal trigeminal nucleus and of the dorsal horn of the upper spinal cord, as well as in the medio-dorsal region of the nucleus of the solitary tract [18, 21, 22].

20.4 Oxytocin, Dopamine and Glutamate

20.4.1 Dopamine/Glutamate Hypothesis of Schizophrenia

For decades, theories of schizophrenia have focused on a single brain neurotransmitter, dopamine [23]. However, it is unlikely that the constellation of symptoms which characterise this disorder may reflect dysfunction of a single neurotransmitter system [24].

Glutamate has been under study for several decades now for a range of neuropsychiatric illnesses [25]. Unlike dopamine, which plays an important role only in circumscribed brain regions, glutamate neurotransmission is involved in a wide array of brain functions across different structures of the brain. Glutamate is the primary excitatory neurotransmitter in the mammalians and approximately 60% brain neurons utilize glutamate as their primary neurotransmitter. Glutamatergic fibers give rise to the major afferent, intrinsic and efferent pathways throughout the cortex and hippocampal formation and are prominent in many sensory organs including the olfactory bulb and retina which may be related to social recognition. Within the brain, thalamocortical fibers, which represent the main input to cortex, are primarily glutamatergic. Furthermore, virtually all pyramidal neurons in the cerebral cortex, which project to both cortical and sub-cortical structures, are glutamatergic. The distribution and involvement of glutamatergic neurotransmission through different pathways and structures in the brain can explain why glutamate is involved in so many complex disorders. Indeed, multiple lines of evidence support the link between glutamatergic neurotransmitter and the pathophysiology of schizophrenia and other neuropsychiatric disorders [26, 27].

Glutamate receptors are divided into two broad families: ionotropic receptors: including N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA), and kainic acid (KA) receptors and metabotropic glutamatergic receptors (mGluRs). A prominent glutamatergic hypothesis argues that hypofunction of NMDA receptors may contribute to schizophrenia symptomology. Phencyclidine (PCP), a NMDA receptor antagonist, induces psychotic symptoms in normal volunteers [28, 29] and exacerbates symptoms in schizophrenia patients.

20.4.2 Oxytocin and Glutamate

It seems that glutamate and oxytocin are localized and synthesized in similar structures and areas in the brain. Both glutamate and its receptors are localized in a variety of hypothalamic nuclei which are considered critical for reproduction and neuroendocrine functions. For example, glutamate has been localized in magnocellular and parvocellular neurons in the paraventricular nucleus, in the ventromedial, arcuate and supraoptic nuclei, in the median eminence and the infundibular stalk [3, 30–35]. In the pituitary, it has been detected in pituicytes and in axonic terminals of the posterior lobe [36]. mGluRs have been found in different regions within the hypothalamus and in the three lobes of the pituitary gland [37]. Glutamate is mainly expressed in hypothalamic areas associated with neuroendocrine regulation. The distribution of glutamate receptors in areas that are important for reproductive processes and neuroendocrine functions may reveal the major role of glutamate in regulation of these events. As described before, oxytocin is mainly synthesized in magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus [38].

Additional evidence in favors of an interaction between glutamate and oxytocin comes from animal studies. Pampillo et al. [39], reported that glutamate can regulate hypothalamic oxytocin release through activation of AMPA and mGluRs in adult male rats. Morsette et al. [40], also showed that mGluR activation increases oxytocin release in rat hypothalamo-neurohypophysial explants.

Animal studies on PCP administration and prepulse inhibition (PPI), which is impaired in adults with schizophrenia and several other neuropsychiatric disorders also suggest a relation between glutamate and oxytocin. Chronic PCP administration to male rats reduces the time animals spend in social interactions [41, 42]. The mechanism by which PCP alters social behavior is unclear, but endogenous oxytocin could serve as a central mediator of social behaviors in rodents. Caldwell et al. found that PCP treatment results in larger PPI deficits in oxytocin knockout mice than in regular mice [43]. Lee et al. [44] showed that chronic PCP administration to rats causes reduction in oxytocin mRNA in the paraventricular nuclei of the hypothalamus and marked changes in oxytocin receptor binding. Moreover, treatment with oxytocin reversed the social deficits caused by chronic PCP treatment.

20.4.3 Oxytocin and Dopamine

Both dopamine and oxytocin receptors are located in brain areas that influence social behavior. The dopaminergic reward pathways activity is associated with socially affiliative behaviors and it is modulated by oxytocin activity [12]. Oxytocin interacts with dopaminergic circuits in the nucleus accumbens shell and in the ventral tegmental area which are also associated with reward. The striatum, which contains oxytocin receptors in addition to dopamine receptors, is involved in reward-related learning. The ventral striatal dopaminrgic circuit is engaged in predicting whether a future reward is likely to be forthcoming from a course of action. The dorsal

striatum monitors the outcome of actions in order to optimize future choices that could lead to a reward [45]. These circuits are also responsible for the complex cognitive perception of trust which is associated with oxytocin administration [12]. As discussed in the next paragraph, trust adaptation differences are associated with specific activity reductions in amygdala, the midbrain regions, and the dorsal striatum in subjects receiving oxytocin, suggesting that neural systems mediating fear processing (amygdala and midbrain regions) and behavioral adaptations to feedback information (dorsal striatum) modulate oxytocin effects on trust [46]. Moreover, increased forebrain dopamine neurotransmission, regulated by the ventral tegmental area dopamine neurons, is thought to contribute to the psychotic symptoms of schizophrenia [47].

20.5 Early Life Neuroprotection and Social Interactions

Well-being and survival in primates, including humans, depends critically on social interactions [48], and disturbed social behavior is a key component of diseases such as autism, schizophrenia, depression and anxiety disorders [49]. Oxytocin plays an important role in early life neuroprotection in both direct neuroprotection of fetus through molecular modulation and indirect neuroprotection through affective behaviors such as maternal care, attachment abilities and social reward feedback.

Delivery is a stressful event associated with high risks to the fetal brain [50] and must be prepared in advance by some mechanisms that alert it to the imminent onset of parturition before the day of birth [51]. One of these mechanisms is thought to be the reversal and transformation of γ-aminobutyric acid (GABA) receptors from excitatory to inhibitory receptors by oxytocin [52]. GABA functions as an inhibitory neurotransmitter in the adult brain. However, during fetal and postnatal periods GABA acts as an excitatory neurotransmitter. The GABA excitatory phase is important in the control of neuronal firing, generation of the primitive patterns of activity and the fetus neurodevelopment. During fetal and postnatal periods activation of GABA receptors increases the firing of action potentials in the majority of CA3 pyramidal cells in rats [53]. However, the proportion of cells activated by GABA sharply decreases during a brief time period shortly before and during the day of birth [52].

Tyzio et al. [52] suggested that oxytocin down-regulates the Na(+)-K(+)-2Cl(–) co-transporter activity. The NKCC1 down regulation reduces the Cl^- elevated levels in immature neurons and hence changes the GABA reversal potential vs. the resting membrane potential from positive to negative. Therefore, this process switches GABA receptors from excitatory to inhibitory activity. This mechanism contributing to reduction of neuronal activity and metabolic demand and helps protect fetal neurons from hypoxic or hypoglycemic insult during delivery. Whether the source of protective oxytocin is maternal or fetal is presently debated [51, 54].

The nature of maternal care is of crucial importance since it can affect brain morphology and physiology and hence, the development of cognitive, emotional and

neuroendocrine responses to stress. In rats, variations in maternal behaviors, such as pup licking during the first weeks of life, are associated with individual differences in stress responsiveness, emotionality and cognitive functioning in the adult offspring. These behaviors also correlate with levels of oxytocin expression in the hypothalamic medial preoptic area [55, 56] and are reduced by oxytocin antagonists [57]. Bonding behaviors reduce fear response under conditions of novelty [58]. In rats, maternal behaviors are rapidly expressed following normal vaginal delivery and are often disturbed by interventions that impair oxytocin release. After delivery rats will take care of offspring of other rats as freely as of their own. In contrast, ewes are able to recognize her own lamb from the moment it is born, and she feeds only her own offspring. This phenomenon is thought to be due to an "olfactory memory" that is fixed by centrally released oxytocin which activates neural pathways at parturition [59]. Oxytocin receptor mRNA expression increases around birth in the olfactory bulbs, medial preoptic area, bed nucleus of the stria terminalis and amygdala and may influence maternal interactions with the newborn, leading to pup acceptance and onset of maternal care [57]. Oxytocin administration to the mother, can facilitate nipple attachment in young rat pups [60]. Rat pups also show preference for specific odors that are associated with exposure to their mothers. On the contrary, preferences for the mother do not develop in animals that are pretreated with oxytocin antagonists [61]. Oxytocin injections can produce rapid effects on the tendency of infants to cry [62]. In humans, the increase in oxytocin from early to late pregnancy correlates with higher maternal-fetal bonding [57]. Overall, oxytocin may act on both mothers and infants to influence the response of young offspring to their maternal environment.

The oxytocin system also regulates maternal aggression which may be exhibited at the end of pregnancy and during early lactation. Rat females with high anxiety-related behavior are more aggressive than rats with low anxiety-related behavior. Oxytocin release within the paraventricular nuclei of the hypothalamus and central nucleus of the amygdala is higher in high anxiety related behavior rats. Blockade of oxytocin receptor in the paraventricular nuclei of the hypothalamus or central nucleus of the amygdala reduces aggression in high anxiety-related behavior rats, whereas infusion of oxytocin into the paraventricular nuclei of the hypothalamus tend to increase maternal aggression in low-anxiety related behavior rats [63]. The endogenous opioid actions on paraventricular nuclei of hypothalamic oxytocin neurons are different in virgin and pregnant rats. In pregnant rats opioids seem to increase oxytocin in paraventricular nuclei of the hypothalamus and decrease CRH release during lactation. This results in a decreased release of CRH in response to stress and decreased anxiety and depression-related behavior in lactating rats. Thus, disruption of these parameters may be a risk factor for postpartum depression, which is associated with increased anxiety and depression, as well as reduced maternal care. Furthermore, the significant attenuation of hormonal stress response in pregnancy and lactation are important for the healthy prenatal development of the offspring by preventing excessive levels of circulating glucocorticoids [64].

During lactation, the offspring suckling causes the release of oxytocin aggregated in the paraventricular and supraoptic nuclei of the hypothalamus. These large pulses of oxytocin into the circulation are essential for suckling induced milk let-down.

This reflex can be blocked by a small amount of oxytocin antagonist injected into the supraoptic nuclei of the hypothalamus [65]. In lactating women, the increase of oxytocin following breast-feeding is associated with dampened levels of adrenocorticotrophic hormone (ACTH) and cortisol [66–69]. In addition, lactation in humans also appears to reduce responses to physical and psychosocial stress exposure. In lactating women, attenuated hypothalamic-pitutary-adrenal (HPA) axis responses can be observed if breast-feeding starts 30–60 min before stress exposure, depending on the kind of stressor [70–72]. As no effect of stress has been found on oxytocin plasma levels, oxytocin does not seem to mediate the attenuation of cortisol stress responses at the adrenal level [73]. Thus, the inhibitory effect of oxytocin on HPA axis responsiveness points to a more central modulation and could, in fact, be localized to the paraventricular nucleus, as demonstrated in rats [74, 75]. Interestingly, breast-feeding mothers with increased plasma oxytocin in response to a speech stressor that immediately followed baby-holding were found to have lower blood pressure than mothers with a decrease in oxytocin after stress [76]. The oxytocin release during lactation may reduce maternal response to stressful life events but also may influence maternal bonding and social recognition. Late pregnancy and lactation in humans is associated with increased calmness and reduced emotional responses to stressful events [72, 77, 78].

The formation of social attachments is a critical component of human relationships and begins from birth, with the infants bonding to their caregivers. These initial social bonds are formed simultaneously with brain development and continue to provide regulatory emotional functions throughout adulthood. Moreover, it seems that the bonding between the infant and caregiver has crucial effects on infant brain development and social abilities. Moreover, the oxytocin system is affected by early social experience and may regulate the transduction of early social experiences into short and long term behavioral and neuroanatomic changes [79].

In rats, oxytocin is present early in the development but shows a fully processed transcript only at the post natal period [80]. Oxytocin levels are increased by socialy pleasant sensory experiences such as comforting touches and smells and are associated with selective infant-parent attachments and the formation of social interactions. Furthermore, exogenous oxytocin administration reduces the separation response of the rat pup [81]. Compared to the adult brain, there is an transient but marked “overproduction” of oxytocin receptors in the limbic brain areas during the first two postnatal weeks [82]. Furthermore, oxytocin receptors being part of the reward circuitry, might be involved in providing a sense of security and protection that makes social interactions rewarding in the infant [83].

In non-human mammals, oxytocin has a central role in general behavioral regulation, particularly in positive social interactions. Oxytocin seems to permit animals to overcome their natural avoidance of proximity and inhibit defensive behavior, thereby facilitating approach behavior. Oxytocin might also promote prosocial approach behaviors—such as trust—in humans [84]. Several studies have shown correlations between oxytocin and social rewarding. Healthy women who showed increased plasma oxytocin levels in response to positive emotions and who maintained oxytocin levels while experiencing negative emotions were less likely to report interpersonal problems associated with intrusiveness. Maintaining oxytocin

levels during sadness has also been associated with lower anxiety in close relationships [85]. Another study compared a warm touch intervention in couples with a monitoring-only control group. Touch resulted in increased salivary oxytocin and a subsequent reduction in sympathetic tone indicated by lower systolic blood pressure as well as reduced alpha amylase [86]. Buchheim et al. [87] showed that single intranasal administration of 24 IU oxytocin increases the subjective experience of attachment security in male students identified with an insecure attachment pattern. Altogether, these human studies results suggest a possible protective effect of endogenous oxytocin [88]. Heinrichs et al. [89] showed that subjects treated with 24 IU intranasal oxytocin 50 min before stress and social support had lower cortisol concentrations during stress exposure compared to subjects who received either social support, oxytocin or none. Notably, there were corresponding results in terms of psychological measures: subjects without social support and with placebo showed the expected decrease in calmness and increase in anxiety during stress, while participants who received either social support or oxytocin or both protective factors showed increasing calmness and decreasing anxiety scores during stress. Thus, oxytocin may enhance the buffering effect of social support on stress responsiveness. In another study, Ditzen and colleagues [90] showed that 40 IU intranasal oxytocin increases positive communication behavior during a couple conflict in both men and women, and significantly reduces cortisol reactivity. Moreover, Kosfeld et al. [84] showed that 24 IU intranasal oxytocin increases trust among humans. In particular, 45% of the participants which inhaled oxytocin showed a maximal trust level compared to only 21% in the placebo group. Importantly, oxytocin did not increase the readiness to bear risks in general but rather specifically increased the individuals willingness to accept social risks within the framework of social interactions. An fMRI study indicated oxytocin effects on the neural circuitry underlying trusting behavior. 24 IU intranasal oxytocin increased the tolerance to betrayal of trust compared to placebo. This difference in trust adaptation was associated with attenuation of activity in areas mediating emotional processing (amygdala, midbrain) and behavioral adaptation to feedback (dorsal striatum) in subjects receiving oxytocin [46]. These findings indicate that oxytocin may reduce social stress and help to diminish social conflicts.

As mentioned previously, the amygdala contains dense concentrations of oxytocin receptors [20, 91] which regulate its activity [92]. Thus, an effect on amygdala may provide a potential mechanism which oxytocin influences prosocial behavior, specifically by suppressed of social avoidance responses [93–95]. Several fMRI studies in humans, have demonstrated that intranasal oxytocin administration may reduce amygdala activity in response to emotional social stimuli such as angry faces. Recent studies indicate that 27 IU intranasal oxytocin reduced both amygdala activity and coupling of the amygdala to brainstem regions implicated in autonomic and behavioral manifestations of fear [4, 92]. Petrovic et al. [96] reported that 32 IU intranasal oxytocin attenuated the effect of aversive conditioning of neutral faces. Moreover, attenuated effect of oxytocin on the activity in the right amygdala and the right fusiform gyrus for direct gaze stimuli as compared to averted gaze stimuli was observed. Domes et al. [97] found that 24 IU intranasal oxytocin reduced amygdala

responses to fearful, angry, and happy faces even when the emotional content of the presented face was not evaluated explicitly. In addition, exploratory whole brain analysis revealed modulatory effects in prefrontal and temporal areas, as well as in the brainstem. In conclusion, recent neuroimaging studies suggest a modulatory role of oxytocin on amygdala responsiveness to unconditioned and conditioned socially relevant stimuli. The attenuating effect on amygdala activity in response to both positive and negative stimuli might reflect reduced uncertainty about the predictive value of a social stimulus and thereby facilitates social approach behavior [88].

Oxytocin may also affect social cognition in both recalling and processing social cues by improving social memory. A single dose of 24 IU intranasal oxytocin enhanced performance on the ability to infer the mental state of another individual from facial cues [98]. Guastella et al. [99] reported an increased number and duration of gazes toward the eye region of emotionally neutral human faces following intranasal oxytocin administration as compared to placebo, indicating that oxytocin has an important role in emotion recognition and interpersonal communication in humans. However, enhanced attention for negative social cues (schematic angry faces) was not confirmed in a recent study [100]. Di Simplicio et al. [101] reported slowed reaction times during facial fear recognition and reduced misclassification of positive facial expressions as negative ones.

Regarding memory, intranasal oxytocin was found to selectively modulate implicit memory depending on the social relevance of semantic word stimuli [73]. Oxytocin enhanced the encoding of positive social memories [102] and immediate (30 min) and delayed (24 h) recognition for face identity even when given after the learning condition [103]. Moreover, oxytocin specifically improved recognition memory for faces, but not for non-social stimuli, which suggests an immediate and selective effect of the peptide on strengthening neuronal systems of social memory [104].

In conclusion, accumulating evidence during the last decade indicate that oxytocin modulates social behavior, perception and cognition as well as response to social stress and reward, thereby promoting attachment and positive social approaches. Recent fMRI studies support the concept that activity of amygdala and associated cortical areas are effected by intranasal oxytocin administration of which may contribute to social stress reduction and enhancement of social interactions.

20.6 Oxytocin and Neuropsychiatric Disorders

20.6.1 Autism Spectrum Disorders (ASD)

Giving the cardinal effects of oxytocin on attachment and social behavior it is not surprising that recently this nanopeptide is massively studied in the context of Autism Spectrum Disorders (ASD). ASD are characterized by abnormalities in social functioning, speech and communication, restricted interests and repetitive behaviors [105]. The impaired social behaviors in ASD include failure in using

standard non-verbal behaviors to regulate social interactions (e.g., gaze aversion when interacting with others, limited range of affective expression), lack of social and emotional reciprocity and deficits in the ability to be empathic. Moreover ASD are also characterized by social cognitive deficits, in particular difficulties recognizing faces [106–108] and difficulties processing the affective states of others through facial displays [109, 110]. As discussed in this chapter, all these impairments are associated with oxytocin modulation.

A number of studies revealed direct associations between oxytocin and ASD and genetic studies also support a role of oxytocin in ASD. Ylisaukko-Oja [111] suggested the region of chromosome 3 in which the oxytocin receptor is located as a susceptibility loci for ASD. Wu et al. [112] found an association between two SNPs (rs2254298, rs53576) of the oxytocin receptor gene and ASD in a sample of Han Chinese family trios. Jacob et al. [113] also found an association between rs2254298, but not with rs53576, and autism in a Caucasian sample.

Moreover, disturbed plasma oxytocin levels have been observed in children with autism compared to age matched controls [114]. The plasma samples obtained from the autistic children were associated with higher oxytocin precursor levels, as well as an increased ratio of oxytocin precursor to oxytocin, suggesting that oxytocin may be processed differently in the brains of children with autism [115]. However, Jansen et al. [116] found increased basal oxytocin levels in ASD adults which hypothetically may be related to developmental factors.

Intravenous infusion of synthetic oxytocin (Pitocin) reduced the frequency of repetitive behaviors and decreased the total number of different repetitive behaviors compared to placebo administration in adults with ASD [117]. In contrast, two studies had failed to found therapeutic effects of oxytocin in treating obsessive-compulsive disorder (OCD) patients [118, 119]. These results may point to differences in the neurobiology of repetitive behaviors in ASD vs. OCD. In an ongoing pilot study intranasal oxytocin administration to eight high functioning ASD patients improved repetitive behaviors symptoms [120]. In addition, analysis of responders and non-responders based on the Clinician's Global Impressions-Improvement (CGI-I) scale ratings suggested that more subjects administrated oxytocin were categorized as responders than placebo subjects [105, 121, 122]. Preliminary analyses indicated that subjects treated with oxytocin showed improvement on the Diagnostic Analysis of Non-Verbal Accuracy (DANVA2) subscale, which measures emotion recognition across multiple modalities (e.g. facial expression, paralanguage, tone of voice and posture) (reviewed in [120]). Taken together these data suggest that oxytocin may be an essential modulator of repetitive behavior and social cognition in ASD.

20.6.2 Anxiety Disorders

Anxiety disorders are the most prevalent class of psychiatric disorders in the U.S. and many other countries [123]. Unlike the relatively mild, brief anxiety caused by a stressful event (such as speaking in public), anxiety disorders last for months and

can get worse if they are not treated. Anxiety disorders commonly occur along with other mental or physical illnesses, including alcohol or substance abuse, which may mask anxiety symptoms or make them worse. In some cases, these other illnesses need to be treated before a person will respond to treatment for the anxiety disorder. Anxiety disorders are associated with serious disability [123, 124], increased rates of chronic medical conditions [125], high rates of comorbidity and phenotypic overlap with a variety of neuropsychiatric conditions (mood and substance abuse disorders in particular).

There is evidence that abnormalities in HPA axis function may characterize a subset of anxiety disorders, and that the nature of these alterations differs from those seen in mood disorder patients groups [126]. Although the set point of HPA-axis activity and of other central systems is programmed by genotype, it can be changed to a different level by developmental influences and early negative life events. CRH and arginine vasopressin, are secreted into the hypophyseal portal blood and act in synergistic fashion on the anterior pituitary to increase adrenocorticotropic hormone release into the circulation and consequent glucocorticoid hormone secretion from the adrenal cortex. Long-lasting hyperactivity of CRH neurons, resulting in increased stress responsiveness and reflecting a glucocorticoid resistant state, is commonly seen in depressed individuals [127–129]. Morover, elevated plasma and salivary cortisol and cortisone levels, increased urinary free cortisol excretion, disturbed dexamethasone suppression, decreased corticosteroid receptor function, an enhanced adrenal response to ACTH, a blunted pituitary ACTH response to CRH as well as adrenal and pituitary enlargement are commonly observed in patients suffering from depression [130–132]. Stressful life events such as bereavement, child abuse, and early maternal separation are also risk factors for depression, anxiety disorder, or both. Childhood physical or sexual abuses are important early stressors that may predispose individuals to adult onset depression accompanied by a permanent hyperactivity of the HPA system. Almost all environmental and genetic risk factors for depression appear to correlate with increased HPA-axis activity in adulthood. On the other hand, when patients or animals are treated with antidepressants, electroconvulsive therapy, or when they show spontaneous remission, the HPA-axis function may return to normal [133].

Administration of intranasal oxytocin in small samples of patients with anxiety disorder, including PTSD [134] and OCD [119], has been explored. In a recent study comparing healthy men with and without exposure to a specific adverse childhood event (i.e. early separation from at least one parent), intranasal oxytocin led to a reduction in salivary cortisol levels that was significantly larger in the non-exposed group than in the group who had experienced this early adverse event [135]. Thus, individual differences in neuroendocrine sensitivity to intranasal oxytocin should be considered for optimization of this potential therapeutic approach. In another recent study by Guastella et al. [136] intranasal oxytocin was given as an adjuvant treatment to exposure therapy for social anxiety disorder (SAD). In this study oxytocin reduced the exaggerated negative mental representations of self which are typically displayed by SAD patients following a speech exposure task. Despite these acute benefits, the addition of oxytocin to exposure therapy did not enhance treatment outcomes for SAD in the long-term [136].

20.6.3 Obsessive-Compulsive Disorder

Given the involvement of characteristic repetitive behaviors in OCD, this disorder is likely associated with disrupted oxytocin function. Indeed, historically, OCD was one of the first psychiatric disorders to be linked to oxytocin and presently a number of indirect findings point to the possible role of oxytocin in this disorder [137]. Animal studies show an increase in stereotyped behaviors following intracerebroventricular administration of oxytocin, including stretching, repetitive grooming, startle and squeaking in mice [81, 138–140], grooming in rats [141, 142] and wing-flapping in chicks [143]. Women with OCD often report OCD onset or worsening of symptoms during pregnancy or the puerperium (reviewed in [137]); and increased CSF oxytocin levels were found in an adult non-tic-related OCD subgroup [144]. Additional evidence are the extensive oxytocin interactions with the 5- HT and dopamine systems, which are known to be disrupted in OCD [137].

The specific role that oxytocin plays in OCD, is however unclear, and findings to date have been inconclusive. One study investigating the long-term effects of clomipramine treatment in children and adolescents with OCD found increased oxytocin levels and decreased stereotypies following treatment [145]. However, a randomized, double-blind, six-week, placebo-controlled study investigating the therapeutic effects of intranasal oxytocin (18 IU/day) in 12 adults with OCD found no reduction of OCD symptoms following oxytocin treatment [118]. Similarly, another trial in seven adults with OCD that used much higher doses of intranasal oxytocin (160 IU/day divided in 4 doses, with 2 of the subjects receiving a 320 IU/day daily dose) did not identify any significant OCD symptom reduction [119]. One potential limitation of this study is the relatively short period (1 week) of oxytocin administration, since it can take up to 10 weeks of pharmacological treatment to induce an effect on OCD behavior [146]. It is conceivable thus that, even at high doses, a week of intranasal oxytocin administration does not sufficiently affect the CNS to result in a change in behavior. Finally, two adult subjects with trichotillomania (a putative obsessive-compulsive spectrum disorder) treated for 1 week with intranasal oxytocin (160 IU/day divided in 4 doses) also did not experience therapeutic benefits [119]; again, the duration of this study may not have been enough to produce behavioral changes.

20.6.4 Posttraumatic Stress Disorder (PTSD)

There are different potential connections between oxytocin function and posttraumatic stress disorder (PTSD). PTSD is a mental disorder that may follow exceptionally stressful events and PTSD symptoms include persistent reexperiencing of the traumatic event, as well as intense psychological and physiological reactivity to events that symbolize or resemble it. Long-term potentiation and desensitization are critically involved in learning and memory formation which are impaired in PTSD [147]. This suggests important roles for emotional memory and conditioning in this disorder. Hormonal effects were demonstrated on memory

and conditioning and maybe involved in the pathophysiology of PTSD [148, 149]. A facilitating effect of stress hormones on memory retrieval in PTSD has been supported by the finding that indirect stimulation of the adrenergic system by means of the α2- adrenergic receptor antagonist yohimbine produces intrusive recollections and flashbacks in Vietnam veterans with PTSD [150]. Animal studies have also demonstrated the modulating effects of hormones such as vassopresin and oxytocin on memory and conditioning. Oxytocin may plays a role in inhibition acquisition of a conditioned response (or memory storage) and conditioned responding (or memory retrieval) [151–155]. It is suggested that endogenous vasopressin and oxytocin may have the same memory-modulating effects as the exogenously administered compound [155]. In a human study [134] intranasal oxytocin (20 IU of Pitocin) was administrated to Vietnam veterans suffering from PTSD. The levels of mean physiologic responses during personal combat imagery were lower for oxytocin vs. placebo or arginine vasopressin administration [134]. The results in this study raise the possibility that oxytocin might have therapeutic value in PTSD. These findings are consistent with a possible role for stress hormones in the pathogenesis of PTSD symptoms and suggest a possible biological mechanism accounting for the observation that nonspecific stress can aggravate PTSD symptoms, and trigger delayed onset PTSD [156]. Furthermore, PTSD may be associated with perturbed HPA axis function leading to hypocortisolemia and a hyperadrenergic state [157–159]. Oxytocin is thought to play a role in reducing stress by dampening HPA axis activity [160]. Moreover, a severe early stress event can theoretically alter brain development through its effect on the oxytocin-vasopressin stress-response system, lying down the substrates for the development of various neuropsychiatric disorders, including PTSD [161].

20.6.5 Affective Disorders

There has been speculation that some of the symptoms commonly reported in depression (i.e. social withdrawal, reduced appetite, cognitive impairment) may reflect central oxytocin function alterations [162]. Lower levels of plasma oxytocin have been reported in humans with major depression [163]. Oxytocin levels are also negatively correlated with self-reported psychological distress, including depressive symptoms [164]. A postmortem study of eight depressed patients reported increased numbers of oxytocin expressing neurons in the paraventricular nuclei of hypothalamus [165]. In addition, a positive correlation has been found between the levels of oxytocin and happiness score as self reported in a daily symptom ratings scale [166, 167]. However, results from studies measuring plasma and CSF oxytocin levels have been inconsistent with reports of reduced [168], similar [169, 170] and increased [166] oxytocin levels in patients with depression compared with controls. A negative correlations between plasma oxytocin levels of depressed patients and depression and anxiety scores would be in accordance with previous work postulating abnormalities of neurohypophysial secretion in major depressive disorder [171].

20.6.6 Schizophrenia

Both schizophrenia and oxytocin seem to be related to the function of dopaminergic and glutamatergic circuitries. It is believed that deficits in the dopaminergic and glutamatergic systems are related to schizophrenia symptoms. It is likely that glutamate can regulate hypothalamic oxytocin release and NMDA receptor antagonists may reduce the oxytocin mRNA levels in the paraventricular nuclei of hypothalamus [39]. Dopamine interacts with oxytocin in neuronal circuits that affect social cognition and social behavior such as reward systems and trust [12]. Moreover, Feifel and Reza [172] found that oxytocin administration restores PPI that has been reduced in rats by administration of a non-competitive NMDA antagonist or an indirect dopamine agonist. These results suggest that oxytocin may play an important role in the modulation of dopaminergic and glutamatergic regulation of PPI, and that it may act as an endogenous antipsychotic.

Schizophrenia patients suffer from long lasting brain deficits that may start in embryo and/or at delivery. Oxytocin acts as a neuroprotector by reversing the GABA receptor function from excitatory during the embryonic period to inhibitory during the delivery period [52]. Thus, oxytocin reduces the neuronal activity and the metabolic demand and helps protect the newborn from hypoxic and/or hypoglycemic insult during delivery.

Oxytocin may also act as a "behavior regulator" since it increases social abilities such as trust and social memory [84, 104]. Moreover, it also increases maternal care, social attachment, trust and social rewarding. These changes may affect gene expression, neuronal development and behavioral patterns. Environmental events such as inappropriate social interactions may lead to differences in oxytocin receptor function throughout life. Maladaptive social interaction may also lead to poor development of the neuronal rewarding circuits. As a result, initiation and social interactions are impaired. Moreover, social interactions become not rewarding and the patient may develop a flat emotional affect, which is a cardinal schizophrenia negative symptom. The oxytocin function deficits may also reflect impairments in neuronal areas such as amygdala. Deficits at amygdala level may cause inappropriate emotional reactions to life events such as social stress.

Beckmann et al. [173] compared CSF oxytocin levels of schizophrenia patients and normal volunteers. The lowest CSF oxytocin levels were found in normal subjects, significantly higher levels were found in schizophrenia patients without antipsychotic treatment and the highest oxytocin levels were found in schizophrenia patients treated with antipsychotic drugs. Treatment with haloperidol for three weeks did not change CSF oxytocin levels significantly. However, Glovinsky et al. [174] found no differences between CSF oxytocin concentrations in neuroleptics-treated schizophrenia patients, neuroleptics-withdrawn schizophrenia patients and normal control subjects. In rats, amperozide and clozapine but not haloperidol increased plasma levels of oxytocin for short periods of time [175]. These finding, taken together with the observation that intranasal oxytocin administration increases positive social behavior such as trust and empathy [84], may indicate that

oxytocin may represent a potential treatment for negative symptoms in schizophrenia. Clinical trials examining these concepts are presently ongoing.

20.7 Conclusions and Future Directions

Oxytocin has an extensive influence on social behaviors starting from the prenatal period throughout life. At delivery, oxytocin has a cardinal neuroprotective function by reversing GABA receptors action from excitatory to inhibitory. Oxytocin increases both social cognition and social behavior. It increases trust, attachment and social rewarding and decrease anxiety and stress. Therefore, oxytocin increases social experience and enhances social activity. During adulthood, oxytocin is also involved in pair bonding, lactation and parental care. Oxytocin function impairments may contribute to neurological and behavioral deficits and as a result to developmental impairments. These impairments may be prominent in neuropsychiatric disorders such as ASD, depression, OCD and schizophrenia. Oxytocin administration may represent a novel treatment paradigm in these disorders. The study of oxytocin role in the pathophysiology and therapeutics of neuropsychiatric disorders has increased during the last decade and represent presently an important area of research in neuroscience.

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Chapter 21
Neuromodulation for Neuropsychiatric Disorders: Novel Techniques -Vagus Nerve Stimulation, Transcranial Magnetic Stimulation, Transcranial Direct Current Stimulation, and Deep Brain Stimulation

Russell J. Andrews

Abstract The last two decades have seen the development of several neuromodulation techniques that have been applied to the problem of severe, medication-refractory depression – notably vagus nerve stimulation, transcranial magnetic stimulation, transcranial direct current stimulation, and deep brain stimulation. The four techniques are here reviewed from the standpoint of the hardware involved, the techniques of application, the biological effects of the stimulation on the brain (with an emphasis on neuroprotection), and the results obtained in neuropsychiatric disorders to date. Vagus nerve stimulation involves stimulation of the vagus nerve in the cervical region via an electrode encircling the nerve, with that stimulation resulting in effects on many regions in the brain. Transcranial magnetic stimulation uses a focused magnetic field to induce, through the intact skull, electrical stimulation in a specific region of the brain. Transcranial direct current stimulation induces a small current in a portion of the brain, also through the intact skull, via cathode and anode electrodes placed on the scalp. Deep brain stimulation employs a small number of electrodes (usually two – one for each hemisphere) placed through hole(s) in the skull into a specific brain nucleus; the effect of deep brain stimulation as it is presently performed is to reversibly ablate the function of that nucleus or region. Given the rapid developments in the neural-electrical interface, anticipated future developments in deep brain stimulation are considered as well.

Abbreviations

ACTH	adrenocorticotrphic hormone
BDNF	brain derived neurotrophic factor
CNT	carbon nanotube
CBF	cerebral blood flow

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5_21,

CE Mark	certification that a product meets the health and safety requirements of the 27 countries of the European Union plus 4 additional countries included in the European Economic Area
DBS	deep brain stimulation
DTI	diffusion tensor imaging
ECT	electroconvulsive therapy
EEG	electroencephalography
fMRI	functional magnetic resonance imaging
FDA	Food and Drug Administration (USA)
HAM-D	Hamilton Depression Rating Scale
Hz	Hertz (cycles per second)
LTP	long term potentiation
MEA	multi-electrode array
MEG	magnetoencephalography
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
NASA	National Aeronautics and Space Administration (USA)
NMDA	N-methyl-D-aspartate
NEI	neural-electrical interface
NGF	nerve growth factor
NTS	nucleus of the tractus solitarius
OCD	obsessive-compulsive disorder
PC12	pheochromocytoma 12
PET	positron emission tomography
rTMS	repetitive transcranial magnetic stimulation
SCC	subcallosal cingulate
SEM	scanning electron micrograph
STN	subthalamic nucleus
TBS	theta burst suppresion
tDCS	transcranial direct current stimulation
TMS	transcranial magnetic stimulation
TRD	treatment resistant depression
UPDRS	Unified Parkinson's Disease Rating Scale
VNS	vagus nerve stimulation
YBOCS	Yale-Brown Obsessive-Compulsive Scale

21.1 Introduction

A broad definition of neuromodulation for neuropsychiatric disorders would include electroconvulsive therapy (ECT). ECT has been an effective treatment option for a variety of neuropsychiatric disorders for over 40 years, utilizes techniques which have been in clinical use for an equally long period, and likely has only modest

neuroprotective effects [1, 2]. For these reasons ECT is not included in this review, which is concerned with emerging techniques aimed at ameliorating brain function in neuropsychiatric disorders from a non-pharmacologic aspect. Also not included are techniques such as motor cortex stimulation (by electrodes placed surgically directly on the brain surface), since the efficacy of such techniques appears marginal for treatment resistant depression (TRD) and the magnitude and invasiveness of the surgical implantation procedure makes it unlikely that such a technique will gain widespread acceptance.

The birth and/or evolution of vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and deep brain stimulation (DBS) over the last 20 years for neuropsychiatric disorders ranging from TRD and obsessive compulsive-disorder (OCD) to eating disorders and Tourette's syndrome has been due to a several factors:

1. The discovery and/or development of the methodology and hardware involved. This is the case for VNS and TMS, where the techniques – hardware development and stimulation methods – were in essence products of laboratory research which has been extended to the clinical setting. This is in part true for tDCS as well, where the basic principle of stimulating the brain transcranially has been understood for over a century (and could be considered a cousin of ECT) but the clinical application lacked appropriate hardware for extensive research.
2. Extension of an existing technique to a new clinical indication. DBS techniques, as presently practiced, have changed little over the past 40 years apart from some increased sophistication of the implanted pulse generators – the clinical effect is essentially a reversible ablation of function in a discreet volume of brain tissue surrounding the active electrode(s).
3. Dramatic advances in neuroimaging and the measurement of cerebral blood flow (CBF) and brain electrical activity, e.g. magnetoenchepalography (MEG). The advent of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) in particular have provided a physiological basis for the neuromodulation techniques reviewed here. Because the findings of the neuroimaging modalities in relation to neuropsychiatric disorders is discussed elsewhere in this volume, and is detailed in a recent review with regard to DBS [3], the findings from this extensive literature will not be reviewed here in detail.

Each of the four techniques for neuromodulation considered here – VNS, TMS, tDCS, and DBS – is considered in turn: the technique's evolution, present method in the clinical setting, and results to date for neuropsychiatric disorders. DBS is more extensively reviewed because of the relatively dramatic findings in the small number of patients who have had DBS systems implanted recently for neuropsychiatric disorders. A section on future directions in neuromodulation for neuropsychiatric disorders follows.

21.2 Vagus Nerve Stimulation (VNS)

The VNS device consists of an electrode, a pulse generator/battery, and a connecting extension or lead implanted subcutaneously as shown in Fig. 21.1. The electrode is three helical coils that encircle the vagus nerve in the mid-cervical region—the positive electrode cephalad, the negative electrode immediately caudad, and an anchoring coil further caudad (Fig. 21.1). The left vagus nerve is stimulated because it has more afferent fibers (and less cardiac effects) than the right vagus nerve. Implantation of the pulse generator/battery (in the infraclavicular region) is identical to that for DBS. The implantation surgery is performed as an outpatient procedure under either local/regional or general anesthesia. The electrical stimulation programmed by the physician is intermittent but continues 24 h a day. Typical stimulation parameters are: current (1–2 milliamp); pulse width (500 μsec); rate (30 Hertz (Hz)); stimulation 'on' time (30 sec); stimulation 'off' time (5 min). In addition, the patient can give him/herself an extra stimulation with a magnet placed for a second over the region of the pulse generator/battery – although this feature is of more benefit in epilepsy patients who have an aura prior to a seizure than it is for TRD patients. The same magnet, when taped or otherwise held over the pulse generator/battery, can turn off the VNS for temporary or extended periods, e.g. for those patients who experience voice changes during VNS and who are involved in singing or public speaking. The major side effects of VNS – voice changes, coughing, and dyspnea – subside greatly during the initial weeks of stimulation for the majority of VNS patients.

VNS has been in clinical use as a treatment for medication-refractory epilepsy for over a decade, having received the CE Mark in Europe in 1994 and Food and Drug Administration (FDA) approval in the USA in 1997. The improvement in mood experienced by many patients who underwent VNS for refractory epilepsy

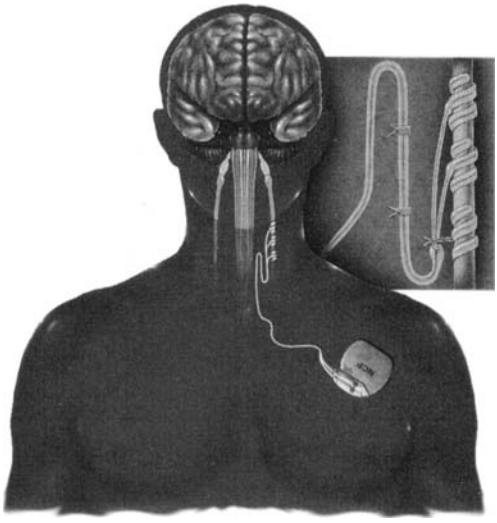

Fig. 21.1 Vagus nerve stimulator subcutaneous implanted device. (Courtesy of Cyberonics)

suggested that VNS might be of benefit in TRD. VNS for TRD has had a checkered history with the FDA in the USA [4]. Although approved for clinical use in the USA, VNS for TRD is currently not reimbursed by government insurance schemes such as Medicare and Medicaid; thus the device and implantation surgery costs of ~$25,000 must be borne by the patient in most cases [4].

The primary projections of the vagus nerve to the brain are via the nucleus of the tractus solitarius (NTS), notably to the thalamus, hypothalamus, amygdala, and other limbic structures [5]. The effect of VNS on the brain in patients with TRD may be different from the effects in patients with epilepsy. Several recent reviews and reports have summarized the literature regarding VNS effects on both CBF and neurotransmitters in various regions of the brain [6–10]. Although VNS in epilepsy patients increases CBF in the thalamus, in TRD patients the CBF effect appears to be primarily in frontal regions of the brain, especially the orbitofrontal cortex. Additional effects of VNS on serotonin and norepinephrine neurotransmitter levels have been noted, but the data between studies in depression and in epilepsy (as well as studies within either disorder) are inconsistent. A recent study in rats subjected to VNS acutely (3 h) showed statistically significant increases in both brain derived neurotrophic factor (BDNF) and fibroblast growth factor messenger ribonucleic acid (mRNA) in both the hippocampus and cerebral cortex, but a decrease in nerve growth factor (NGF) mRNA in both the hippocampus and cerebral cortex (the decrease in the cerebral cortex not reaching statistical significance) [11]. Prefrontal cortex norepinephrine levels (by microdialysis) increased 70% over baseline within $2\frac{1}{2}$ h [11]. The implications of such findings regarding VNS as a neuroprotective intervention are uncertain.

A well-established finding regarding VNS, both for epilepsy and for TRD, is that the beneficial effect increases over time for a year or more after implantation. This was found in a recent multicenter European study involving 74 patients followed for one year after VNS implantation. Response was defined as a >50% reduction (compared with preoperative baseline) on the Hamilton Depression Rating Scale (HAM-D); remission as a score of <10. At 3 months the response rate was 37% and the remission rate 17%; at 12 months the response rate was 53% and the remission rate 33%. The primary side effects were typically much less common at 12 months than at 3 months: voice changes decreased from 63% of patients to 2%; cough decreased from 26 to 3%; dyspnea remained stable at 10%. It should be noted that such side effects can be reduced or eliminated by adjusting the stimulation parameters (most commonly by reducing the current setting).

In summary, vagus nerve stimulation for TRD is accumulating substantial evidence for significant benefit in 50% or more of patients implanted. The clinical benefit of VNS improves over the first year at least, and appears to be maintained for several years at least (few data are yet available for longer periods). One paper, sponsored by the manufacturer of the VNS device, presents data suggesting that VNS for TRD is cost-effective, with the break-even device life being between 3.18 and 4.57 years (much shorter than the battery life of up to 8 years, and the battery can be replaced by a simple outpatient surgical procedure) [12].

21.3 Transcranial Magnetic Stimulation (TMS)

TMS, as the name implies, involves stimulating a region of the brain through the intact skull by means of a rapidly cycling magnetic field. A pulse generator creates a high current (5,000 amperes or more) for a brief period (~ one msec) that is passed through a coil placed on the scalp. The rapidly alternating current creates a strong but focal magnetic field (one to two tesla, i.e. comparable to that of clinical magnetic resonance imaging (MRI) scanners), which is unimpeded by the intervening scalp and skull (see Fig. 21.2). The rapidly alternating magnetic field induces electrical currents intracranially. A relatively focused electrical stimulation of the cortex – to a depth of several centimeters – results from configuring two TMS coils in figure-of-eight fashion. By repeating the stimulations for several seconds (i.e. repetitive transcranial magnetic stimulation (rTMS)), greater clinical effect can be achieved: TMS at frequencies > 1 Hz (typically 5–20 Hz) are excitatory; TMS at frequencies of 1 Hz or less are inhibitory. The stimulation is usually set at 100–120% of the threshold to stimulate a motor response (e.g., twitch in the contralateral hand).

Although TMS (usually as rTMS) has been in clinical trials for various disorders – from Parkinson's disease and chronic pain to schizophrenia and TRD – for over 20 years, both the hardware and the treatment methodologies continue to evolve. Together with the less frequently employed tDCS, TMS has been the subject of a recent monograph [14]. Although still investigational in the USA, it has become an approved treatment modality for TRD in the European Union, Australia, Canada, Israel, and New Zealand. Some of the issues involved include:

- Precise placement of the coil on the scalp for repetitive treatment sessions. Image guided techniques such as those used in neurosurgery have been employed, but clearly extend the treatment time – important in regimens that utilize recurrent daily sessions for several weeks;

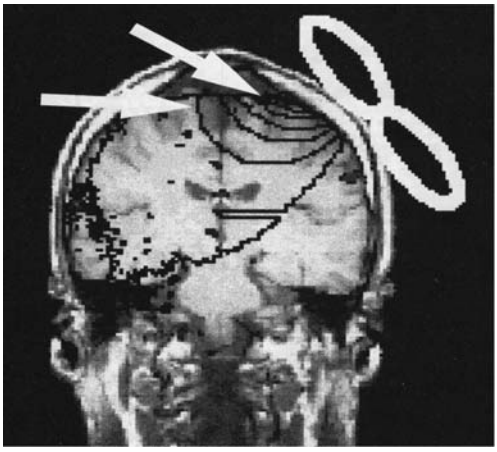

Fig. 21.2 Coronal MRI of subject with TMS coil above left hemisphere motor area. The *black* (gauss) *lines* denote the magnetic field produced by the TMS coil. (Reproduced from George et al. [13] with permission)

- Number and timing (daily? weekly?) of treatment sessions to optimize cost/benefit;
- Correlation of treatment regimens with changes in neuroimaging (PET, fMRI);
- Optimal coil shape for a specific disorder;
- Optimal stimulation site (frontal? temporal?); unilateral or bilateral?
- High frequency (>5 Hz, excitatory) versus low frequency (<1 Hx, inhibitory) stimulation.

These last two points appear inter-related: somewhat similar beneficial effects of high frequency rTMS of the left dorsolateral prefrontal cortex have been obtained with low frequency rTMS of the right dorsolateral prefrontal cortex [15]. The laboratory findings in rTMS are quite variable, as might be expected from the numerous variables listed above. However, several findings are of especial interest for TRD [16].

- Prefrontal rTMS increased dopamine release in the striatum and nucleus accumbens (the latter being a region where dopamine is relevant for anhedonia). The nucleus accumbens is one of the sites considered in DBS for TRD, as discussed below;
- rTMS appears to affect brain noradrenergic and serotonergic systems in a manner similar to antidepressants such as desipramine;
- rTMS appears to affect the hypothalamic-pituitary-adrenocortical system in a manner similar to antidepressants, however, it is unclear if the mechanisms are similar;
- Similar to VNS in rodent models (as well as antidepressants), rTMS appears to increase hippocampal BDNF mRNA;
- The effect of rTMS on hippocampal neurogenesis is unclear, despite the similarity of rTMS to antidepressants in effect on stress-induced adrenocorticitrphic hormone (ACTH) and corticosteroid levels.

The clinical efficacy of rTMS in TRD and other neuropsychiatric disorders (OCD, schizophrenia), although relatively consistent across studies, has been modest at best [17, 18]. A recent variation on the standard rTMS paradigm, in an attempt to improve the neuromodulatory effect (i.e. effect on neural plasticity, including long term potentiation (LTP)), is theta burst suppression (TBS) – the use of bursts (typically 3 pulses) of high frequency stimulation (50-200 Hz) repeated at 5 Hz intervals (5 Hz being in the theta range in EEG) [19]. The intent is to maximize LTP. Two variations of TBS rTMS have been described: (1) intermittent TBS (3-pulse bursts at 5 Hz for 2 sec – 10 bursts – repeated every 10 sec for 20 cycles, i.e. total 600 pulses); (2) continuous TBS (3-pulse bursts at 5 Hz for 40 sec, i.e. total 600 pulses). It is hoped that the TBS paradigm for rTMS might reduce the treatment time needed to achieve lasting clinical effects, e.g. in TRD [19].

21.4 Transcranial Direct Current Stimulation (tDCS)

Neuromodulation by tDCS for TRD has been in clinical research trials for nearly 50 years, although the techniques used have been modified somewhat [20–24]. Typically two electrodes 25–35 cm^2 are placed on the scalp, and a current of 1–2 mA is passed from the cathode to the anode (or to a distant reference electrode, e.g. on a lower extremity) (see Fig. 21.3) for up to 20 min – with a resulting current density of approximately 0.03–0.08 mA/cm^2. Although stimulation of the underlying brain is not as efficient as in TMS, recent modeling studies indicate the current reaching the brain is significant [25]. Interestingly, early studies employed long stimulation trials – up to 11 h a day, 5 days a week, for 6 months [20, 21].

The effects of tDCS on neural plasticity have been attributed to modification of the N-methyl-D-aspartate (NMDA) receptor (the effects being blocked by

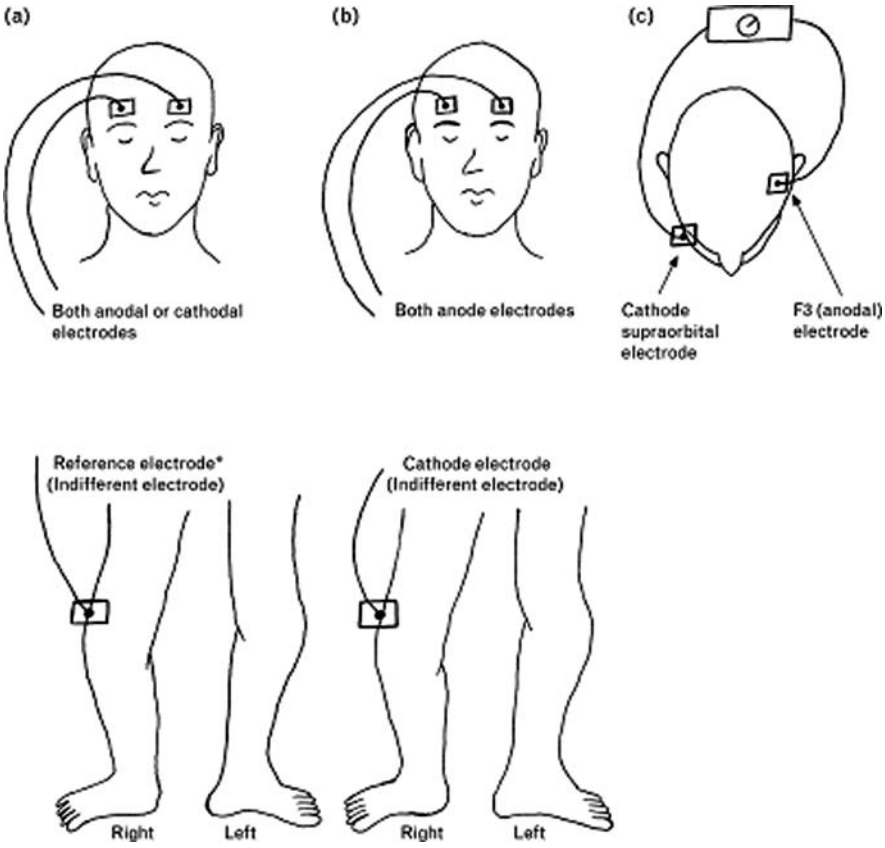

Fig. 21.3 Electrode montages for tDCS clinical studies on depression. (Reproduced from Murphy et al. [24] with permission)

the NMDA receptor anatagonist dextromethorphan) [23]. Quite extensive recent research appears to confirm that tDCS increases cortical excitability beneath the anode and decreases excitability beneath the cathode [23]. Clinical effects of tDCS may be somewhat similar to rTMS (although rTMS has both neurostimulatory and neuromodulatory effects, whereas tDCS has only neuromodulatory effects), but tDCS is less focal in the volume of brain treated [24].

Regarding the efficacy of tDCS in TRD, two recent studies can be considered. One compared sham tDCS, occipital cortex tDCS (2 mA for 20 min for 10 days), and left dorsolateral prefrontal cortex tDCS in 40 patients with mild-to-moderate major depressive disorder [26]. The average reduction in HAM-D scores was 40% for the dorsolateral prefrontal tDCS group, 21% for the occipital tDCS group, and 10% for the sham tDCS group. The second study compared tDCS with fluoxetine in 42 patients with major depressive disorder [27]. Although the beneficial effect of the medication and tDCS were similar in the end, the improvement was seen after 2 weeks in the tDCS group but not until 6 weeks in the fluoxetine group.

Given the modest clinical efficacy of tDCS presently for TRD, it is likely – thanks to the simple, relatively inexpensive nature of tDCS – that it will be primarily an adjunct to more effective, but costly and/or complicated, techniques for TRD. For example, tDCS might be instituted initially in a patient with TRD while the patient is being evaluated for more involved, invasive techniques such a VNS or DBS. Alternatively, tDCS could be employed to provide more rapid response in patients with major depression than they would receive from medication(s) alone – as in the study noted in the previous paragraph, where benefit for major depression appeared one month earlier in tDCS-treated patients than in those treated with fluoxetine alone.

21.5 Deep Brain Stimulation (DBS)

Deep brain stimulation (DBS) employs electrodes approximately one millimeter in diameter implanted into a specific region of the brain to – in effect – reversibly 'ablate' the function of that volume of brain tissue. The advantage, e.g. of thalamic DBS over thalamotomy, is that if the clinical result were unsatisfactory the electrode could be turned off (or even removed) without significant long-term sequellae for the patient. DBS has been employed since the 1960s by a few neurosurgeons to stimulate the periaqueductal/periventricular grey for the treatment of chronic pain [28]. For the decade prior to the discovery of L-dopa for Parkinson's disease in 1968, thalamotomies were performed in large numbers to control Parkinsonian tremor. With the discovery by the French neurosurgeon Benabid in the late 1980s that thalamic DBS was as successful as thalamotomy at controlling tremor (both essential tremor and Parkinsonian tremor), DBS began a steady course toward more widespread application for movement disorders – initially Parkinson's disease and essential tremor, and later dystonia [29, 30]. Thalamic DBS received the CE Mark in 1993

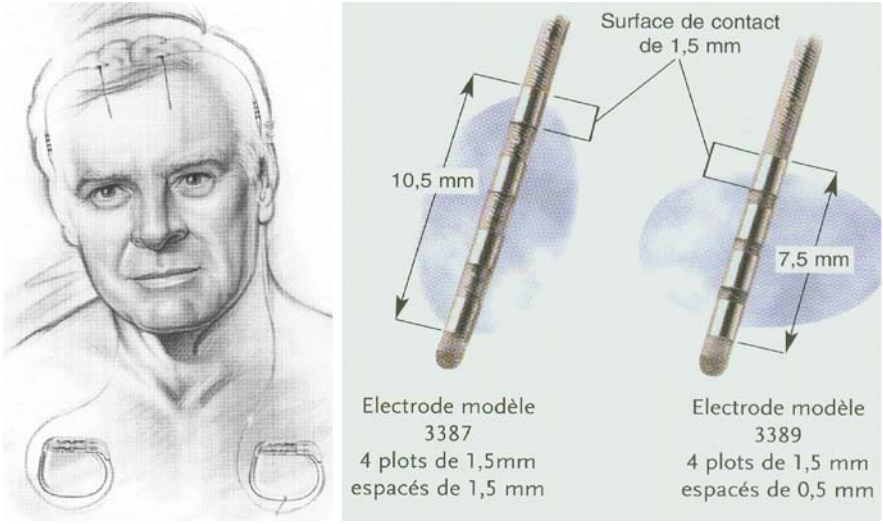

Fig. 21.4 Deep brain stimulator (DBS) with subcutaneous implanted pulse generators and electrodes placed in the brain. (Courtesy of Medtronic)

and FDA approval in 1997; subthalamic and globus pallidus DBS for movement disorders other than (or in addition to) tremor received the CE Mark in 1998 and FDA approval in 2002.

DBS implanted components consist of (1) an electrode (1.27 mm diameter with four contacts); (2) a pulse generator/battery (similar to a cardiac pacer, both in action and in placement subcutaneously inferior to the clavicle); and (3) an extension connecting the two (see Fig. 21.4). Electrode implantation for movement disorders is usually conducted under intravenous sedation so the patient can be tested during the procedure; MRI-based stereotactic image guidance is customarily used. Frequently microelectrode recording (with a microelectrode < 100 microns diameter) is employed to identify structures with characteristic electrical activity (such as the subthalamic nucleus (STN)), and microelectrode (and/or macroelectrode – with the implanted 1.27 mm diameter electrode) stimulation to confirm appropriate placement and avoid side effects [31, 32]. In DBS for TRD (and OCD, etc.), the targeting is based primarily on MRI image guidance since in general there are not characteristic microelectrode recording patterns for the nuclei or regions targeted in TRD/OCD DBS (except, e.g. the STN for OCD) [33].

The results of DBS for advanced Parkinson's disease, particularly with stimulation of the STN bilaterally, have been quite impressive in appropriately-selected patients. A multicenter randomized-pairs trial of 156 patients with advanced Parkinson's disease followed for 6 months after STN DBS or continued optimal medical management showed the DBS group to be superior on the Parkinson's Disease Questionnaire and the Unified Parkinson's Disease Rating Scale (UPDRS), part III [34]. Five-year follow-up of 49 patients undergoing bilateral STN DBS

showed only very modest deterioration in the UPDRS scores over the 5 years, remarkable in that patients are customarily referred for DBS when their functionality begins to deteriorate markedly [35].

Neurosurgical interventions for neuropsychiatric disorders have had a distinctive history, which will not be considered here but has been detailed in the literature [36]. Lesions performed for mood disorders and OCD have included the following [36]:

- Capsulotomy (anterior limb of the internal capsule)
- Cingulotomy (anterior cingulum)
- Subcaudate tractotomy (frontobasal white matter)
- Limbic leucotomy (combination of cingulotomy and subcaudate tractotomy)

Success rates for these ablative procedures varied widely, with the percentage of patients demonstrating improvement being in the 30–70% range in most studies (several of which had over 100 patients), and very rarely over 80%. This is very different from the situation in DBS (or ablative procedures, performed by an experienced neurosurgical team) for movement disorders, where the vast majority of patients are significantly improved.

DBS for neuropsychiatric disorders has been the subject of recent review articles [37–39]. The initial targets have, not surprisingly, included those listed above which were targets for ablative procedures. Additional targets include the nucleus accumbens, habenula, and thalamus, as well as the STN for OCD [33, 37]. Models for the various depressive states to guide DBS have been developed incorporating anatomic, neurotransmitter, and pharmacologic response data [38]. Sophisticated imaging techniques (e.g. PET and diffusion tensor imaging (DTI) MRI) have been employed to determine the connections of these various targets, and possible differences between normal individuals and TRD/OCD patients [38, 40].

The number of TRD and/or OCD patients treated with DBS remains small, however – it is the very rare published series to date that reports on more than 10 patients [37]. However, one recent study of 20 TRD patients who underwent bilateral SCC gyrus DBS is informative [41]. DBS treatment response was defined as a 50% or greater reduction in score on the 17-item HAM-D; remission was defined as a HAM-D score of 7 or less. Although at one month following DBS implantation the response and remission rates were 35 and 10%, respectively, at 6 months the response and remission rates were 60 and 35%, respectively; the benefits were maintained at one year following DBS implantation. In another study, one patient underwent cingulotomy with transient benefit – HAM-D score dropped from 22 to 5, but within 10 months had returned to 19 [42]. The benefit following bilateral subcallosal cingulated (SCC) gyrus DBS was maintained for 30 months (HAM-D score 7). Another promising DBS target for TRD is the nucleus accumbens, although the number of patients reported so far is small [43].

For OCD, the published literature has been summarized, with various targets used (STN, anterior limb of the internal capsule, nucleus accumbens, and ventral capsule/striatum) [37]. The response rate (typically using the Yale-Brown Obsessive-Compulsive Scale (YBOCS)) ranged from 25 to 75%, with less than 40 patients

total. In a double-blind, crossover, multicenter study involving 8 patients, the STN has been targeted with encouraging results (YBOCS mean improvement from 28 to 18, $P \sim 0.01$) [33]. There has been an encouraging collaboration and standardization across four centers (3 in the US, one in Belgium); over 8 years 26 OCD patients, using uniform selection criteria, have undergone DBS implanted into the region of the ventral anterior limb of the internal capsule and adjacent ventral striatum [43]. It is of interest that the optimal target was felt to be more posterior (and slightly more medial) in the patients treated later; when divided into 3 groups (first 9, second 9, third 8), the last group had distal electrode placements that were ~ 6 mm more posterior and ~ 3 mm more medial than the first group (based on postoperative MRI). The resultant voltage and pulse width settings for the last group were much lower than the first group (suggesting more optimal targeting), which also benefits the time before pulse generator or battery replacement is required. Another interesting result was the continued improvement in YBOCS over the first two years (similar to that seen with VNS): at one month after DBS implantation only 28% of patients showed a 35% or greater decrease in YBOCS; at one year 48%; at two years 65%.

Refractory Tourette's syndrome has also been treated recently with DBS, although there has yet to be consensus on the optimal site(s). Recent reviews of DBS in Tourette's syndrome – the world's published experience consisting of less than 50 patients – emphasize the heterogeneity of Tourette's syndrome patients, when the associated syndromes such as OCD are considered [44, 45]. Thus it is not surprising that various targets have been used – notably the centromedian-parafascicular thalamus, the globus pallidus internus, and anterior limb of the internal capsule/nucleus accumbens region. Results have been modest at best to date, which is underscored by the fact that several groups have placed two electrodes in both right and left hemispheres (typically in both the centromedian-parafascicular thalamus and the globus pallidus internus) in an effort to achieve greater clinical success [44, 45]. Unfortunately, for the immediate future it appears that the type of multicenter, collaborative study that has been undertaken in DBS for OCD with some success is unlikely to occur for Tourette's syndrome patients – because of both the heterogeneity of the patients and the modest clinical benefits of DBS to date (which underscores the present incomplete understanding of the pathophysiology of Tourette's syndrome).

21.6 Future Trends in Neuromodulation for Neuropsychiatric Disorders

For two of the techniques of neuromodulation considered above – VNS and tDCS – it is unlikely that the future will bring major technological advances. It is unlikely there will be dramatic alterations in how the vagus nerve can be stimulated, and for tDCS the issues of current transmission through the scalp and skull will remain.

For TMS it appears there will be modest refinements in technique that may enhance the efficacy of TMS for neuromodulation in neuropsychiatric disorders.

Coils are likely to be further refined so that the magnetic field – and hence the electrical stimulation induced by that magnetic field – is more focused, enhancing the precision of TMS. The potential to stimulate deeper regions of the brain than is possible with present TMS techniques is also being investigated [46]. Details of the Hesed coil are beyond the scope of this review, but have been presented; this technique raises the possibility of stimulating subcortical regions of the brain without undue effects on the cerebral cortex (or pain, muscle twitching, etc.) [46].

Given that the brain communicates both chemically and electrically, the future of neuromodulation will likely lie in combining modulation of both the electrical and the chemical environments of the brain. This is especially true in neuropsychiatric disorders, where neurotransmitter imbalances appear to be a very important if not essential aspect of the pathophysiology. As stated in a paper on DBS of the nucleus accumbens for TRD [47].

> Psychotropic drugs work by altering neurochemistry to a large extent in widespread regions of the brain, many of which may be unrelated to depression. We believe that more focused, targeted treatment approaches that modulate specific networks in the brain will prove a more effective approach to help treatment-resistant patients. [47, p. 1]

A recent laboratory study in rats has characterized the dopamine microenvironments within the nucleus accumbens, using techniques previously unavailable [48]:

> The brain is comprised of billions of neurons that are in constant communication with one another by the secretion of neurotransmitters. These neurons are organized into specific networks that regulate the behavioral state of the animal. The electrical firing of individual neurons can be monitored with electrophysiological microelectrodes, and such recordings have shown that many neurons fire randomly in animals at rest, while the firing patterns of neurons within circuits becomes synchronized during certain behaviors. Until recently the chemical communication that is an anticipated consequence of increased neuronal firing was only inferred. However, voltammetric microelectrodes provide an approach to monitor neurotransmitter concentrations in the extracellular fluid of the brain in real time. [48, p. 2046]

> The results of this study . . . reveal that dopamine concentrations within the nucleus accumbens are spatially heterogeneous and, in some sites, exhibit high-frequency fluctuations, and in others exhibit no activity even though dopamine release can be evoked in these locations. These unforeseen results provide the first direct evidence that dopamine transmission within the nucleus accumbens depends upon the activation of discrete subpopulations of dopamine terminals in this brain region. [48, pp. 2051–2052]

As noted above, the present techniques for DBS (employing macroelectrodes stimulating a volume of brain tissue several millimeters in diameter) in effect results in reversible ablation of the electrical activity within that volume – plus whatever effect that stimulation has on adjacent processes (axons and dendrites) of distant neurons. The effect of DBS on glial cells and their interaction with neurons has only begun to be investigated [49]. If neuromodulation is to live up to the goal in neuropsychiatric disorders of restoring brain function toward normal, it will require techniques that can interact with the nervous system on the microenvironment level (i.e. at the micron or submicron level rather than the millimeter level), as noted

in the quote above. Additionally, it will require addressing both the electrical and chemical aspects of brain function, the latter having been essentially ignored to date in neuromodulation.

Nanotechniques are being developed which can address these issues for neuromodulation in the future [50]. Some of the major issues are the following:

- Improvement in charge transfer from the device to the brain tissue to minimize the risk of injury from charge injection (i.e. improvement in the neural-electrical interface (NEI));
- Improvement in the precision of neuromodulation (i.e. stimulation at the level of the cell or a small group of cells, or perhaps at the level of the synaptic cleft or even intracellularly);
- Inclusion of recording information regarding brain function with the same precision as the stimulation;
- Monitoring and modulating local brain chemical activity (i.e. neurotransmitter concentrations) with the same precision as the recording/stimulating of local brain electrical activity.

The remainder of this section will briefly summarize ongoing research in these areas.

Regarding improvement in charge transfer from the neuromodulatory device to the brain tissue, remarkable advances are being made through the use of nanomaterials and conducting polymer coatings. A 10 to 100-fold reduction in impedance and similar increase in capacitance can be achieved (i.e. greatly improved NEI). Findings initially reported in cell cultures regarding the dramatic improvement in NEI with the use of conducting polymer-coated (e.g. polypyrrole) carbon nanotube (CNT) arrays as an electrode have recently been demonstrated in vivo in rodent and primate models, in which standard microelectrodes were compared with similar microelectrodes that had been CNT-coated (Fig. 21.5) [51, 52].

The CNT arrays allow much greater precision in the stimulation and recording of brain electrical and chemical (neurotransmitter) activity than possible with macroelectrodes or microelectrodes – down to the cellular level, or even intracellularly if necessary (Fig. 21.6) [53, 54]. Furthermore, it is possible with CNT arrays to detect and monitor certain electrochemically-active neurotransmitters, including dopamine, with a response rate and detection level exceeding the capabilities of carbon fiber microelectrodes noted above in the research on dopamine microenvironments in the rat nucleus accumbens (unpublished data, National Aeromautics and Space Administration (NASA) Ames Nanotechnology Group, 2006).

Computational analysis techniques have recently been applied to DBS in order to improve efficacy [55–57]. The trends for future DBS from computational analysis can be summarized:

- High frequency DBS (>100 Hz) will be replaced by low frequency DBS (<30 Hz);
- Feedback monitoring of brain electrical activity will guide stimulation;

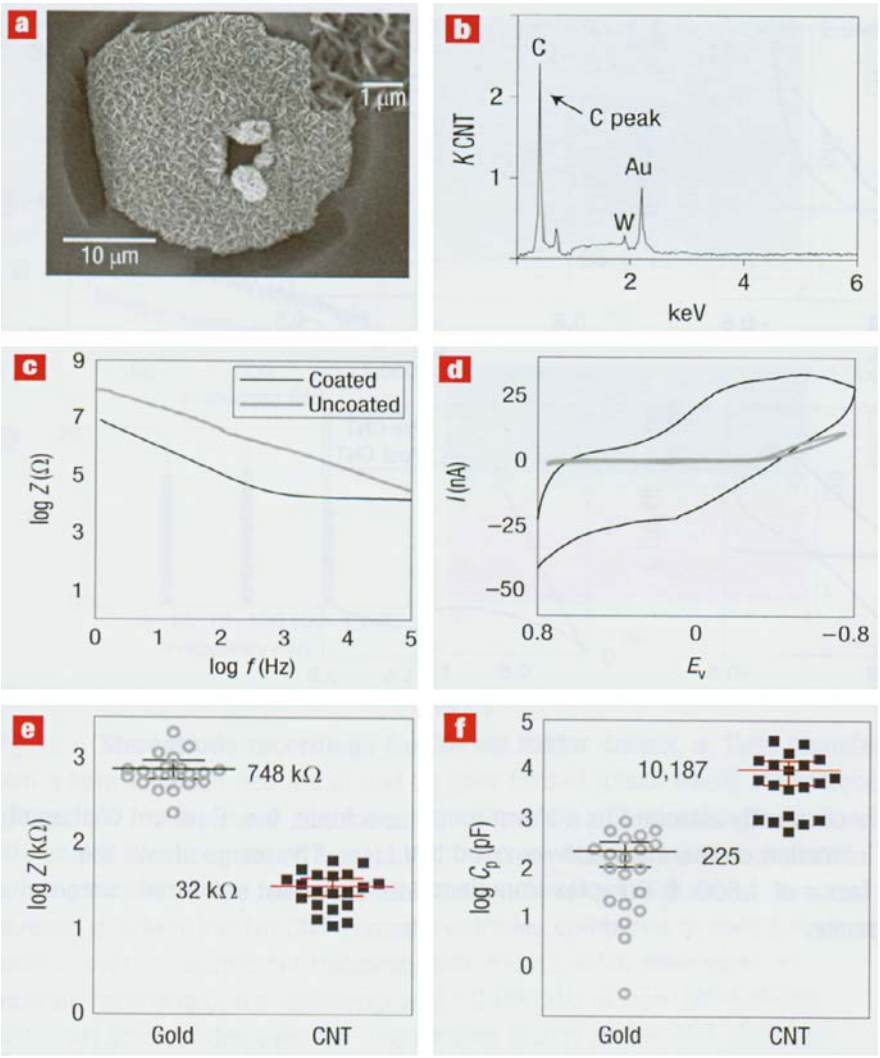

Fig. 21.5 CNT-coated MEA electrodes. (**a**) SEM of CNT-coated MEA electrode (magnified inset show porous nature of CNT coating). (**b**) Energy-dispersive X-ray analysis confirms carbon in the MEA coating. (**c**) Impedance spectroscopic scan – CNT coating results in decreased impedance at all frequencies. (**d**) Cyclic voltammetry scan – CNT coating results in increased charge transfer. (**e**) CNT coating – 23-fold decrease in impedance. (**f**) CNT coating – 45-fold increase in charge transfer. (Reproduced from Keefer et al. [52] with permission)

- Stimulation will be more focused using much smaller microelectrodes;
- Undesirable side effects will be much less common with more precise stimulation;
- Multiple recording and stimulation sites will become routine;

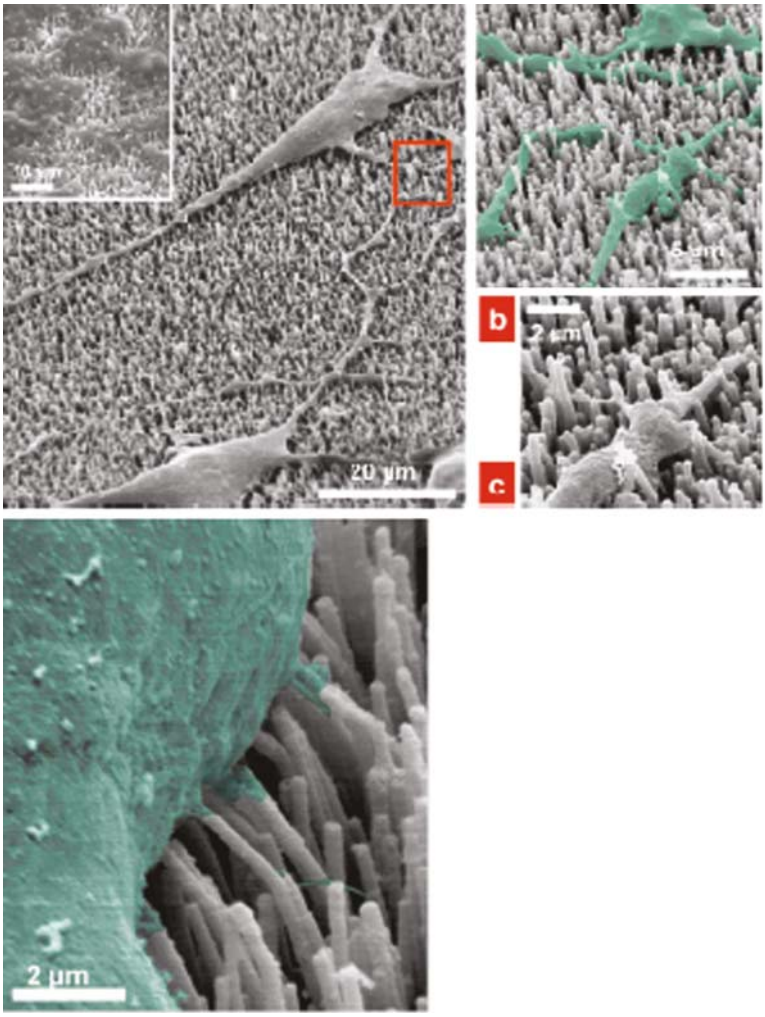

Fig. 21.6 *Left*: SEM images of PC12 neural network interpenetrating with freestanding CNTs with polypyrrole coating. (**b**) Polypyrrole-coated CNTs support a close NEI as the neuritis extend through the bed of CNTs. (**c**) Magnification of PC12 cells on CNT array. Right: Polypyrrole-coated CNTs are sufficiently rigid to maintain vertical alignment yet flexible enough to bend in compliance with the force exerted by the cell body. (Reproduced from Nguyen-Vu et al. [53] with permission)

- The greatly reduced current requirements will make large subcutaneous batteries (pulse generators) and connecting leads unnecessary;

- Most importantly: the abnormally synchronized brain electrical activity will be 'tweaked' back to normal (in the parlance of epilepsy, 'unkindling' of the abnormal firing patterns – in contrast to the 'kindling' of epileptic firing patterns that becomes ingrained over time).

The question then becomes:

How do we get many (perhaps dozens or hundreds) of micron-sized recording and stimulating devices precisely placed in the appropriate regions of the brain in a minimally-invasive manner?

For this, a solution has been demonstrated by proof-of-principle: one can access any region of the brain with micron-level accuracy by using the vascular supply to the brain – down to the capillary level. Endovascular techniques are rapidly evolving, and it has been shown that neuronal recording and stimulation can occur through the capillary wall as effectively as via intraparenchymal electrodes (Fig. 21.7) [58].

21.7 Conclusions

Neuromodulation for neuropsychiatric disorders has progressed significantly from the period of large numbers of frontal leucotomy/lobotomy procedures performed with little scientific understanding and perhaps even less ethical guidance. The techniques described above range from minimally-invasive TMS and tDCS (not, however, without certain risks, e.g. induced seizures) to VNS (quite a safe surgical implantation when performed by a competent surgeon) to DBS (with a significant risk of intracranial hemorrhage even when performed in the most experienced centers). As both the techniques and our understanding of their neuromodulatory effects evolve – increasingly based on laboratory research and computational modeling rather than clinical serendipity – the scientific basis for novel techniques and novel targets is enhanced. Additionally, ethical review panels and multicenter collaborative studies ensure the clinical trials are based on scientific evidence and humanitarian goals. Given the devastating effects of many neuropsychiatric disorders – especially when 'treatment-resistant' to the usual behavioral and pharmacologic interventions – the increasingly limited risk of intracranial procedures warrants further efforts at neuromodulation, even if invasive. A life of depression, isolation, maladaptive behavior, or suicide benefits neither the individual nor society.

Of the techniques described, DBS – as it evolves into more sophisticated methods of neuromodulation such as noted in the previous section – has the brightest future for enhancing neuromodulation for neuropsychiatric disorders. The goal of coaxing the central nervous system from abnormal, counterproductive communication patterns back to productive patterns – both electrically and chemically, one cell (neuronal or glial) at a time if need be – can be realized. As new techniques for understanding the nervous system are developed, the efficacy of DBS will improve dramatically. One such technique, optogenetics, uses light-sensitive ion

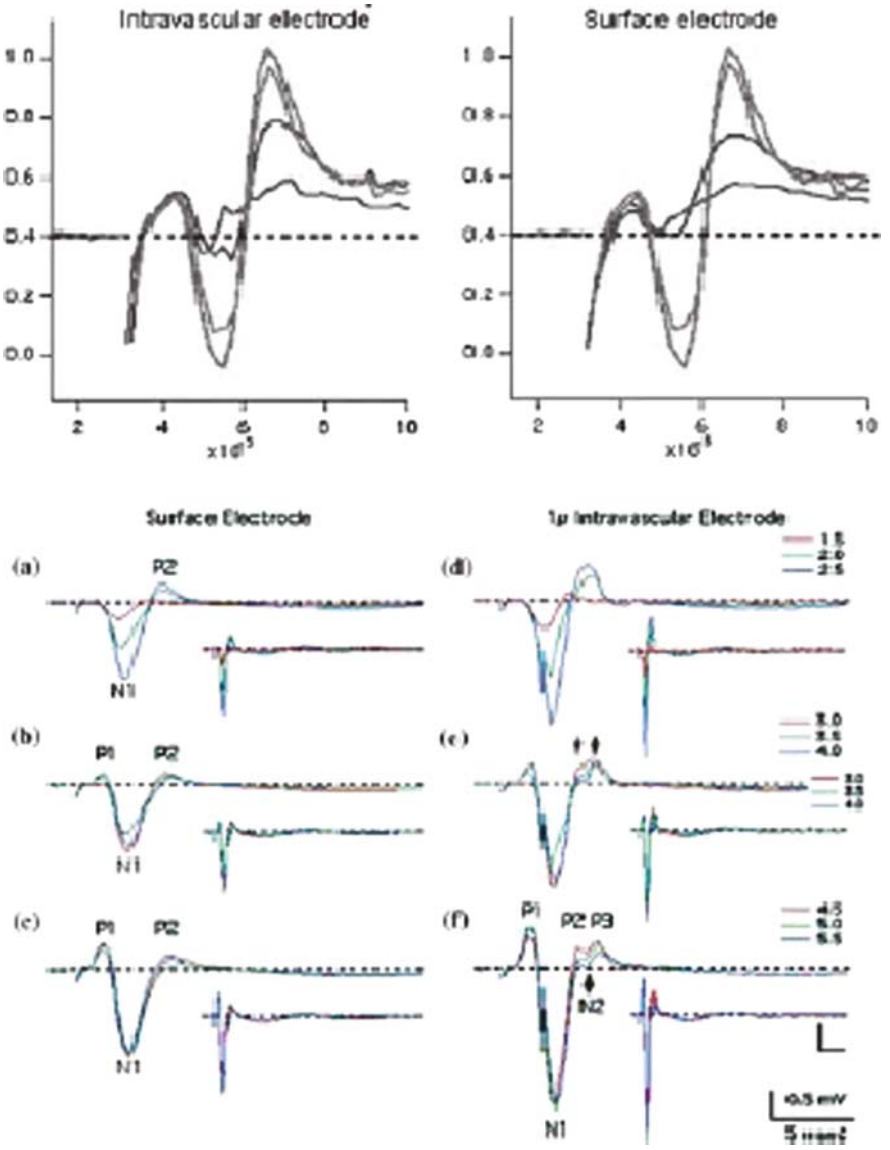

Fig. 21.7 Spinal vessel recording with 1 μm intravascular electrode vs surface electrode. Several tracings as the stimulus amplitude is increased. Amplitude: mV; time: msec. (Reproduced from Llinas et al. [58] with permission)

conductance-regulating proteins to selectively excite or inhibit neurons: by illuminating the region of brain with either a fiberoptic or light-emitting diode (LED) at 470 nm (nanometers) wavelength (blue light) or 580 nm wavelength (yellow light)

either excitation or inhibition can be induced by the movement of either sodium ions or chloride ions into the neuron [59].

It is truly an exciting time in the development of neuromodulation for neuropsychiatric disorders. The combination of cellular-level technologies such as CNTs and optogenetics are creating an understanding of normal and abnormal brain function not previously possible. Computational analysis techniques allow modeling of therapeutic modalities involving multisite neuromodulators in ways also not previously possible. We are beginning to communicate with the nervous system on its own terms – and will soon have the methods to 'persuade' aberrant brain firing patterns to resume normality.

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M.S. Ritsner (ed.), *Brain Protection in Schizophrenia, Mood and Cognitive Disorders*,
DOI 10.1007/978-90-481-8553-5, © Springer Science+Business Media B.V. 2010