REVIEW

Consensus paper of the WFSBP Task Force on Biological Markers: Biological Markers in Depression

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Abstract

Biological markers for depression are of great interest to aid in elucidating the causes of major depression. We assess currently available biological markers to query their validity for aiding in the diagnosis of major depression. We specifically focus on neurotrophic factors, serotonergic markers, biochemical markers, immunological markers, neuroimaging, neurophysiological findings, and neuropsychological markers. We delineate the most robust biological markers of major depression. These include decreased platelet imipramine binding, decreased 5-HT1A receptor expression, increase of soluble interleukin-2 receptor and interleukin-6 in serum, decreased brain-derived neurotrophic factor in serum, hypocholesterolemia, low blood folate levels, and impaired suppression of the dexamethasone suppression test. To date, however, none of these markers are sufficiently specific to contribute to the diagnosis of major depression. Thus, with regard to new diagnostic manuals such as DSM-V and ICD-11 which are currently assessing whether biological markers may be included in diagnostic criteria, no biological markers for major depression are currently available for inclusion in the diagnostic criteria.

Introduction

The aim of this article is to review recent evidence on biological markers of depression that could be detected in different ways – by testing body fluids or cells, by brain imaging or other methods. Depressive disorders are heterogeneous and diagnosed on the basis of a patient’s symptoms, not on the basis of a laboratory test. So the search for biological markers for depression is partially due to the need of finding diagnostic adjuncts.

The treatment of depressive disorders varies both in type and effectiveness. Antidepressant drugs have been used for over 40 years, but they still present the problem of delayed onset of action. Normally it takes about 14 days for the patients responding to a given antidepressant to improve during which time they are still depressed and suicidal. So a biological marker would be useful to distinguish early improvement under drug treatment (first days, even hours).

Biological markers may give an insight into the underlying biological basis of depression, which can be used to develop more effective drug treatments.

The idea behind identifying biological markers is that by using them, psychiatrists could reveal the specific depression profile of each patient and select the optimal treatment. The term ‘biological marker’ is used here to describe a biological change associated with depression that could be used to indicate the presence and severity of the condition and predict drug or other treatments’ response as well as the clinical prognosis.

Neurodevelopment/neurotrophic factors

Neurodevelopment factors

The human brain, the most complex of all organs, contains 100 billion neurons and 10 times as many
glial cells. These cells of the human brain connect and organize into functional units with specific roles to sense, perceive, process and act on information from outside and inside the individual to promote survival and other actions of the human being.

Each of these cells contains the same genetic material (genotype). However, each cell type utilizes actively different portions of it so each individual neuron expresses different properties and functions. Differentiation is the process by which cells become specialized, expressing those components of the genome, which confer special properties associated with the functions of the neuron in the mature brain. This process takes place throughout development. While the majority of neurons exist already by birth (neurogenesis), most of them have not completed their individual growth and specialization. Over the 3 years following birth, the important processes of neuronal migration, axo-dendritic projection, myelinization, synaptogenesis, and neurochemical differentiation continue to take place. As the brain develops, neurons migrate and differentiate in response to chemical, ‘microenvironmental’ stimuli (morphogens), which confer information to and direct specific differentiation of the cell. So, the neuron’s unique structure, biochemistry and function are the result of its unique environmental history, i.e. the specific pattern of exposure, timing and quantity of these microenvironmental stimuli. Some of the most important microenvironmental stimuli are receptor-mediated signals from neurotransmitters and hormones, which act as morphogens. That is why the ‘stress response’ is extremely important for brain organization (Perry and Pollard 1998). This well-characterized set of adaptive physiological responses to real or perceived danger involves a series of complex, interactive neurophysiological reactions in the brain, the autonomic nervous system, the hypothalamic–pituitary adrenocortical (HPA) axis and the immune system. The neurophysiological activation (e.g., increased release of norepinephrine) seen during acute stress is usually rapid and reversible. When the stressful event is of a sufficient duration, intensity, or frequency, however, the brain is altered. Stress-induced ‘sensitization’ may occur. The neurochemical systems mediating the stress response (e.g., locus coeruleus noradrenergic systems) change, becoming more ‘sensitive’ to future stressors related to the original experience. The molecular mechanisms underlying this phenomenon are not well understood but are related to the same cascade of molecular processes involved in learning and memory. The stressful experience, via a cascade of neurochemical events, alters the microenvironmental milieu of the CNS, resulting in altered gene expression. The new gene products may then result in ‘permanent’ or structural changes which are associated with sensitization, learning, memory and, in the developing brain, differentiation. In the adult, mature brain, increases in, or unusual patterns of, catecholamine activity may result in sensitization. In the developing brain, however, neurotransmitters, in addition to their roles in cellular communication, play an important role in the basic neurodevelopmental processes (Lauder 1988). Trauma-related alterations in catecholamine activity during childhood, therefore, may alter brain development, resulting in altered functional capabilities of the ‘traumatized’ brain. Changes in the pattern, timing and quantity of catecholamine (or any critical neurotransmitter system) activity during development might be expected to result in altered development of catecholamine receptor/effector systems and the functions mediated, in part, by these systems. A trauma-induced prolonged stress response will result in an abnormal pattern, timing and intensity of catecholamine activity in the developing brain. The time during development that this prolonged or abnormal catecholamine activity is present, determines to some degree the nature and severity of the disrupted development. In general, the earlier and the more pervasive the trauma, the more neurodevelopment will be disrupted.

There is some evidence to suggest that prenatal or maternal traumatic stress has significant impact on neurodevelopment. The development of the human brain continues beyond birth and its development remains vulnerable to the abnormal patterns of neurotransmitter and hormone activity associated with traumatic stress. Young children victimized by trauma are at risk for developing permanent changes in brain development with potential impact on all aspects of emotional, cognitive and behavioural functioning. There are times in development, called critical periods, during which a set of signals must be present for the neurons to differentiate normally. In addition, there are times when an undifferentiated neuron is especially ‘receptive’ or sensitive to a set of signals. At these times, termed sensitive periods, the neuron will use this set of signals to facilitate further specialization as part of a larger functional subsystem in the brain. For humans, some extreme illustrations of the principles of development during these sensitive periods have been described in the literature. Children raised with little or no exposure to verbal language never develop the neural apparatus needed for optimal speech or language development (Freedman 1981); children raised in sensory-deprived settings have major deficits in developing integrated neurosensory processing; children with various visual deficits (e.g., strabismus), for example, develop abnormal visual and perceptual
capabilities (Lipton 1970). In humans there is very little information regarding these ‘windows’ of vulnerability; the majority of the irreversible sensory processing deficits have resulted from deprivations during the first three years of life. There are, of course, critical and sensitive periods for the development of important brain systems and functions other than neurosensory processing. There is overwhelming evidence suggesting sensitive, if not critical, periods for brain functions associated with ‘mental health’ including attachment, affect modulation, anxiety regulation, and behavioural impulsivity (Provence 1983), all of which utilize to varying degrees the same neurobiological subsystems which mediate the ‘stress response’. The sensitive periods for the stress response ‘apparatus’ in the brain — developmental phases during which an individual is most vulnerable to traumatic stressors — occur when the stress-mediating catecholamine systems are undergoing neurogenesis, migration, synaptogenesis and neurochemical differentiation. The functional capabilities of the CNS systems mediating stress in the adult are determined by the nature of the ‘stress’ experiences during the development of these systems, i.e. in utero, during infancy and childhood. Increased psychiatric symptoms and disorders are observed in adults who have severe, unpredictable early life stressors (Rutter 1984). A provocative study (Breier et al. 1988) reported the effects of parental loss during childhood on the development of psychopathology in adulthood. They examined a number of adults who had suffered a parental loss during childhood and found that the subjects with psychiatric disorders and symptoms had significant biological and immunological changes related to early parental loss relative to control groups. The authors concluded that early parental loss (a traumatic event) accompanied by the lack of a supportive relationship subsequent to the loss (an external stress reducing factor) is related to the development of adult psychopathology. Other studies have documented relationships between developmental trauma and borderline personality disorders, depressive disorders (Kaufman 1991), dissociative disorders and a variety of other medical and psychiatric conditions. According to Gale and Martyn (2004) impaired neurodevelopment during foetal life may increase susceptibility to depression.

Clearly, these many studies provide correlative data indicating that developmental stress is a major expressor of any underlying constitutional or genetic vulnerability and may be the primary aetiological factor in the development of certain neuropsychiatric disorders. The abnormal pattern of stress-mediating neurotransmitter and hormone activations during development alters the brains of traumatized children. The specific nature of these functional alterations is seen in all of the brain functions, which are directly or tangentially related to CNS catecholamine systems. Unfortunately, the CNS catecholamines (and likely other important neurotransmitter systems altered by these experiences) are involved in almost all core regulatory activities of the brain. The brainstem and midbrain catecholamines are involved in regulation of affect, anxiety, arousal/concentration, impulse control, sleep, startle, autonomic nervous system regulation, memory and cognition. Clearly the physical signs and symptoms seen in traumatized children include dysfunction and dysregulation in these domains. Indeed, the ‘core’ symptoms seen in severely traumatized children may be traced back to dysregulation of these root neurophysiological regulatory functions.

It has been proposed that stress-induced changes in the hippocampus may be central to the development of depression in genetically vulnerable individuals. New evidence implicates the prefrontal cortex (PFC) in addition to the hippocampus as a site of neuropathology in depression. The PFC may be involved in stress-mediated neurotoxicity because stress alters PFC functions and glucocorticoid receptors, the PFC is directly interconnected with the hippocampus, and metabolic alterations are present in the PFC in depressed patients. Postmortem studies in major depression and bipolar disorder provide the first evidence for specific neuronal and glial histopathology in mood disorders. Three patterns of morphometric cellular changes are noted: cell loss (subgenual PFC), cell atrophy (dorsolateral PFC and orbitofrontal cortex), and increased numbers of cells (hypothalamus, dorsal raphe nucleus). The relevance of cellular changes in mood disorders to stress and prolonged PFC development and a role of neurotrophic/neuroprotective factors are suggested, and a link between cellular changes and the action of therapeutic drugs is discussed (Rajkowska 2000).

**Neurotrophic factors**

***Neurogenesis.* The development and maintenance of the vertebrate nervous system requires continuous supply of a number of polypeptide hormones known as neurotrophic factors. During the period of target innervation, limiting amounts of neurotrophic factors regulate neuronal numbers by allowing survival of only some of the innervating neurons, the remaining being eliminated by programmed cell death. Increasing evidence indicates that several neurotrophic factors also influence the proliferation, survival and differentiation of precursors of a number of neuronal lineages. In the adult, neurons
continue to be dependent on trophic factor support. Thus, neurotrophic factors are important signalling molecules for the development and maintenance of neurons, acting throughout development on many types of neuronal populations (Ibanez 1995; Mamounas et al. 1995). They increase cell survival by providing necessary trophic support for growth, but also by exerting inhibitory effects on cell death cascades (Thoenen 1995; Pettmann and Henderson 1998; Riccio et al. 1999).

Proliferation and maturation of neurons have been demonstrated to occur at a significant rate in discrete regions of adult brain, including the hippocampus and subventricular zone, although many of the newborn cells degenerate 1–2 weeks after birth. Very little is known about the intracellular signal transduction pathways that control adult neurogenesis. There are indications that the cAMP–CREB pathway regulates the survival, differentiation and function of newborn neurons (Nakagawa et al. 2002). Adult neurogenesis is an extremely dynamic process that is regulated in both a positive and negative manner by neuronal activity and environmental factors. It has been suggested to play a role in several important neuronal functions, including learning, memory, and response to novelty. In addition, exposure to psychotropic drugs or stress regulates the rate of neurogenesis in adult brain by regulating the expression and function of growth factor cascades, suggesting a possible role for neurogenesis in the pathophysiology and treatment of depression and other illnesses (Smith et al. 1995; Nibuya et al. 1999; Duman et al. 2001). Several neurotrophic factors (such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF)), as well as cytokines and insulin-like growth factor-1 (IGF-1), increase cell survival (Mamounas et al. 1995; Pettmann and Henderson 1998). This occurs through binding of these factors to membrane receptors and regulation of intracellular signal transduction pathways that can control apoptosis, including regulation of Bcl-2 family members. Signal transduction cascades that are currently believed to mediate many of the effects of neurotrophic factors include the mitogen-activated protein kinase (MAPK) cascade, the phosphatidylinositol-3 kinase (PI3K)–Akt pathway, and the phospholipase C cascade (Segal and Greenberg 1996). Recent studies have demonstrated that the activation of the MAPK pathway can inhibit apoptosis by inducing the phosphorylation of Bad (a major proapoptotic protein) and increasing the expression of Bcl-2 (a major antiapoptotic protein), the latter effect likely involves the cAMP response element–binding protein (CREB) (Bonni et al. 1999; Riccio et al. 1999). Accumulating data suggest that not only is Bcl-2 neuroprotective, but it also exerts neurotrophic effects and promotes neurite sprouting, neurite outgrowth, and axonal regeneration (Chen et al. 1997; Chen and Tonegawa 1998; Holm et al. 2001). It is important to note that both lithium and valproate increase Bcl-2 (Moore et al. 2000). Estrogen is also a neurotrophic factor. Estradiol can induce BDNF expression, and recent data suggest that structural and electrophysiological effects of estradiol in the hippocampus might be mediated by BDNF (Scharfman and Maclusky 2005). Disruption of the relationship between estrogen and BDNF could contribute to neurological and psychiatric disorders that have been associated with the hippocampus, such as depression and Alzheimer’s disease. The cAMP response element-binding protein (CREB) is a critical integrator of neural plasticity that is responsive in a brain region-specific manner to a variety of environmental and pharmacological stimuli, including widely prescribed antidepressant medications. CREB is an ubiquitous key-element of intracellular signal transduction cascades that may contribute to symptoms of depression (Newton et al. 2002). Its transcriptional activity depends on phosphorylation at Ser-133. By measuring CREB-phosphorylation in T-lymphocytes it was found that the responders to psychopharmacological treatment with different treatment regimens showed a significant increase in CREB-phosphorylation compared to the non-responders. These results indicate for the first time that the increase in CREB-phosphorylation might be a molecular state marker for the response to antidepressant treatment (Koch et al. 2002). However, other studies do not support such a hypothesis (Lai et al. 2003). Furthermore, activation of both protein kinases, PKA and PKC was associated with reduced CREB-P in melancholics relative to normal controls. Finally, PKA activity was found to correlate positively with Hamilton depression scores after 16 weeks of treatment with serotonin reuptake inhibitor antidepressants. These data further implicate signal transduction abnormalities in melancholic major depression, particularly PKA and PKC. This suggests an abnormality of factors controlling the expression or degradation of these enzymes (Akin et al. 2005).

**Brain-derived neurotrophic factor (BDNF).** BDNF is the neurotrophic factor in the focus of intense research for the last years. BDNF belongs to the neurotrophin family of growth factors and affects the survival and function of neurons in the central nervous system. Its normal physiological role is to encourage the outgrowth of dendrites from nerve
endings, and to help stabilize connections between neurons. BDNF is, however, an unusual neurotrophic factor because its widespread functions in the brain go beyond the traditional role of a growth factor to promote growth, survival, and maintenance of cells. Beyond promoting neuronal survival and resilience to injury, BDNF also has a powerful role in facilitating activity-dependent plasticity, which underlies the capacity for learning and memory. Brain regions where plasticity is particularly important include the hippocampus and cortex, critical centers for learning and memory. The hippocampus is a central component for encoding new information, and damage there severely impairs learning. From a plasticity point of view, insufficient BDNF would weaken synaptic encoding strength or capacity, while from the neurotrophic angle, reduced BDNF makes hippocampal neurons more vulnerable to insult and degeneration. BDNF is produced by neurons, particularly in the hippocampus and cortex. Neuronal activity, i.e., during encoding of information, stimulates BDNF gene transcription, transport of BDNF mRNA into dendritic spines, and BDNF protein release into the synaptic cleft (Hartmann et al. 2001). BDNF improves survival of cholinergic neurons of the basal forebrain, as well as neurons in the hippocampus and cortex.

BDNF acts on neurons at both presynaptic and postsynaptic sites by binding to its tyrosine kinase receptor TrkB, and internalization of the BDNF–TrkB complex-signalling endosome (Lu 2003). By enhancing synaptic transmission and neuronal excitability, BDNF modulates synaptic change, including hippocampal long-term potentiation (LTP), a neural mechanism associated with learning and adaptive behaviours in adult animals (Poo 2001; Tyler et al. 2002). BDNF-deficient mice show decreased synaptic innervation and reduced levels of synaptic vesicle proteins (Martinez et al. 1998; Pozzo-Miller et al. 1999), demonstrating that BDNF is important for normal synaptic signalling (Martinez et al. 1998). Two potent stimuli that rapidly increase BDNF levels in the hippocampus are exercise and learning. In rodents, voluntary daily wheel running consistently increases BDNF mRNA and protein levels in the hippocampus and other brain regions, including parts of the cortex (Cotman and Berchtold 2002). In addition, learning itself increases brain BDNF levels, particularly in the hippocampus. Interestingly, in humans, regular exercise is associated with benefits to brain health and cognitive function, which may in part be due to increased availability of BDNF. Indeed, physically active adults not only have a lower risk of cognitive impairment, but also a lower risk of depression and of developing AD or dementia of any type (Friedland et al. 2001; Laurin et al. 2001). Studies demonstrating that neurogenesis is increased by conditions that stimulate neuronal activity (such as enriched environment, learning, and exercise) suggest that this process is also positively regulated by, and may even depend on, neuronal plasticity (Kempermann 2002). BDNF and serotonin (5-hydroxytryptamine, 5-HT) are known to regulate synaptic plasticity, neurogenesis and neuronal survival in the adult brain. These two signals co-regulate one another such that 5-HT stimulates the expression of BDNF, and BDNF enhances the growth and survival of 5-HT neurons (Mattson et al. 2004).

Our contemporary knowledge on neurotrophic factors and depression is based on a number of postmortem studies and animal models of depression and antidepressants’ action. Some observations from animal studies suggest that BDNF and NGF may play a role in depression and (because of the different brain regional concentrations of BDNF and NGF found in male and female animals) may be relevant to gender differences in vulnerability to depression (Angelucci et al. 2000). There is emerging evidence – primarily from postmortem studies – that supports a role for abnormalities in neurotrophic signalling pathways in depression. Decreased levels of CREB, BDNF, and the TrkB receptor have been reported in suicide victims (Dwivedi et al. 2003a,b; Yamada et al. 2003). It has been indicated from postmortem studies that a transcription factor likely mediates neural plasticity in the mammalian brain and neural tissues (Yamada et al. 2003).

A large body of evidence has established a link between stressful life events and development or exacerbation of depression. Recent studies suggest that stress-induced atrophy and loss of hippocampal neurons may contribute to the pathophysiology of depression. At the cellular level, evidence has emerged indicating neuronal atrophy and cell loss in response to stress and in depression. At the molecular level, it has been suggested that these cellular deficiencies, mostly detected in the hippocampus, result from decreased expression of BDNF associated with elevation of glucocorticoids. However, as also revealed by converging lines of evidence, high levels of glucocorticoids down-regulate hippocampal synaptic connectivity (‘negative’ metaplasticity), whereas an increase in expression of BDNF up-regulates connectivity in the hippocampus (‘positive’ metaplasticity) (Garcia 2002). Stress hormones and 5-HT may act in an opposite manner – stress hormones decrease BDNF levels while 5-HT increases them. The reduction of BDNF could be the result of the decrease in CREB as shown in animal studies (Trentani et al. 2002). The loss of BDNF in chronic stress leads to an altered
output from areas such as the hippocampus to the pre-frontal cortex. It is well known that the glucocorticoid and mineralocorticoid hormones, released from the adrenal glands during stress, contribute to depression by acting on the hippocampus. They bind to specific receptors in this region and thus cause a drop in both BDNF mRNA and protein in neurons. In view of the opposite effects of stress and antidepressants on hippocampal neurogenesis, it is quite plausible that alterations in hippocampal neurogenesis are fundamental to the clinical syndrome of depression (Jacobs et al. 2000; Manev et al. 2001; D’Sa and Duman 2002; Kempermann 2002).

A growing body of evidence suggests that antidepressants may regulate neurotrophic signalling cascades. Different classes of long-term antidepressant treatments – including norepinephrine (NE) reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), and electroconvulsive therapy – up-regulate CREB and BDNF expression, which indicates that CREB and BDNF are common postreceptor targets of these therapeutic agents (Nibuya et al. 1995; Nibuya et al. 1996). More evidence that links up-regulation of these pathways and antidepressant activity comes from behavioural models (Duman et al. 1999). It was observed that CREB overexpression in the dentate gyrus or BDNF injection results in an antidepressant-like effect in behavioural models of depression and antidepressant efficacy in rats (Siuciak et al. 1997; Chen et al. 2001a; Shirayama et al. 2002). Chronic, but not acute, antidepressant treatment also increases the neurogenesis of dentate gyrus granule cells (Jacobs et al. 2000; Manev et al. 2001; D’Sa and Duman 2002). Lithium, one of the most effective antidepressant potentiating agents, also increases neurogenesis in the dentate gyrus (Chen et al. 2000). In contrast, increased neurogenesis is not observed in response to long-term administration of non-antidepressant psychotropic drugs.

New research in animals is beginning to change radically our understanding of the biology of stress and the effects of antidepressant agents. Stress and antidepressants have reciprocal actions on neuronal growth and vulnerability (mediated by the expression of neurotrophins) and synaptic plasticity (mediated by excitatory amino acid neurotransmission) in the hippocampus and other brain structures. Antidepressant treatments might not only restore cell density but also regulate higher-order synaptic plasticity in the hippocampus by abolishing ‘negative’ metaplasticity, and thus restore hippocampal cognitive processes that are altered by stress and in depressed patients (Garcia 2002). Stressors have the capacity to progressively disrupt both the activities of individual cells and the operating characteristics of networks of neurons throughout the life cycle, while antidepressant treatments act to reverse such injurious effects. A central role for the regulation of synaptic connectivity in the pathophysiology of depressive disorder is thus proposed (Reid and Stewart 2001). Chronic administration of several different classes of antidepressant, but not non-antidepressant, agents was found to increase BrdU-labelled (bromodeoxyuridine, a marker of cell division) cell number, indicating that this is a common and selective action of antidepressants. These findings raise the possibility that increased cell proliferation and increased neuronal number may be a mechanism by which antidepressant treatment overcomes the stress-induced atrophy and loss of hippocampal neurons and may contribute to the therapeutic actions of antidepressant treatment (Malberg et al. 2000). Disrupting antidepressant-induced neurogenesis blocks behavioural responses to antidepressants, seen in a study, in which X-irradiation of mouse hippocampus prevented the neurogenesis and behavioural effects of two classes of antidepressants. These findings suggest that the behavioural effects of chronic antidepressants may be mediated by the stimulation of neurogenesis in the hippocampus (Santarelli et al. 2003).

Several studies with contradicting results suggest that these changes occur in the context of broader impairments of cellular plasticity and resilience, rather than in that of a limited neurogenesis model (van der Hart et al. 2002; Morcuende et al. 2003; Vollmayr et al. 2003).

**BDNF as a biological marker in depression.** In post-mortem studies of depressed patients with or without antidepressive treatment it was shown that there are abnormalities in the cAMP signalling pathway, and its downstream neurotrophic factor BDNF, which are major targets of antidepressant medications (Chen et al. 2001b). Increased BDNF expression was found in dentate gyrus, hilus and supragranular regions in subjects treated with antidepressant medications at the time of death, compared with antidepressant-untreated subjects. Furthermore, there was a trend toward increased BDNF expression in hilar and supragranular regions in depressed subjects treated with antidepressants, compared with the subjects not on these medications at the time of death. These findings are consistent with recent studies measuring CREB levels in this same subject sample, and support current animal and cellular models of antidepressant function.

Several lines of research show that the BDNF molecule is probably the “final common pathway”
for different antidepressant approaches. These include antidepressants (Nibuya et al. 1995), ECT (Nibuya et al. 1995; Duman and Vaidya 1998; Altar et al. 2003), exercise (Oliff et al. 1998; Garcia et al. 2003) and rTMS (repetitive transcranial magnetic stimulation) (Müller et al. 2000). All of these treatments increase BDNF at least in rats, and likely in humans, as supported by multiple threads of evidence, e.g., direct measurements of BDNF in the bloodstream (Karege et al. 2002, 2005; Shimizu et al. 2003). Treatment of depressed patients with antidepressants increases the reduced pretreatment serum BDNF levels close to the levels of normal controls (Aydemir et al. 2005; Gervasoni et al. 2005; Gonul et al. 2005). Some results suggest that the combination of exercise and antidepressant treatment may have significant neurochemical and behavioural effects. In addition, they support the possibility that the enhancement of BDNF expression may be an important element in the clinical response to antidepressant treatment (Russo-Neustadt et al. 2001). The results suggest that noradrenergic activation via β-adrenergic receptors may be essential for both exercise- and antidepressant-induced BDNF regulation. 5-HT (1A) and 5-HT(2A/C) receptor activation, on the other hand, appear to be most important for antidepressant-induced BDNF regulation, but may also participate significantly in exercise-induced regulation in the CA4 region of the hippocampus (Ivy et al. 2003).

Studies on human blood levels show that BDNF is low in depressed patients (Karege et al. 2002). These results suggest that major depression is characterized by low serum BDNF levels and support the hypothesis of neurotrophic factor involvement in affective disorders. In another study, serum BDNF was found significantly lower in antidepressant-naïve patients with major depression than in the treated group or in the control group. A significant negative correlation was found between serum BDNF and HAM-D scores in all patients. In a preliminary examination, reduced BDNF values of three drug-naïve patients recovered to basal levels after antidepressant treatment. This study suggests that low BDNF levels may play a pivotal role in the pathophysiology of depression and that antidepressants may increase BDNF in depressed patients (Shimizu et al. 2003). Low serum BDNF could thus be a marker of depression if the results are replicated in larger studies.

Recent findings from animal studies have suggested a possible role for BDNF in depression. BDNF protects against stress-induced neuronal damage, and it might affect neurogenesis in the hippocampus, which is thought to be involved in the pathogenesis of mood disorders (Hashimoto et al. 2004). Expression of CAMKII-α and TBR1 mRNAs was significantly increased in bipolar patients but not in major depressed patients, and there was a trend toward reduced BDNF expression in both groups (Molnar et al. 2003).

Exogenous delivery of BDNF or neurotrophin-3 (NT-3) promotes the function, sprouting and growth of 5-HT-containing neurons in the brains of adult rats. Antidepressants increase BDNF mRNA in the brain, via 5-HT2A and β-adrenoceptor subtypes and prevent the stress-induced decreases in BDNF mRNA. So, the existing treatments of depression might work by increasing endogenous brain levels of BDNF or NT-3, which in turn could promote monoamine-containing neurons growth and function (Altar 1999).

BDNF exerts direct antidepressant activity in animal models of depression (Shirayama et al. 2002). Other studies demonstrate that chronic antidepressant treatment increases the rate of neurogenesis in the adult hippocampus. They also show that antidepressants up-regulate the cyclic adenosine monophosphate (cAMP) and the neurotrophin signalling pathways involved in plasticity and survival. In vitro and in vivo data provide direct evidence that the transcription factor, cAMP response element-binding protein (CREB) and BDNF are key mediators of the therapeutic response to antidepressants (Nibuya et al. 1996). These results suggest that depression may be associated with a disruption of mechanisms that govern cell survival and neural plasticity in the brain. Antidepressants could mediate their effects by increasing neurogenesis and modulating the signalling pathways involved in plasticity and survival (D’Sa and Duman 2002).

Allelic variation of BDNF expression. Genetic abnormalities in BDNF and CREB may also occur in depression. Recent findings from family-based association studies have suggested that the BDNF gene is a potential risk locus for the development of bipolar disorder. They suggest that BDNF plays a critical role in the pathophysiology of mood disorders and in the activity of therapeutic agents in patients with mood disorders (Hashimoto et al. 2004). Sequence variations in the CREB1 gene have been reported to cosegregate with depressive disorders in women (Zubenko et al. 2003). A BDNF coding variant may be associated with the personality trait of neuroticism, which is a risk factor for depression (Sen et al. 2003). This polymorphism has been associated with alterations in BDNF trafficking and secretion in vitro and with alterations in hippocampal working memory in humans (Egan et al. 2003). Endogenous levels of BDNF protein
were measured in serum samples of healthy volunteers. A negative correlation between BDNF serum concentration and the depression related factor neurotropicism was found. Low BDNF levels in healthy humans with depressive personality traits might constitute a risk marker, reflecting a personality profile, which is linked to vulnerability to mood disorders. These results provide further support for the hypothesis that BDNF may be central to the development of depressive mood states (Lang et al. 2004). In addition, two studies (Neves-Pereira et al. 2002; Sklar et al. 2002) suggest that the BDNF locus is associated with bipolar disorder.

Other neurotrophic factors: fibroblast growth factors. Many studies show us that BDNF is not the sole neurotrophin engaged in depression. Several data show the importance of fibroblast growth factors (FGFs). FGFs are a family of molecules that stimulate cell growth in many areas of the body, and are involved in the growth of multiple tissues and in growth that takes place at various stages of life. They have potent effects during embryonic, foetal and child development, and can modify the size and structure of particular brain regions. They are also involved in the repair of adult tissues after injury and may mediate the cross-talk between different cell types in the brain. As a result, they can be seen as mediators of the property that neuroscientists call “neural plasticity” – the ability of the brain to adapt to stress, experience, disease and the effects of drugs. Dysregulation of several fibroblast growth factor (FGF) system transcripts may occur in frontal cortical regions of brains from human subjects with major depression (Evans et al. 2004). This altered gene expression was discovered by microarray analysis of frontal cortical tissue from major depression, bipolar, and nonpsychiatric control subjects and was verified by quantitative real-time PCR analysis and, importantly, in a separate cohort of major depression subjects. Furthermore, it was shown, through a separate analysis of selective serotonin reuptake inhibitor (SSRI)-treated and non-SSRI-treated major depression subjects that the observed changes in expression of FGF transcripts are not secondary to drug treatment. Rather, changes in specific FGF transcripts are attenuated by SSRIs and may thus be partially responsible for the mechanism of action of these drugs (Evans et al. 2004). The most significant differences were in levels of mRNA for one of the FGFs, called FGF1, and for the two receptors, FGFR2 and FGFR3 (Evans et al. 2004). The brains of bipolar patients in the study did not show the decreased FGF gene activity, although both groups were severely depressed at the time of death. This is yet another indication that bipolar illness, though classified with depression as a mood disorder, is biologically a very different disease (Evans et al. 2004). Thus if these results are confirmed in the future by other investigators, we can gain access to a marker, differentiating between unipolar and bipolar depression.

Serotonergic markers in depression

Imipramine binding

One of the most robust biological markers in psychiatry is the binding of imipramine to blood platelets. Imipramine binds to the high-affinity serotonin transporter (5-HTT) on platelets, which represent a model system for serotonergic neurons. Imipramine binding to blood platelets is generally decreased in depression, as indicated by a decreased maximal binding capacity ($B_{\text{max}}$). Similarly, 5-HT uptake in blood platelets ($V_{\text{max}}$) is decreased. These findings correspond to a decrease of maximal binding capacity of imipramine to brain tissue. A meta-analysis by Ellis and Salmond (1994) has shown that imipramine binding to platelets is indeed a robust biological marker of depression.

5-HT receptors

5-HT1A receptor. Due to the efficacy of serotonergically acting drugs in major depression, some 5-HT receptors have been extensively studied in major depression. Of the 18 different types and subtypes of 5-HT receptors, the most extensively studied 5-HT receptor is the 5-HT1A receptor. Table I gives an overview of 5-HT1A receptor levels in major depression. As can be seen, almost all the studies point to a decreased or unchanged expression of the 5-HT1A receptor. These studies have employed a number of different methods. Only one study found an increased 5-HT1A receptor expression. Thus, a trend of decreased 5-HT1A receptor expression appears to be a robust finding in major depression. A functional genetic variant of the 5-HT1A receptor, the C-1019G promoter polymorphism, has been investigated in major depression. The −1019G allele was more frequent in major depression (Lemonde et al. 2003). Replication studies are eagerly awaited.

5-HT1B receptor. Among the genetic variants of the 5-HT1B receptor, the G861C polymorphism is the most frequently investigated variant. G861C, or most likely a variant in linkage disequilibrium with G861C, exhibits functional effects. Investigating variants of the 5-HT1B receptor in major depression, most of the studies do not find an association between the 5-HT1B receptor and major depression...
A single postmortem study investigating prefrontal cortical 5-HT1B receptor binding did not find differences between major depression and controls (Huang et al. 1999). A cofactor of the 5-HT1B receptor, S100A10 (also termed p11) has been suggested to play a role in depression (Svenningsson et al. 2006); however, its effect may also relate to anxiety.

5-HT1D receptor. In a single postmortem study, a higher number of 5-HT1D receptors was found in globus pallidus (Lowther et al. 1997a). No 5-HT1D receptor variants have been investigated in major depression. In suicide victims, the frequency of a synonymous 5-HT1D receptor variant was similar to controls (Turecki et al. 2003).

5-HT1E and 1F receptor. Investigations of the 5-HT1E and 1F receptors have generally yielded negative results (Lowther et al. 1997a; Turecki et al. 2003).

5-HT2A receptor. 5-HT2A receptor polymorphisms are generally not associated with major depression (Zhang et al. 1997; Frisch et al. 1999; Tsai et al. 1999; Minov et al. 2001; Oswald et al. 2003; Choi et al. 2004; Khait et al. 2005; Levinson 2005).

5-HT2C receptor. A polymorphism of the 5-HT2C receptor, the Cys23Ser variant, has been investigated in a number of studies. Two out of four studies found an increased frequency of the serine variant, indicating that this variant may be associated with major depression (Frisch et al. 1999; Lerer et al. 2001; Holmes et al. 2003; Köks et al. 2006). 5-HT2C receptor RNA undergoes adenosine-to-inosine RNA modification. This results in amino acid changes in the second intracellular loop of the receptor protein. No consistent RNA editing changes of the 5-HT2C receptor were observed in major depression (Niswender et al. 2001; Gurevich et al. 2002; Iwamoto and Kato 2003).

Other 5-HT receptors. Variants of the 5-HT3 receptor and the 5-HT4 receptor have not been investigated in major depression. However, 5-HT4 receptor binding was assessed in depressed suicide victims (Rosel et al. 2004). A significantly higher number of 5-HT4 receptors was found in frontal cortex and caudate nucleus, compared to controls. No variants of the 5-HT5 receptor have been investigated in major depression, and no association of a common 5-HT5 receptor polymorphism was found in suicide victims (Turecki et al. 2003). A variant of the 5-HT6 receptor was assessed in major depression, with negative results (Hong et al. 1999; Lee et al. 2005). No investigations of the 5-HT7 receptor have been performed in major depression.

Overview of serotonergic polymorphisms in major depression

Table II gives an overview of serotonergic polymorphisms in major depression. In addition to 5-HT receptors, the serotonin transporter (Levinson 2005) and monoamine oxidase A are also shown. Findings concerning the recently discovered 5-HT-synthesizing enzyme of the brain, tryptophan hydroxylase 2 (TPH2) (Walther and Bader 2003; Walther et al. 2003) are currently under intense investigation and controversial. However, functional variants of TPH2 have been shown to be important in the pathogenesis of obsessive-compulsive disorder (Mössner et al. 2006), making it unlikely that TPH2 variants will yield biological markers of depression.

<table>
<thead>
<tr>
<th>Investigated region</th>
<th>Method</th>
<th>Change</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>mRNA</td>
<td>Decreased</td>
<td>Lopez-Figueroa et al. (2004)</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>Binding</td>
<td>Unchanged</td>
<td>Arranz et al. (1994)</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>Binding</td>
<td>Unchanged</td>
<td>Stockmeier et al. (1997)</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>PET</td>
<td>Unchanged</td>
<td>Meltzer et al. (2004)</td>
</tr>
<tr>
<td>Cortex</td>
<td>Binding</td>
<td>Unchanged</td>
<td>Lowther et al. (1997b)</td>
</tr>
<tr>
<td>Cortex</td>
<td>PET</td>
<td>Decreased</td>
<td>Bhagwagar et al. (2004)</td>
</tr>
<tr>
<td>Cortex</td>
<td>PET</td>
<td>Decreased</td>
<td>Sargent et al. (2000)</td>
</tr>
<tr>
<td>Cortex</td>
<td>PET</td>
<td>Decreased</td>
<td>Drevets et al. (2000)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>mRNA</td>
<td>Decreased</td>
<td>Lopez-Figueroa et al. (2004)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Binding</td>
<td>Unchanged</td>
<td>Stockmeier et al. (1997)</td>
</tr>
<tr>
<td>Raphe</td>
<td>Binding</td>
<td>Increased</td>
<td>Stockmeier et al. (1998)</td>
</tr>
<tr>
<td>Raphe</td>
<td>PET</td>
<td>Decreased</td>
<td>Arango et al. (2001)</td>
</tr>
<tr>
<td>Raphe</td>
<td>PET</td>
<td>Decreased</td>
<td>Bhagwagar et al. (2004)</td>
</tr>
<tr>
<td>Raphe</td>
<td>PET</td>
<td>Decreased</td>
<td>Meltzer et al. (2004)</td>
</tr>
</tbody>
</table>
Biochemical markers

Lipids

The search for biochemical markers of depression has been particularly intense. Several studies have searched for lipids as biological markers of depression. Hypocholesterolemia has been associated with depression, suicide and affective disorders. Rapidly accumulating evidence suggests that low or lowered cholesterol may be associated with increases of suicides and accidents. Lipid-induced changes in brain chemistry affect the lipid environment of the brain and modulate neurotransmitter action and function of neuronal proteins. The tertiary and quaternary structures of neuronal proteins as receptors, ion channels and protein kinases depend upon the electrical and fluidity parameters determined by electrolytes and the lipid environment of the brain. Lipid and electrolyte abnormalities have a marked impact on neuronal disturbance and ultimately, mood and behaviour. The biochemistry of depression is often characteristic in the blood chemistry as low levels of cholesterol, iron, potassium, albumin and nitrogen markers and elevation of triglycerides. Cholesterol is the substrate for all steroidal and gonadal hormones. There is virtually no system of the body that does not require attenuation of specific fatty acid substrates and coenzymes to maintain health and repair of bodily tissues. Cholesterol levels were identified as a blood marker for depression and anxiety in a normal population in a primary care setting (Rafter 2001).

People with low cholesterol scored significantly higher on the Hamilton depression scale. A hypertriglyceridemia-driven metabolic cause of depression has also been demonstrated in controlled clinical trials, showing that triglyceride lowering alleviates the symptoms of depression. The connection of hypertriglyceridemia and depression involves insulin resistance, as the ingestion of high glycemic food releases insulin which immobilizes the modulation of essential fatty acid metabolism, negatively impacting the production of prostaglandins, cytokines, hormones, membrane traffic and brain chemistry (Glueck et al. 1993). Examination of red cell membrane fatty acid profiling is reflective of long-term insufficiencies and imbalances in fatty acid metabolism. Plasma fatty acids reflect dietary intake of a few days’ duration rather than metabolic conversion observed in fatty acids incorporated into red cell membranes. Examination of patients with affective disorders, depression, schizophrenia, anxiety, autism, seizure disorders, aggression, attention deficit disorder as well as physically oriented acute and chronic illness reveal characteristic patterns that may be addressed with lipid manipulation with targeted fatty acids through competitive inhibition. Brain function depends on the organic metal constituents of the central nervous system as well as lipids but the convergence of these systems occurs in the case of depression for cholesterol. Thus, for example, manganese is required to stimulate the synthesis of cholesterol. A large part of the brain, especially the membranes of the nerves, is made up of essential fatty acids. Roles for fish oil in various brain-related disorders including Alzheimer’s disease, ADHD, autism, schizophrenia, hostility, anxiety, borderline, and bipolar disorders are observed. It has been proposed that the Omega 6 to Omega 3 ratio should be 1:1 (Simopoulos et al. 1999). Recent evidence has suggested an important role for lipids in the aetiology and treatment of depression (Ross et al. 2004): methylnicotinate-induced vasodilation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Functional</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT1A</td>
<td>C-1019G</td>
<td>Yes</td>
<td>Yes (replication required) binding mostly decreased in depression</td>
</tr>
<tr>
<td>5HT1B</td>
<td>Several</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5HT1D</td>
<td>Several</td>
<td>Not known</td>
<td>Binding increased in depression No association in suicide</td>
</tr>
<tr>
<td>5HT1E</td>
<td>C117T</td>
<td>Not known</td>
<td>No association in suicide</td>
</tr>
<tr>
<td>5HT1F</td>
<td>C-78T</td>
<td>Not known</td>
<td>No association in suicide</td>
</tr>
<tr>
<td>5HT2A</td>
<td>T102C</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5HT2B</td>
<td>Not known</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>5HT2C</td>
<td>Cys23Ser</td>
<td>Yes</td>
<td>Probably yes</td>
</tr>
<tr>
<td>5HT3</td>
<td>Pro16Ser</td>
<td>Yes</td>
<td>Not assessed</td>
</tr>
<tr>
<td>5HT4</td>
<td>G-19C</td>
<td>Not known</td>
<td>No association in suicide</td>
</tr>
<tr>
<td>5HT5</td>
<td>C267T</td>
<td>Not known</td>
<td>No</td>
</tr>
<tr>
<td>5HT6</td>
<td>Not known</td>
<td>Not known</td>
<td></td>
</tr>
<tr>
<td>5HT7</td>
<td>Not known</td>
<td>Not assessed</td>
<td></td>
</tr>
<tr>
<td>MAOA</td>
<td>MAOA-LPR</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5HTT</td>
<td>5HTTLPR</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table II. Serotonergic polymorphisms in major depression.
was used to investigate lipid-dependent signalling mechanisms involving the phospholipase A2–cyclooxygenase pathway, an important signalling system, involved in the action of several neurotransmitters including serotonin. Methylnicotinato-induced erythema was reduced in subjects with unipolar depression compared to controls at 5 min after application and it returned to normal after 15 min. Thus, although the maximal response to methylnicotinate appears normal, patients with unipolar depression exhibit an apparently delayed response. In that study, all unipolar patients were medicated at the time of testing. The results support the hypothesis that unipolar depression may be associated with abnormalities in lipid-associated signalling systems, and may provide insight into how lipid intake may modulate depressive symptoms.

Nitrogen

Macronutrients such as consumption of high quality proteins and the supplementation of creatine, ammonium chloride, urea, glutamine, and niacin, as indicated in the patient's blood chemistry will attenuate nitrogen retention. Nitrogen markers are blood urea nitrogen (BUN), creatinine, and uric acid. It is interesting to remember that the inhalation of nitrous oxide (laughing gas) may evoke the emotion of pleasure with the increase in nitrogen, while exposure to high altitudes, atmospheric low pressure or a full moon, where a downward nitrogen shift is created, may evoke feelings of depression or exacerbate mood disorders. Nitrogen retention is dependent upon dietary consumption of nitrogen rich foods along with lipid consumption, electrolyte stability, and mineral density.

Analysis of polymorphisms of nitric oxide synthases have not yielded any conclusive evidence for their involvement in the pathogenesis of major depression (Yu et al. 2003; Reif et al. 2006). In a small sample of 15 subjects with major depression, decreased plasma nitric oxide metabolite levels and platelet endothelial nitric oxide synthase activity were observed (Chrapko et al. 2004). Conversely, however, increased nitric oxide metabolite levels in plasma were suggested to be associated with suicide attempt, especially in major depressive patients (Lee et al. 2006).

Electrolytes and trace metals

Acute and chronically ill patients frequently become severely depressed and lose their will to live when serum potassium levels drop to below the low end of the laboratory reference range. Subnormal levels of zinc are associated with treatment resistant depression (Maes et al. 1997b). Deficiencies of magnesium can provoke a wide range of psychiatric symptoms related to depression, ranging from apathy to psychosis (Rasmussen et al. 1989). Research on manic patients, on the other hand, has revealed elevated vanadium in the hair, significantly higher levels than those measured in both a control group and a group of recovered manic patients (Naylor et al. 1984). Numerous studies have found long-term chronic low doses of mercury cause neurological, memory, behaviour, sleep, and mood problems (Smith 1978; Kishi et al. 1994; Sibulrud et al. 1994; Bittner et al. 1998; Echeverria et al. 1998). Mercury has been found to strongly inhibit the activity of dipeptidyl peptidase (DPP IV), which is required in the digestion of the milk protein casein. Studies involving a large sample of psychiatric patients found that a large part of those tested had high levels of the milk protein β-casomorphin-7 in their blood and urine and defective enzymatic processes for digesting milk protein. Elimination of milk products from the diet has been found to improve the condition. As mercury levels are reduced the protein binding is reduced and improvement in the enzymatic process occurs. Additional enzymatic effects at the cellular level via the binding of mercury to proteins include the blockade of sulphur oxidation processes and neurotransmitters (Stefanovic et al. 1998), enzymatic processes involving vitamins B6 and B12 (Srikantaiah and Radhakrishnan 1970), effects on the cytochrome-C energy processes, along with mercury's adverse effects on cellular mineral levels of calcium, magnesium, zinc, chromium, and lithium (Chetty et al. 1990; Rajanna et al. 1995; Freitas et al. 1996). Mercury causes decreased lithium levels, and lithium is an important treatment option for depression. Lithium protects brain cells against excess glutamate and calcium (Rossi et al. 1993; Nonaka et al. 1998). Mercury inhibits macrophage and neutrophil defense against candida by affecting Th1 and Th2 cytokine effects (Perlingeiro and Queiroz 1994; Mathieson 1995; Hua et al. 1996). Candida overgrowth results in production of the highly toxic candidotoxin and ethanol which are known to cause fatigue, toxicity, and depressive symptoms (Crook 1984; Edwards 1985). There is also evidence that mercury affects neurotransmitter levels which have effects on conditions such as depression. There is evidence that mercury can block the dopamine–β-hydroxylase (DBH) enzyme (Manzo et al. 1996). This enzyme synthesizes noradrenaline, and low noradrenaline can cause fatigue and depression. Mercury molecules can block all copper-catalysed dithiolane oxidases, such as coproporphyrin oxidase and DBH. Mercury and other toxic metals have been
Folate deficiency is known to lower levels of 5-HT in the CNS. At the same time, such a deficiency of 5-methyltetrahydrofolate causes reduced turnover of serotonin, and perhaps dopamine, in the central nervous system. It has been concluded that the mechanism by which deficiency of 5-methyltetrahydrofolate causes reduced 5-hydroxytryptamine and dopamine turnover is unlikely to be mediated by $S$-adenosylmethionine (Surtees et al. 1994). A consistent finding in major depression has been a low plasma and red cell folate, which has also been linked to poor response to antidepressants (Coppen and Bailey 2000). There was a significantly greater improvement in the fluoxetine plus folic acid group. Folic acid is a simple method of improving the antidepressant action of fluoxetine and probably other antidepressants. Folic acid should be given in doses sufficient to accumulate in the pineal gland and reduce melatonin levels, which is thought to be a significant factor in mercury’s toxic effects (Baccarelli et al. 2000).

**Vitamins: folic acid**

Several cross-sectional studies have focused on the low blood folate levels of depressed patients. Folate is a cofactor in 1-carbon metabolism, during which it promotes the remethylation of homocysteine (a cytotoxic sulfur-containing amino acid, that can induce DNA strand breakage, oxidative stress and apoptosis). Dietary folate is required for normal development of the nervous system and plays important roles in regulating neurogenesis and programmed cell death. Recent epidemiological and experimental studies have linked folate deficiency and resultant increased homocysteine levels with several neurodegenerative conditions, including Alzheimer’s disease, Parkinson’s disease, and stroke. Genetic and clinical data suggest roles for folate and homocysteine in the pathogenesis of psychiatric disorders (Mattson and Shea 2003). In a large Finnish study, depressed patients in the general population with energy-adjusted folate intake below the median had a higher risk of getting a discharge diagnosis of depression during the follow-up period than those who had a folate intake above the median. A low dietary intake of folate may be a risk factor for severe depression (Tolmunen et al. 2004). There has been some discussion in the recent literature regarding the possible relationship between peripheral levels of folate and serotonin deficiency in the CNS. At the same time, such a serotonin deficiency has been implicated in the biology of suicidal behaviour. Thus, decreased peripheral folate levels may be expected in patients who commit violent suicide. In this respect, the red-cell and serum folate levels in nine persons who later committed suicide were compared with those in age- and sex-matched control groups. However, no significant difference between the groups was found (Wolfersdorf et al. 1995). Although one of the first biological treatments of a major psychiatric disorder was the dietary treatment of pellagra, the use of diet and dietary components in the study of psychopathology has not aroused much interest in recent years (Young 1993). Folic acid deficiency causes a lowering of brain serotonin in rats, and of cerebrospinal fluid 5-hydroxyindoleacetic acid in humans. There is a high incidence of folate deficiency in depression, and there are indications in the literature that some depressed patients who are folate deficient respond to folate administration. Folate deficiency is known to lower levels of $S$-adenosylmethionine, which is an antidepressant that raises brain serotonin levels. These data suggest that low levels of serotonin in some depressed patients may be a secondary consequence of low levels of $S$-adenosylmethionine. They also suggest that the dietary intake and psychopharmacological action of methionine, the precursor of $S$-adenosylmethionine, should be studied in patients with depression. The limited available evidence suggests folate may have a potential role as a supplement to other treatments for depression. It is currently unclear if this is the case both for people with normal folate levels, and for those with folate deficiency (Taylor et al. 2004). In the study of Botez et al. (1982), folate-deficient patients were classified according to whether they exhibited a neuropsychiatric syndrome, consisting of organic mental changes, polyneuropathy, and depression, which responded to folate administration. CSF 5-hydroxyindoleacetic acid (5-HIAA) was low in the vitamin B12-deficient patients and in those folate-deficient patients whose symptoms were not related to folate deficiency. CSF 5-HIAA returned to normal with folate treatment in the patients exhibiting folate-responsive neuropsychiatric signs. The data indicated a close association between folate-responsive neuropsychiatric symptoms and changes in serotonin metabolism in the central nervous system (Botez et al. 1982). Similarly, in another study, a subgroup of severely depressed patients with red-cell folate deficiency had significantly lower CSF 5-HIAA compared to neurological controls. For all depressed patients red-cell folate was significantly correlated with CSF 5-HIAA and homovanillic acid (HVA). CSF tetrahydrobiopterin was significantly correlated with CSF 5-HIAA and HVA and red-cell folate. These observations provided further evidence of the links between folate, biopterin and monoamine metabolism in depression (Bottiglieri et al. 1992). Folate deficiency, or inborn errors of folate metabolism, cause reduced turnover of serotonin, and perhaps dopamine, in the central nervous system. It has been concluded that the mechanism by which deficiency of 5-methyltetrahydrofolate causes reduced 5-hydroxytryptamine and dopamine turnover is unlikely to be mediated by $S$-adenosylmethionine (Surtees et al. 1994). A consistent finding in major depression has been a low plasma and red cell folate, which has also been linked to poor response to antidepressants (Coppen and Bailey 2000). There was a significantly greater improvement in the fluoxetine plus folic acid group. Folic acid is a simple method of improving the antidepressant action of fluoxetine and probably other antidepressants. Folic acid should be given in doses sufficient...
Folate deficiency and low folate status have been linked to depression, persistent depressive symptoms, and poor antidepressant response. In another study, 31% of all examined psychiatric patients had red cell folate below 200 ng/ml and 12% had concentrations below 150 ng/ml. The mean red cell folate in the depressed patients was significantly lower than in the euthymic, manic and schizophrenic groups. Alcoholics had a similar mean red cell folate to depressed patients, which was not quite significantly lower than the other groups. The mean serum B12 level in the alcoholics was, however, significantly raised. There were no significant differences in red cell folate or serum B12 between lithium-treated and untreated euthymic patients. The highest proportions of values below 200 and 150 ng/ml were found in depressed and alcoholic patients. Endogenous depressives had the highest percentage of values below 150 ng/ml (folate-deficient) of all psychiatric groups and alcoholic patients (Carney et al. 1990). In another study, patients with depressive disorder had lower serum folate levels than healthy controls, but showed no differences in red cell folate levels. Patients with schizophrenia also had lower serum folate levels than age- and sex-matched controls, while red cell folate levels did not differ (Herran et al. 1999). Another investigation also showed that patients with major depressive disorder had significantly lower serum and red cell folate concentrations than normal controls. Lower serum folate concentrations were associated with greater severity of depression. There was no association between serum and red cell folate concentrations and endogenicity of depression or the presence of weight loss (Abou-Saleh and Coppen 1989). Another study in the same year found that the duration of the current depressive episode was significantly inversely correlated with folate levels, age at onset of illness was significantly correlated with B12 and in a subgroup of recurrent depressives, current age and age at onset of depressive illness were positively correlated with folate (Levitt and Joffe 1989). Depressed geriatric patients have lower levels of folate than controls and folate supplement can reduce depressive morbidity (Alpert et al. 2003). Higher folate levels at baseline predicted greater improvement with antidepressant treatment, especially for SSRI response, based on a self-rating depression scale. A relation between low folate status and depression has been recognized since the 1960s. Since 1998, flour in the United States has been fortified with folic acid, and the prevalence of folate deficiency has decreased dramatically (Ramos et al. 2004). The data indicate that, despite folic acid fortification, low folate status is associated with depressive symptoms in elderly Latino women (but
not elderly Latino men). Recently, a review of the effectiveness, adverse effects and acceptability of folate in the treatment of depression was performed. Electronic databases (Cochrane Controlled Trials Register and the Cochrane Collaboration Depression, Anxiety and Neurosis Controlled Trials Register) and reference lists were searched, and authors, experts and pharmaceutical companies contacted to identify randomized controlled trials that compared treatment with folic acid or 5-methyltetrahydrofolic acid to an alternative treatment, for patients with a diagnosis of depressive disorder (Taylor et al. 2004). The identified trials did not find evidence of any problems with the acceptability or safety of folate. The limited evidence available suggests folate may have a potential role as a supplement to other treatments for depression. The relationship between serum folate, vitamin B12, and homocysteine levels and the rate of relapse in outpatients with remitted major depressive disorder during a 28-week continuation phase of treatment with fluoxetine was assessed (Papakostas et al. 2004a). The presence of low serum folate levels, but not low B12 or elevated homocysteine levels, was associated with relapse during continuation treatment with fluoxetine. The relapse rates for patients with and without low folate levels were 42.9 versus 3.2%, respectively. Low serum folate levels were found to place patients with remitted major depression at risk for relapse during the continuation phase of treatment with fluoxetine. Those patients who previously failed to respond to open treatment with fluoxetine 20 mg/day were enrolled in a 4-week, double-blind trial of either (1) fluoxetine dose increase, (2) lithium augmentation of fluoxetine, or (3) desipramine augmentation of fluoxetine (Papakostas et al. 2004b). Low serum folate levels, but not elevated homocysteine or low vitamin B12 levels, were associated with poorer response to treatment. The response rates for patients with and without low folate levels were 7.1 versus 44.7%, respectively. Low serum folate levels were found to be associated with further treatment resistance among patients with fluoxetine-resistant major depression.

Vitamins B6 and B12

A group of Danish investigators have suggested that a low level of vitamin B6 is associated with symptoms of depression. A low level of vitamin B6 might theoretically cause depression as vitamin B6 is a cofactor in the tryptophan-serotonin pathway. A low plasma level of the vitamin B6 derivative, pyridoxal phosphate (PLP) was significantly associated with the depression score. That study suggested that a low level of plasma PLP is associated with symptoms of depression (Hvas et al. 2004a). However, a recent systematic review did not find a meaningful treatment effect of vitamin B6 for depression in general (Williams et al. 2005).

In a study of the 6-month treatment outcome in patients with major depressive disorder, higher vitamin B12 levels were significantly associated with a better outcome. The association between the folate level and treatment outcome was weak and probably not independent. The vitamin B12 level and the probability of recovery from major depression may thus be positively associated (Hintikka et al. 2003).

An association between depression and folate status has been demonstrated in clinical studies, whereas data are sparse on the relationship between depression and other components of 1-carbon metabolism such as vitamin B12, homocysteine, and the methylenetetrahydrofolate reductase 677C→T polymorphism. Overall, hyperhomocysteinemia and TT methylenetetrahydrofolate reductase genotype, but not low plasma folate or vitamin B12 levels, are significantly related to depression without comorbid anxiety disorder. Plasma folate level were inversely associated with depression only in the subgroup of middle-aged women (Bjelland et al. 2003). In a large group of elderly people (3884 probands), hyperhomocysteinemia, vitamin B12 deficiency, and, to a lesser extent, folate deficiency were all related to depressive disorders (Tiemeier et al. 2002). For folate deficiency and hyperhomocysteinemia, the association with depressive disorders was substantially reduced after adjustment for functional disability and cardiovascular disease, but for vitamin B12 this appeared independent. The association of vitamin B12 and folate with depressive disorders may have different underlying mechanisms. Vitamin B12 may be causally related to depression, whereas the relation with folate could be due to physical comorbidity (Tiemeier et al. 2002). Vitamin B12 and folic acid play a major role, since deficiency of both vitamins is associated with the pathogenesis of different diseases such as declining neurocognitive function and atherosclerotic lesions (Wolters et al. 2004). Vitamin B12 and folic acid act as coenzymes and show a close molecular interaction on the basis of the homocysteine metabolism. In addition to the serum concentrations of the vitamins, the metabolites homocysteine and methylmalonic acid are sensitive markers of cobalamin and folate status. A high prevalence of poor cobalamin status is caused by the increasing prevalence of atrophic gastritis type B in the elderly, occurring with a frequency of approximately 20–50% in elderly subjects. Atrophic gastritis results in declining gastric acid and pepsinogen secretion, and reduced absorption of vitamin
B12. Folic acid intake among elderly subjects is generally well below the recommended dietary reference values. Even moderately increased homocysteine levels or poor folate and vitamin B12 status are associated with vascular disease and neurocognitive disorders. Results of a meta-analysis of prospective studies revealed that lowering homocysteine level by 25% was associated with lowering ischemic heart disease risk by 11% and lowering stroke risk by 19%. Homocysteine initiates different proatherogenic mechanisms such as the formation of reactive oxygen species and an enhanced fibrin synthesis. Supplementation of folic acid reduces the homocysteine concentration by 25%. Additional vitamin B12 induces further reduction by 7%. In secondary prevention, supplementation has already led to clinical improvements. Depression, dementia, and mental impairment are often associated with folate and vitamin B12 deficiency. The biochemical reason of this finding may be the importance of folic acid and vitamin B12 for the transmethylation of neuroactive substances (myelin, neurotransmitters), which is impaired in vitamin deficiency (“hypo-methylation hypothesis”) (Wolters et al. 2004). In contrast to other studies that have found folate deficiency in 10–50% of psychiatric patients, one study observed that less than 2% of the patients had serum folate levels below 3 ng/ml, while low vitamin B12 levels (below 200 pg/ml) were seen in about 12% of the patients (Woltersdorf and Konig 1995).

In one study, 28% of all depressed subjects were deficient in B2 (riboflavin), B6 (pyridoxine), and/or B12 (cobalamin), but none in B1 (thiamine) or folate. The geriatric sample had significantly higher serum folate levels. Psychotic depressive patients had lower B12 than did non-psychotic depressives. The data support the hypothesis that poorer status in certain B vitamins is present in major depression, but blood measures may not reflect central nervous system vitamin function or severity of affective syndromes (Bell et al. 1991). In another study of geriatric patients by the same research group, only 3.7% of patients were B12 deficient and 1.3% were folate deficient; 4% were anemic (Bell et al. 1990b). Nevertheless, those with below-median values of both vitamins had significantly lower Mini-Mental State scores than patients higher in one or both vitamins. These data suggest that biochemically interrelated vitamins such as B12 and folate may exert both a separate and a concomitant influence on affect and cognition and that poorer vitamin status may contribute to certain geriatric psychiatric disorders that lack a familial predisposition. Similarly, in a study examining whether community-dwelling older women with metabolically significant vitamin B12 or folate deficiency are particularly prone to depression, serum homocysteine levels, folate levels, and the prevalences of folate deficiency and anemia were not associated with depression status (Penninx et al. 2000). However, the depressed subjects, especially those with severe depression, had a significantly higher serum methylmalonic acid level and a nonsignificantly lower serum vitamin B12 level than the nondepressed subjects. After adjustment for sociodemographic characteristics and health status, the subjects with vitamin B12 deficiency were twice as likely to be severely depressed as were the non-deficient subjects. In another study, a psychotic depression subgroup demonstrated numerous significant positive correlations between vitamin B12 level and cognitive subtests not seen in other diagnostic subgroups, especially those of IQ, and verbal and visual memory (Bell et al. 1990a). The last study of this research group is an augmentational open one with tricyclic antidepressants combined with 10 mg each of vitamins B1, B2, and B6 in geriatric inpatients with depression (Bell et al. 1992). The active vitamin group demonstrated a trend toward greater improvement in scores on ratings of depression and cognitive function, as well as in serum nortriptyline levels compared with placebo-treated subjects. Without specific supplementation, B12 levels increased in the subjects receiving B1/B2/B6 and decreased in those on placebo. These findings implicate a possible role of B complex vitamin augmentation in the treatment of geriatric depression.

Detection of cobalamin deficiency is clinically important for a better understanding of neuropsychiatric diseases, and why the deficiency occurs more frequently than previously anticipated. However, serum cobalamin measurements have a limited ability to diagnose a deficiency state (Gultepe et al. 2003). However, a good correlation exists between urinary methylmalonic acid (MMA) and serum vitamin B12 determinations. When cobalamin deficiency is suspected in neuropsychiatric patients, urinary MMA determination analysis can be the first diagnostic test used. If the MMA concentration is above the reference value, serum cobalamin levels can be determined for further diagnosis.

Vitamin B12 deficiency is a common problem in elderly subjects (Wolters et al. 2004). An already moderately reduced vitamin B12 level is associated with vascular disease and neurocognitive disorders such as depression and impaired cognitive performance. Furthermore, a poor vitamin B12 status is presumed to be involved in the development and progression of dementia. This is especially observable if the folic acid status is reduced as well. Due to the insecure supply, the cobalamin status of elderly
persons should be regularly controlled and a general supplementation with vitamin B12 should be considered (Wolters et al. 2004). Associations between vitamin B12 deficiency and impaired cognitive function and depression have been reported. In a randomized placebo controlled study of depressed patients with vitamin B12, no improvement was found in cognitive function comparing the treatment and placebo group, nor among individuals with only slightly impaired cognitive function (Hvas et al. 2004b). Finally, an open trial of treatment resistant major depression found a response rate of 50% and a remission rate of 43% following augmentation of antidepressive therapy with S-adenosyl-1-methionine (Alpert et al. 2004).

G-proteins

Abnormal signal transduction pathways have been implicated in the pathogenesis of major depression and bipolar disorder (Zill et al. 2000). G-proteins are key elements of these pathways in the regulation of cellular responses by transmission of signals from receptors to effector proteins. In recent years several studies have reported altered levels and activities of G-protein subunits in depressive patients. A polymorphism of a G-protein β3 subunit (C825T) has been shown to be associated with increased signal transduction and ion transport activity. A significantly higher frequency of the T allele was found in depressive patients than in healthy controls. Moreover, a significant association between TT homozygosity and response to antidepressant treatment after 4 weeks was observed. Thus, the G-protein β3 subunit appears to be a susceptibility factor for major depression (Zill et al., 2000). In a Korean sample, significantly more carriers of the 825T allele were found in major depressive disorder patients than in normal controls (Lee et al. 2004). The T-allele carriers showed higher scores than those with the CC genotype in the baseline total and in some subcategories of the Hamilton Depression Rating Scale. A statistically significant association between T-allele carriers and antidepressant treatment response was found. These results suggest that the T allele of the C825T polymorphism is associated with major depressive disorder. It was also demonstrated that major depressive disorder patients bearing the T allele had a more severe symptomatology and a better response to antidepressant treatment than patients without the T allele. Subjects with TT variants showed better response to treatment and this effect was independent from analysed demographic and clinical variables (Serretti et al. 2003). 825T allele carriers displayed higher levels of depression, as measured by questionnaire (Exton et al. 2003). Regression analysis demonstrated that allele type was the single predictor of depression. The G protein β3 subunit 825T allele is predictive of depressive mood in a young, healthy population.

For depressed patients under the age of 25 years, the T allele of the G protein β3 subunit was associated with a markedly poorer response to nortriptyline, while serotonin transporter polymorphisms did not predict antidepressant response (Joyce et al. 2003). However, in patients 25 years or older, the G protein β3 polymorphisms did not predict antidepressant response, while the short/short genotype of the serotonin transporter promoter polymorphism was associated with a poorer response to both fluoxetine and nortriptyline. These differential pharmacogenetic predictors of antidepressant response by age may provide clues to understanding the discontinuities in pharmacological responsiveness of child/adolescent and adult depressive disorders.

Although it is well established that depression is a major risk factor for the development of coronary artery disease and that cerebrovascular disease can be a major contributing factor for the development of depression, the information about the interplay between the central nervous system and cardiovascular disease is still limited. In an investigation of the G-protein β3-subunit C825T polymorphism and the angiotensin I converting enzyme (ACE) ID polymorphism, analysis of both genes showed that the combined actions of ACE and C825T genotypes accumulate in carriers of the ACE D allele (ID and DD) and C825T TT homozygotes with ID/DD TT carriers showing a more than five-fold increase in risk for major depression (Bondy et al. 2002). As the study was carried out with depressive patients without serious cardiac impairment at the time of the investigation, it was impossible to predict whether this combined action of the ID/DD TT genotype is increasing the risk for both disorders. Nevertheless that study reports for the first time that the same allelic combination of two genes that have been shown to increase the risk for myocardial infarction (Naber et al. 2000) increase the vulnerability for depressive disorder. The C825T polymorphism was associated with seasonal affective disorder in another study sample (Willeit et al. 2003). This finding strengthens the evidence for the involvement of G protein-coupled signal transduction in the pathogenesis of affective disorders.

Hypothalamic-pituitary-adrenal axis

Cortisol hypersecretion, non-suppression or the dexamethasone suppression test (DST) and a
blunted thyrotropin response to protirelin are factors which have been studied extensively (Roy et al. 1987; Joyce and Paykel 1989). None of these tests, however, is positive or diagnostic in the majority of all depressed patients.

The dexamethasone/CRH test is one of the most reliable neuroendocrine function tests for hypothalamic–pituitary–adrenocortical (HPA) system dysregulation in depression. Persistent overdrive of HPA system activity after successful antidepressant treatment predicts an enhanced risk for relapse of a depressive episode. As the renin–angiotensin system influences the HPA axis, a study investigated the angiotensin-converting enzyme gene insertion (I)/deletion (D) polymorphism. This polymorphism determines ACE plasma concentrations. The DD genotype showed a higher cortisol stimulation during the first dexamethasone/CRH test after admission than the II genotype. After successful antidepressive treatment and attenuation of HPA system overdrive these differences were no more detectable. The HPA axis stimulating properties of higher ACE and consecutively higher angiotensin and lower substance P concentrations may be crucial factors for the HPA system hyperactivity during major depressive episodes (Baghai et al. 2002, 2006).

Regarding an animal model of dysregulation of the HPA axis and the stress-response system, corticotropin-releasing hormone receptor-2 (CRHR2)-deficient mice display a stress-sensitive and anxiety-like phenotype suggesting that the CRHR2 is a plausible functional candidate gene influencing the reactivity of the HPA axis and therefore the liability to develop affective disorders. However, a SNP analysis of the CRHR2 gene did not reveal consistent changes in major depression patients (Villafuerte et al. 2002). The availability of free corticotropin releasing hormone in the central nervous system is tightly regulated by the expression of corticotropin-releasing hormone binding protein. Therefore, the gene encoding corticotropin releasing hormone binding protein is a functional candidate gene for major depression. Two single nucleotide polymorphisms within the corticotropin-releasing hormone binding protein gene were significantly associated with the disease (Claes et al. 2003). The corticotropin-releasing hormone binding protein gene is likely to be involved in the genetic vulnerability for major depression. Moreover, variants in a glucocorticoid receptor-regulating protein, FKBP5, have been found associated with increased recurrence of depressive episodes (Binder et al. 2004).

**Neuroimaging markers**

Positron emission tomography (PET) imaging studies have revealed multiple abnormalities of regional cerebral blood flow (CBF) and glucose metabolism in limbic structures and the prefrontal cortex (PFC) in mood disorders. Although disagreement exists regarding the specific locations and the direction of some of these abnormalities, in unmedicated subjects with major depression, regional CBF and metabolism are consistently increased in the amygdala, orbital cortex, and medial thalamus, and decreased in the dorsomedial/dorsal anterolateral PFC and anterior cingulate cortex ventral to the genu of the corpus callosum (subgenual PFC) relative to healthy controls (Drevets 2001; Manji et al. 2001). These abnormalities implicate limbic–thalamic–cortical and limbic–cortical–striatal–palilidal–thalamic circuits, involving the amygdala, orbital and medial PFC, and anatomically related parts of the striatum and thalamus in the pathophysiology of major depression. These circuits have also been implicated more generally in emotional behaviour by the results of electrophysiological, lesion analysis, and brain mapping studies of humans and experimental animals. Structural imaging studies have demonstrated reduced gray-matter volumes in areas of the orbital and medial PFC, ventral striatum, and hippocampus, and enlargement of the third ventricle in individuals with mood-disorders relative to healthy control samples (Drevets 2001). Complementary postmortem neuropathological studies have shown abnormal reductions in cortex volume, and either glial cell counts, neuron size, or both, in the subgenual PFC, orbital cortex, dorsal anterolateral PFC, and amygdala (Ongur et al. 1998; Rajkowska 2000; Manji et al. 2003). It is not known whether these deficits constitute developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, or the sequelae of recurrent affective episodes per se. Nevertheless, the marked reduction in glial cells in these regions has been particularly intriguing, in view of the growing appreciation that glia play critical roles in regulating synaptic glutamate concentrations and central nervous system energy homeostasis and in releasing trophic factors that participate in the development and maintenance of synaptic networks formed by neuronal and glial processes (Coyle and Schwarz 2000; Ullian et al. 2001). Abnormalities of glial function could thus prove integral to the impairment of structural plasticity and overall pathophysiology of major depression. Taken together with other clinical and preclinical data regarding these structures’ specific roles in emotional processing, the neuroimaging and
neuropathological abnormalities in major depression suggest that major depression is associated with activation of regions that putatively mediate emotional and stress responses (for example, amygdala), whereas areas that appear to inhibit emotional expression (such as posterior orbital cortex) contain histological abnormalities that may interfere with the modulation of emotional or stress responses (Drevets 2001). For example, in major depression, the elevation of CBF and metabolism in the amygdala is positively correlated with depression severity, consistent with this structure's role in organizing the autonomic, neuroendocrine, and behavioural manifestations of some types of emotional responses. In contrast, some of the medial and orbital PFC areas, where metabolism is abnormal in major depression, appear to play roles in reducing autonomic and endocrine responses to stressors or threats and in extinguishing behavioural responses to fear-conditioned stimuli that are no longer reinforced (Drevets 2001). Activation of the orbital cortex during depressive episodes may thus reflect endogenous attempts to interrupt unreinforced, aversive thoughts and emotions. However, the histopathological abnormalities identified in these areas in major depression post-mortem suggest that the ability to mediate these functions may be impaired. The hypothesis that dysfunction of these regions may contribute to the development of depression is consistent with evidence that lesions (such as strokes or tumours) involving either the PFC or the striatum (a major target of efferent projections from the PFC), as well as degenerative diseases affecting the striatum (for instance, Parkinson’s and Huntington’s diseases), are associated with an increased risk for developing depression.

PET imaging of 5-HT1A receptor binding in the dorsal raphe nucleus and prefrontal cortex may be assessed with the radioligand $[^{11}C]$WAY 100635. Significantly diminished binding in the dorsal raphe nucleus in elderly depressed patients was observed relative to control subjects (Meltzer et al. 2004). These findings support evidence of altered 5-HT1A autoreceptor function in depression. In a PET study in healthy volunteers, a preferential occupancy by pindolol of 5-HT1A autoreceptors as compared to postsynaptic receptors was demonstrated (Rabiner et al. 2004). This preferential occupancy was attenuated in depressed patients. A possible mechanism underlying this preferential occupancy and the attenuation of this phenomenon in depressed patients on SSRIs may include changes in the proportion of high affinity autoreceptor 5-HT1A sites in the midbrain raphe.

Another study investigated changes of the 5-HT2A receptor in older depressed patients, employing the PET ligand $[^{18}F]$altanserin. Depressed subjects had significantly less hippocampal 5-HT2A receptor binding than controls. Moreover, depressed patients not previously treated for depression had significantly less hippocampal 5-HT2A receptor binding than previously treated subjects. It may be that prior medication leads to a partial compensatory upregulation of the 5-HT2A receptor. In a PET study employing a selective serotonin transporter (5-HTT) ligand ($[^{11}C]$McN5652), 5-HTT sites were increased in the frontal and cingulate cortices of depressed patients (Reivich et al. 2004). These alterations in 5-HTT sites may be of pathophysiological significance in the pathophysiology of depression.

PET studies have reported altered resting regional brain glucose metabolism in mood disorders. Unlike healthy male subjects who have significant increases in regional glucose metabolism in prefrontal and parietal cortical regions after receiving the serotonin-releasing agent fenfluramine, depressed male subjects have no significant increases in regional glucose metabolism. This blunting is consistent with the serotonin hypothesis of depression (Anderson et al. 2004). Two other articles provide intriguing data on the functional neuroanatomy of depression (Brody et al. 2001; Martin et al. 2001). Using single-photon emission computed tomography (SPECT) and $[^{18}F]$fluorodeoxyglucose PET neuroimaging methods, outpatients with major depressive disorder were studied before and after treatment. What distinguishes these reports from earlier neuroimaging investigations using pretreatment and posttreatment designs is that both studies compared the effects of pharmacotherapy with those of interpersonal psychotherapy, one of the better studied psychosocial treatments of depression. Results indicate that both treatments were associated with increased blood flow to the left temporal or right basal ganglia regions.

There is growing evidence from neuroimaging and postmortem studies that severe mood disorders, which have traditionally been conceptualized as neurochemical disorders, are associated with impairments of structural plasticity. Both first- and multiple-episode depressed groups have hippocampal dysfunction apparent on several tests of recollection memory. However, only depressed subjects with multiple depressive episodes have hippocampal volume reductions, with an association between illness duration and hippocampal volume. Reductions in hippocampal volume may not anedate illness onset, but volume may decrease at the greatest rate in the early years after illness onset (MacQueen et al. 2003). Remitted patients with a history of depression have smaller hippocampal
volumes bilaterally than controls. These “post-depressives” also have smaller amygdala core nuclei volumes, and these volumes correlate with hippocampal volumes. In addition, post-depressives score lower in verbal memory, a neuropsychological measure of hippocampal function, suggesting that the volume loss is related to an aspect of cognitive functioning. In the absence of a significant correlation between hippocampal volume and age in either post-depressive or control subjects, a significant correlation with total lifetime duration of depression was found. This suggests that repeated stress during recurrent depressive episodes may result in cumulative hippocampal injury as reflected in volume loss (Sheline et al. 1999). Longer durations during which depressive episodes went untreated with antidepressant medication were associated with reductions in hippocampal volume. There was no significant relationship between hippocampal volume loss and time depressed while taking antidepressant medication or with lifetime exposure to antidepressants. Thus, antidepressants may have a neuroprotective effect during depression (Sheline et al. 2003).

Depression is associated with interpersonal difficulties related to abnormalities in affective facial processing. Average activation (capacity) and differential response to variable affective intensity (dynamical range) were estimated in fMRI time series (Fu et al. 2004). Over time, depressed subjects showed reduced capacity for activation in the left amygdala, ventral striatum, and frontoparietal cortex and a negatively correlated increase of dynamic range in the prefrontal cortex. Symptomatic improvement was associated with reduction of dynamic range in the pregenual cingulate cortex, ventral striatum, and cerebellum. Thus, antidepressant treatment reduces left limbic, subcortical, and neocortical capacity for activation in depressed subjects and increases the dynamic range of the left prefrontal cortex. Changes in anterior cingulate function associated with symptomatic improvement indicate that fMRI may be a useful surrogate marker of antidepressant treatment response. Biological markers of treatment response may include structural brain changes seen on neuroimaging. Depressed patients with small rightsided and total hippocampal volumes were less likely to achieve remission (Hsieh et al. 2002). Thus, left-right hippocampal volume differences appear to exist in depression. Neuroimaging technology has provided unprecedented opportunities for elucidating the anatomical correlates of major depression (Drevets 2001). The knowledge gained from imaging research is catalysing a paradigm shift in which primary mood disorders are conceptualized as illnesses that involve abnormalities of brain structure, as well as of brain function. Dysfunction within the prefrontal cortical and striatal systems that normally modulate limbic and brainstem structures involved in mediating emotional behaviour is postulated to be of particular importance in the pathogenesis of depression.

Finally, a very recent PET study demonstrated MAOA (monoamine oxidase A) levels elevated by 34% throughout the brain in untreated depressed patients (Meyer et al. 2006). This promising finding will certainly be assessed in confirmatory studies, since analyses of functional MAOA polymorphisms in depression have generally yielded negative results.

**Immunological markers in major depression**

**Immune cells**

Early studies showed an increase of T-helper cells (CD4+ cells) and an increased CD4+/CD8+ ratio in depressive disorders (Syvalähti et al. 1985; Maes et al. 1992; Müller et al. 1993). Further investigations of the cellular components of the immune system focussed on monocytes and macrophages. Increased numbers of peripheral mononuclear cells have been described by different groups of researchers (Herbert and Cohen 1993; Seidel et al. 1996; Rothermundt et al. 2001). Neopterin is a sensitive marker of cell-mediated immunity. The main source of neopterin is monocytes/macrophages. In agreement with the findings of increased monocytes/macrophages, an increased secretion of neopterin has been described in patients suffering from major depression by several groups of researchers (Duch et al. 1984; Dunbar et al. 1992; Maes et al. 1994; Bonaccorso et al. 1998).

**Interleukin-6**

As a product of monocytes and macrophages, interleukin-6 (IL-6) is one of the most frequently investigated immune parameters in major depression patients. Most publications report a marked increase of in vitro IL-6 production (Maes et al. 1993) or serum IL-6 levels in depressed patients (Maes et al. 1995b, 1997a; Sluzewska et al. 1995, 1996; Berk et al. 1997; Frommberger et al. 1997; Song et al. 1998). Most of these studies also report elevated plasma levels of acute phase proteins – markers of the unspecific (innate) immune system. Contradictory results are very few, indicating reduced (Katila et al. 1989), or unchanged serum IL-6 levels (Maes et al. 1995b; Brambilla and Maggioni 1998). An age-related increase of IL-6 serum values was reported in patients with major depression (Ershler et al. 1993). From a methodological point of view, the potential influence of possibly interfering
variables such as smoking, gender, recent infections and prior medication on IL-6 release and concentration must be considered (Haack et al. 1999).

Prostaglandin E2 (PGE₂) stimulates IL-6. Therefore an increased secretion of PGE₂ would be expected in depressive disorders, too. Early studies described increased PGE₂ both in the cerebrospinal fluid and in the serum of depressed patients (Linnoila et al. 1983; Calabrese et al. 1986). Increased concentrations of PGE₂ in saliva of depressed patients have been repeatedly described (Ohishi et al. 1988; Nishino et al. 1989). Moreover, in vitro studies show an increased PGE₂ secretion from lymphocytes of depressed patients compared to healthy controls (Song et al. 1998).

Data on IL-6 and the IL-6 system in the CSF are still rare. Markedly decreased levels of IL-6 and its soluble receptor subunit IL-6Rα in elderly patients with major depression compared to matched healthy control persons have been described (Stübner et al. 1999). IL-6 is a highly important inducer of antibody production (Th2 immune response) and indeed, some data show increased antibody titres in major depression. As in schizophrenia, a great heterogeneity of antigen-specificity of the antibodies, such as anti-nuclear anti-phospholipid, antithyroidal or anti-viral antibodies was found (Haggerty et al. 1987; Maes et al. 1991; Amsterdam and Hernz 1993).

There is no doubt that IL-6 is involved in modulation of the hypothalamic–pituitary–adrenal (HPA) axis (Plata-Salaman 1991). Activation of the HPA axis is one of the best-documented changes in major depression (Roy et al. 1987). Furthermore, the relationship between psychological or physical stress and an enhanced IL-6 secretion in the peripheral immune system seems to be well established (LeMay et al. 1990; Salas et al. 1990; Zhou et al. 1993; Miyahara et al. 2000). An impaired ability to cope with stress is often observed in depressed patients. Thus, the high number of reports showing elevated peripheral IL-6 levels in major depression patients may be related to psychological stress. On the other hand, there is evidence for a relationship between high peripheral IL-6 levels and elevated central nervous system serotonin availability. Intravenous or intraperitoneal administration of IL-6 in an animal model induced not only an activation of the HPA axis, but also an increase in brain tryptophan and serotonin metabolism, whereas norepinephrine metabolism was unaffected (Wang and Dunn 1998). Accordingly, IL-6 may be a mediator of activation of the HPA axis and of the central nervous serotonin system after administration of the endotoxin LPS (Wang and Dunn 1999). Thus, elevated plasma levels of IL-6 do not fit with the hypothesis of a serotonin deficiency in major depression. On the other hand, there is also a report showing a correlation between increased IL-6 production in culture supernatants of mitogen-stimulated peripheral leukocytes in vitro and decreased tryptophan levels in depressed patients that emphasizes the influence of IL-6 on serotonin metabolism (Maes et al. 1993). Serotonin synthesis in the CNS is at least partly dependent on the availability of tryptophan in the blood (Fernstrom 1977).

**Interleukin-2**

Data on IL-2 in major depression are mainly restricted to measurements of its soluble receptor in peripheral blood. The blood levels of sIL-2R were repeatedly found to be increased in major depression patients (Maes et al. 1995b,a; Sluzewskia et al. 1996). One single study investigated CSF levels of sIL-2R and reported a highly significant reduction in major depression patients compared to healthy control subjects (Levine et al. 1999). Production of both IL-2 and IFN-γ is the typical marker of a type-1 immune response. IFN-γ is produced in higher amounts by lymphocytes of patients with major depression than in healthy controls (Seidel et al. 1996). Higher plasma levels of IFN-γ in depressed patients, accompanied by lower plasma tryptophan availability, have been described (Maes et al. 1994). Mendlovic et al. (1999) discriminated between suicidal and non-suicidal major depression patients in a small study. They found distinct associations between suicidality and type-1 immune response on the one hand and a predominance of type-2 immune parameters in non-suicidal patients on the other hand (Mendlovic et al. 1999).

**Tryptophan metabolism**

The essential amino acid tryptophan is the precursor of two distinct metabolic pathways, leading to the products serotonin or kynurenine. The enzyme indoleamine 2,3-dioxygenase (IDO) metabolizes tryptophan to kynurenine, which is then converted to quinolinic acid by the enzyme kynurenine hydroxylase. Both IDO and kynurenine hydroxylase are induced by IFN-γ. The activity of IDO is an important regulatory component in the control of lymphocyte proliferation (Mellor and Munn 1999). It induces a halt in the lymphocyte cell cycle due to the catabolism of tryptophan (Munn et al. 1999). The type-2 cytokines IL-4 and IL-10 inhibit the IFN-γ-induced tryptophan catabolism by IDO (Weiss et al. 1999). The enzyme IDO is located in several cell types including monocytes, microglial
cells and astrocytes (Alberati-Giani et al. 1996; Schwarz and Pellicciari 2002). An IFN-\(\gamma\)-induced, IDO-mediated decrease of central nervous tryptophan availability may lead to a serotonergic deficiency. While in schizophrenia the further metabolism of kynurenine to kynurenic acid may be an important factor influencing the dopaminergic neurotransmission via type-2 immune response, in depression the deficiency of serotonin via activation of the type-1 immune response, especially IFN-\(\gamma\), seems to be the link between immunity, inflammation, and serotonergic or dopaminergic neurotransmission, respectively.

**Neurophysiological findings**

Generally, electrophysiological measurements like event-related potentials (ERPs) are suitable as biological markers of physiological and pathological brain function, as they allow to measure neuronal activity related to distinct cognitive and emotional processes. The basic idea behind this method is to repeatedly present a subject with different classes of stimuli, which are supposed to activate distinct brain processes while the ongoing electrical activity in the brain is measured by means of scalp electrodes. The occurrence of each stimulus is marked in the ongoing EEG. This procedure allows to average all behaviourally correct, sufficiently artefact-free EEG epochs locked to one class of stimuli, which results in an event-related potential (ERP). This ERP is dominated by the specific neuronal response of the brain to the stimulus while the background EEG activity is diminished by the averaging process. ERPs have the clear advantage of an optimal resolution in the time domain. This means that neuronal activity associated with emotional or cognitive processes can be assessed with accuracy in the order of milliseconds, which is far superior to metabolic measurements like the blood oxygen level dependent (BOLD) effect in functional magnetic resonance imaging (fMRI). However, the precision of the ERPs in terms of spatial resolution is hampered by the so-called inverse problem, which makes a mathematically exact and unique source localisation impossible for a given distribution of ERP-amplitudes measured at scalp electrodes. In comparison, the spatial resolution of the BOLD response in fMRI investigations is far superior. Other features which make ERPs attractive in the search for biological markers in psychiatric diseases like depressions are that they are absolutely non-invasive, repeatable, easy to apply, very cheap, and, most importantly, well accepted also by psychiatric patients.

Since the first description of the P300 component elicited in an acoustic odd-ball paradigm (Sutton et al. 1965), numerous studies aimed at measuring event-related potentials (ERPs) as biological markers of diagnosis, therapeutic response and outcome in all major psychiatric diseases including depressive disorders. Due to limitations in space, we have to focus on four ERP measures reflecting information processing in the brain, which are possibly relevant in depressive disorders: These are the classical P300 amplitude in the acoustic oddball paradigm (Sutton et al. 1965), the loudness dependence of the auditory evoked N1/P2-response (LDAEP) (Hegerl et al. 2001), the error negativity (Ne/ERN) (Falkenstein et al. 1997) elicited by a visual Erikson flanker task, and the NoGo-antiorisation (NGA) (Fallgatter et al. 1997) provoked by a visual Go–NoGo paradigm. Other electroencephalographic approaches that cannot be dealt with here particularly comprise frequency-analytic approaches, such as studies on the frontal asymmetry in depression that are based on Davidson’s concept of cerebral asymmetry and emotion/affective style (Davidson 1998).

In the classical acoustic P300 paradigm, the subject is presented in random order via headphones with higher frequency, rare target tones (typically 2000 Hz, 20% probability) or lower frequency, more frequent distractor tones (1000 Hz, 80%). The subject has to respond to each target tone either with a button press or with silent counting. Averaging of the correct and artefact-free EEG-epochs typically reveals significantly higher P300 amplitudes at parietal electrode leads for the rare target as compared to the frequent distractor tones, which is commonly interpreted as an indication of stronger neuronal activity in the target condition. This P300 component has been repeatedly shown to have reduced amplitudes and prolonged latencies in patients with depressive disorders as compared to healthy age- and gender-matched controls (Roth et al. 1981; Pfellerbaum et al. 1984; Blackwood et al. 1987; Urretavizcaya et al. 2003; Kawasaki et al. 2004). Although altered P300 amplitudes and latencies are reported quite robustly across studies, the problem of these measures is their low specificity. Identical findings have been reported for all major psychiatric disorders, including schizophrenias, dementias and addictions (Polich and Herbst 2000).

The loudness dependence of the auditory evoked N1/P2-response (LDAEP; slope of amplitude increases with louder tones) is associated with the serotonergic neurotransmission in the primary auditory cortex. A pronounced LDAEP is supposed to reflect low serotonergic neurotransmission and vice versa (Hegerl et al. 2001). A diminished serotonergic neurotransmission is considered as one biological basis of depressive disorders and is the
rationale for treatments with serotonergic antidepressants. Consequently, strong loudness dependence in depressive patients indicative for a weak serotonergic neurotransmission has been shown to predict a favourable response to selective serotonin reuptake inhibitors (SSRI) (Hegerl et al. 2001; Linka et al. 2004). Furthermore, in withdrawn alcohol-dependent patients a highly significant inverse correlation was found between the loudness dependence and the personality trait “Harm Avoidance” from Cloninger’s Temperament and Character Inventory, which is supposed to be associated with both a low serotonergic neurotransmission and an increased risk to develop depressive disorders and alcohol dependence (Herrmann et al. 2001).

The error negativity (Ne, ERN) is another interesting ERP parameter, which occurs within 100 ms after an erroneous response and is supposed to reflect response monitoring processes within prefrontal brain areas (Herrmann et al. 2004). As perceived failure is reported to have harmful effects on subsequent performance in patients with depressive disorders, the Ne/ERN is a potentially interesting ERP parameter in this patient group. Interestingly, another study showed a less negative Ne/ERN in patients with major depressive disorders as compared to healthy controls (Ruchsort et al. 2004). This finding was interpreted as a sign of impaired response monitoring processes in this group of patients.

The NoGo-anteriorisation (NGA) (Fallgatter et al. 1997) is another interesting ERP measure, which is calculated as the spatial difference between the centre of gravity of the electrical P300-field in the NoGo- as compared to the Go-condition of a cued Go/NoGo-task. The NGA has been shown to be a very stable (Fallgatter et al. 1997, 2000; Fallgatter and Strik 1999), reliable (Fallgatter et al. 2001) and age-independent (Fallgatter et al. 1999) ERP phenomenon, which reflects pronounced activity in medial prefrontal brain areas including the anterior cingulate cortex (ACC) during a response inhibition process (Fallgatter et al. 2002). Positron emission tomography (PET) studies suggest that depressive patients might be heterogeneous regarding the level of activity in such medial prefrontal brain areas including the ACC during the acute depressive state with one group being hyperactive while others display a normal or even reduced metabolism. Interestingly, an initial hypermetabolism in these medial prefrontal brain areas predicted a favourable therapeutic response to antidepressive medication (Mayberg et al. 1997) as well as sleep deprivation (Wu et al. 1999), whereas those patients with low initial metabolism in this brain region tended to respond worse. Corresponding results were obtained in another study (Pizzagalli et al. 2001) with a three-dimensional EEG source location analysis (low resolution electromagnetic tomography, LORETA) (Pascual-Marqui et al. 1994) aiming on Theta-EEG activity in the ACC. Based on these findings we investigated the NGA as an ERP-marker of prefrontal brain function in 28 depressive in-patients (18 female, 10 male, mean age 42.8 ± 12.0 years) of the Department of Psychiatry and Psychotherapy at the University of Würzburg, Germany. The NGA was measured at two time-points, shortly after admission to the hospital (t1) and again after about 4 weeks (26.4 ± 9.1 days) of an antidepressive treatment (t2) in a naturalistic design consisting of individually chosen psychopharmacological treatment and supportive psychotherapy. The hypotheses were (1) that the variance of the NGA in acutely depressive patients would be higher than in a healthy control sample matched for sex, age, and handedness (pooled from Fallgatter et al. 1997, 2000; Fallgatter and Strik 1999) and (2) that the therapeutic response as measured by improvements of the depressive symptomatology in terms of investigator- (21-item version of the Hamilton Depression Rating Scale, HDRS) (Hamilton 1967) and patient-ratings (Becks Depression Inventory, BDI) (Beck et al. 1961) would be predicted by the initial NGA. The first hypothesis was supported by the data, since the variance of the NGA at t1 was significantly higher in the group of depressive patients compared to the healthy control sample as indicated by Levene’s test for the equality of variances (mean NGA 0.87 ± 0.34 vs. 0.77 ± 0.68 for healthy controls versus depressive patients; \( F = 7.61, P < 0.01 \) (Figure 1). The second hypothesis was not supported by the data, since there was no significant correlation between initial NGA and therapeutic outcome (Pearson coefficients of \( r = 0.009 \) and 0.024 for the correlation between the initial NGA and changes in the BDI and HDRS score, respectively; n.s.). However, a classification of the patient sample based on the initial NGA revealed subgroup-specific treatment effects in the direction of normalisation: both the patients with initial high and with initial low NGA tended to have an NGA in the normal range after 4 weeks of treatment, whereas the group with a normal initial NGA did not change notably (Figure 2). However, further studies with bigger and less heterogeneous patient samples and more standardized treatment protocols are necessary, to further clarify the usefulness of the NGA as a predictor of therapeutic response in depressive patients.
In summary, there are several ERP measures of information processing which are suitable candidates for biological markers of depressive disorders. However, their value in this respect has to be further established, before a useful clinical application is possible. Moreover, some of these measures (LDAEP, NGA) might have the potential for a prediction of therapeutic response of different antidepressive therapies.

Cognitive deficits in major depression

There is growing evidence that several cognitive domains are significantly impaired in patients with major depression, including attention, memory and executive functioning. Patients with major depression manifest significant impairments in their ability to maintain attention on tasks requiring effortful mental operations, i.e. tasks that require selective and sustained attention, or which imply great resources allocation capacities (Tancer et al. 1990). Contrary to schizophrenia and bipolar disorder, sustained attention (evaluated by Continuous Performance Task) deficits seem state-dependent indicators for major depression (Liu et al. 2002). Several studies have reported that depressed patients are impaired on verbal fluency tasks (Landro et al. 2001; Moritz et al. 2002; Ravnikilde et al. 2002), Trail Making Tests (Austin et al. 1999; Grant et al. 2001; Moritz et al. 2002), Wisconsin Card Sorting Test (Ilonen et al. 2000; Grant et al. 2001; Moritz et al. 2002), and on the Tower of London task (Beats et al. 1996; Elliott et al. 1996). There is, however, some inconsistency, the deficit appearing only in endogenous cases in some studies (Austin et al. 1999; Rogers et al. 2004), while other authors failed to evidence executive functioning deficits in major depression regardless of the severity (Ravnikilde et al. 2002). These discrepancies may reflect a number of methodological issues, including variation in diagnostic criteria, test selection and strategies used to perform this task. Executive processes are also likely to contribute to impairments in memory performance (Baudic et al. 2004), as depressed participants have been found to show greater impairment in recalling information that benefits from semantic organisation compared to information that does not (Channon et al. 1993).

Cognitive deficits appear particularly pronounced in elderly patients and among major depression patients with severe illness, or with melancholic/psychotic features (Beats et al. 1996; Schatzberg et al. 2000). However, the relationship between cognitive performances and major depression melancholic subtype disappeared after covarying for Hamilton score (Austin et al. 1999) and could thus implicate severity itself rather than subtype. On the other hand, while one study failed to observe cognitive differences between unipolar and bipolar patients (Neu et al. 2001), other studies suggest greater cognitive impairment during an acute depressive episode (Borkowska and Rybakowski 2001).
and greater time course deterioration in patients with bipolar compared to patients with unipolar disorder (Burt et al. 2000). Several factors could explain these discrepancies, and among them psychotic features. Indeed, it was observed that patients with first episode of psychotic unipolar depression had a pattern of neuropsychological dysfunction similar to but less severe than that of patients with schizophrenia (Hill et al. 2004). This suggests that these psychotic disorders may have common pathophysiological features.

Because of widely reported but generally modest correlations between symptom severity and neuropsychological deficits in depressed patients, as well as studies showing improvement in function after treatment (Calev et al. 1986; Goldberg et al. 1993), it has traditionally been accepted that cognitive deficits in mood disorders are related to the acute state of illness. However, this point is considered controversial since some authors found cognitive disabilities in euthymic depressive patients which led to the hypothesis that cognitive deficits might persist late after the period of illness. Indeed, some studies have failed to demonstrate any residual memory impairment after clinical recovery, whereas others show persistent impairment, particularly in aspects of executive functioning (Beats et al. 1996; Paradiso et al. 1997). Thus, executive deficits could represent a relatively stable trait marker, whereas mnemonic impairment seems to be related to clinical state. This view is not supported by some studies reporting impaired verbal memory in unipolar depressive patients in remission (Tham et al. 1997; Kessing 1998). This discrepancy could be explained by the number of previous depressive episodes. Indeed, in one of the studies (Tham et al. 1997), cognitive dysfunctions were observed in a subgroup of patients with more frequent relapses and episodes of hospitalization, even when euthymic.

Cognitive functioning in general might be disturbed rather than distinct abilities. However, not all major depression patients show cognitive deficits and there is marked disagreement about the reasons why certain patients develop more severe cognitive problems than others. These discrepancies may

Figure 3. Biological markers of depression. Both genetic and environmental factors during neurodevelopment or later in life influence nervous system function and plasticity and modulate immune and endocrine cascades associated with pathophysiology of depression. Nervous, immune and endocrine systems have feedback regulations and are linked to each other. For example: an increase of cortisol may activate monocytes to increase pro-inflammatory cytokines which may lead to a deficiency of 5-HT due to the favoured metabolism of tryptophan by increased activity of IDO to the neurotoxins kynurenine and quinolinic acid. Such regulations can be measurable and may be used as indicators (robust, good or weak) of the presence, severity and prognosis of depression as well as prediction of drug/other treatment and are characterized as biological markers of depression. Li, limbic areas; ↑, increase; ↓, decrease; s, serum; c, CSF; sa, saliva; l, lymphocytes; b, blood; t, thrombocytes.
reflect a number of methodological problems. We suggest that cognitive deficits may depend on age, illness severity and psychotic or melancholic features. Furthermore, these deficits have not been well characterized in younger patients with mild to moderate depression. The cognitive deficits in depression may be associated with both trait and state factors and raise questions about the long-term cognitive functioning of patients with major depression. These deficits may be explained by structural or functional changes associated with illness severity, aging effects, or a possible cumulative pathologic effect of depression on brain structure and function across recurrent episodes of illness.

**Summary**

In summary, amongst the plethora of potential biological markers of major depression including neurotrophic factors, serotonergic markers, biochemical markers, immunological markers, neuroimaging, neurophysiological findings, and neuropsychological markers (Figure 3), some stand out as more robust. These are:

- decreased platelet imipramine binding (5-HTT expression);
- decreased 5-HT1A receptor expression;
- increase of sIL-2R and IL-6 in serum
- decreased BDNF and FGF-1 in serum
- hypocholesterolemia
- low blood folate levels
- cortisol hypersecretion; non-suppression of the dexamethasone suppression test

However, none of these markers have been shown to be sufficiently specific to allow inclusion into diagnostic manuals of major depression.

**Statement of interest**

The authors have no conflict of interest with any commercial or other associations in connection with the submitted article.

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