Chapter 8

Neurotransmitters and neuropeptides in depression

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INTRODUCTION

Structural brain differences have been found in mood disorders, which indicate that they may have a neurodevelopmental underpinning. However, these structural differences are neither specific nor informative about the molecular processes involved, and are not relevant for diagnostic purposes. Therefore we will focus in this chapter on the neuropathological macro- and microscopic alterations in mood disorders on molecular neuropathology, i.e., the neurotransmitter and neuromodulator alterations in the brain in mood disorders that give functional information and may lead to new rational therapeutic strategies and targets. The conclusion of this review is that a multitude of different genetic and developmental causes may lead to alterations in a network of stress- and reward-related neurotransmitter and neuromodulator systems that, in different ways, cause individuals to be at risk for a depressive disorder in case of the occurrence of stressful environmental events. The hypothalamo–pituitary–adrenal (HPA) axis has a prominent position in this network (Fig. 8.1).

Neuropathology: structural differences

A number of macro- and microscopic structural alterations have been observed in cortical and subcortical brain regions that are considered to be involved in the pathogenesis of mood disorders, or reflect a more general developmental disorder resulting in an increased risk for depression.

CORTICAL NEURONAL AND GLIAL CHANGES IN MOOD DISORDERS

Patients with bipolar disorder (BD) or with major depressive disorder (MDD) have reduced prefrontal cortex (PFC) gyration, affecting both ventral and dorsal subregions, which are significantly associated with generalized cognitive impairments. However, such alterations are not disease-specific, since similar abnormalities were observed in schizophrenia (McIntosh et al., 2009; Zhang et al., 2009). The left subgenual anterior cingulated cortex (ACC) shows gray- and white-matter volume reductions in early BD stages that probably have a neurodevelopmental origin (Fountoulakis et al., 2008). The subgenual region of Brodmann’s area 24 was found to be reduced in volume in patients with familial forms of MDD and BD. There was no change in neuronal size or number in this brain area, but the glial cell number was reduced in both mood disorder groups, especially in the familial forms (Drevets et al., 1998; Ongur et al., 1998). In male BD patients who experienced their first episode of psychosis, an increased thickness of the subcortical ACC was found at the right side of the brain (Fornito et al., 2009). In contrast, persons at high familial risk for depression had a cortical thinning in the right hemisphere that was proposed to produce behavioral disturbances that, in turn, may increase the risk for depression (Peterson et al., 2009). Both current and remitted MDD patients showed significant volume reduction of the left anterior insular cortex as compared with healthy controls (Takahashi et al., 2009b). Furthermore, microscopic studies have revealed both neuronal and glial deficits in the

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Control

![Diagram of the HPA axis in control](image)

Depression

![Diagram of the HPA axis in depression](image)

Light

![Diagram of the HPA axis with light](image)

Anti-depressants

![Diagram of the HPA axis with anti-depressants](image)

**Fig. 8.1.** Schematic illustration of the mechanisms that may cause a mood disorder by impaired input of neurotransmitters (monoamines and amino acids) and/or neuropeptides (arginine vasopressin (AVP), oxytocin (OXT)) on the hypothalamo-pituitary-adrenal (HPA) axis. In addition, the main targets of light and antidepressive medication are indicated.

In the control situation (normal mood), the corticotropin-releasing hormone (CRH) neurons of the stress axis (HPA axis) are inhibited by a γ-aminobutyric acid (GABA)-ergic input from (extra-)hypothalamic areas and by an AVP input from the suprachiasmatic nucleus (SCN). Some monoamines and neuropeptides (e.g., OXT) also inhibit the HPA axis.

When there is depression, the HPA axis is activated by: (1) diminished GABAergic input; and/or (2) increased glutamergic input from (extra-)hypothalamic sites; and/or (3) diminished inhibition by the SCN; and/or (4) stimulatory influence on the HPA axis by alterations in the monoamine or neuropeptide input; and/or (5) a deficient cortisol feedback effect due to the presence of glucocorticoid resistance. The resulting disinhibition of the paraventricular nucleus (PVN) causes a chronic rise in CRH and cortisol levels in depression, which causes mood changes through their action on the brain. The hyperactivity of the HPA axis may be due to a multitude of risk factors such as genetic polymorphisms, development sequelae, and environmental factors. A decreased amount of AVP mRNA of the SCN was found in depression, which seems to be the basis of the impaired circadian regulation of the HPA system in depression and a decreased inhibition of CRH neurons.

Light therapy activates the SCN, directly inducing an increased synthesis and release of AVP that will inhibit the CRH neurons in the PVN.

Antidepressant medication generally inhibits the activity of CRH neurons in the PVN.

→ stimulation; –→ inhibition. The thickness of the lines indicates the strength of the effects. ACTH, adrenocorticotropic hormone.

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**Subcortical changes in mood disorders**

Significantly reduced medial and lateral habenula volumes were found in depression, together with a reduction in neuronal numbers and size in these nuclei (Ranjit et al., 2009). A glial cell reduction was found in the amygdala in MDD patients and not in BD. No reduction was observed in neuron numbers. The glial cell reduction was primarily due to oligodendrocytes and largely occurred in the left hemisphere (Bowley et al., 2002; Hamidi et al., 2004). The interthalamic adhesion was shorter in patients with MDD than in controls and its size was found to be negatively correlated with the severity of the symptom “loss of interest” (Takahashi et al., 2009a). For a recent review of the structural abnormalities in mood disorders, see Price and Drevets (2010), and for a meta-analysis of brain structures affected in BD, see Kempton et al. (2008).

**The hippocampus in mood disorders**

Various macroscopic, microscopic, and molecular alterations have been reported in the hippocampus of patients with mood disorders. Hippocampal volume reduction has been found in both early-onset and late-life depression. An increased prevalence of white-matter lesions was observed only in late-life depression (Jansen et al., 2004). Increased mossy fiber staining in the supragranular layer, suggestive of neuronal sprouting, was observed in subjects with BD (Dowlatashahi et al., 2000). A number of molecular changes indicated a dysfunction of inhibitory γ-aminobutyric acid (GABA)-ergic interneurons in the hippocampus in BD and MDD (Knable et al., 2004). Diminishment of the brain-derived neurotrophic factor was found in the right
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hippocampus but not in the left one in MDD and BD, while the p75 receptor was impaired only in BD (Dunham et al., 2009). Two isoforms of the neuronal cell adhesion molecules were increased in the hippocampus of BD patients, but the synaptic protein marker, synaptophysin, was not changed (Vawter et al., 2000). The presence of synaptic pathology in BD was, however, supported by the decreased expression of complexins (Eastwood and Harrison, 2000).

A major question concerning the hippocampal alterations in mood disorders is whether they may be caused by the glucocorticoid cascade. In depression, the HPA axis is strongly activated and the adrenal cortex hypersecretes glucocorticoids. On the basis of animal experiments, overexposure to glucocorticoids during prolonged periods of stress was expected to be damaging to the brain, particularly affecting the hippocampus. In a series of studies in rats, Landfield et al. (1981) produced experimental evidence demonstrating that cumulative exposure to corticosteroids influences hippocampal neuronal viability as well as function, which was expected to compromise memory function and cognition seriously (Landfield et al., 1981).

Subsequently, Sapolsky and McEwen (1986) provided evidence that chronic stress, with its ensuing increase in corticosteroid levels, caused degenerative loss of pyramidal neurons in the hippocampus and subsequent deficits in memory function and cognition in rats. Damage to the hippocampus was proposed to cause a disinhibition of the glucocorticoid negative feedback, which would lead to a further activation of the HPA axis, to subsequent rises in glucocorticoid levels, and thus to accumulating damage to the hippocampus. This hypothetical feed-forward cascade became known as the “glucocorticoid cascade hypothesis” and was proposed as a major pathogenetic mechanism also in human neurodegenerative diseases associated with HPA axis alterations. Since the HPA axis is indeed activated in depression, a glucocorticoid cascade was proposed also to be causally involved in hippocampal damage in this disorder (Sapolsky and McEwen, 1986).

Because of the presumed neurotoxicity of cortisol in depression, Swaab’s group studied the postmortem hippocampal tissue of a group of patients that were well established to have long-term severe mood disorders, a group of glucocorticoid-treated patients and matched controls using hematoxylin and eosin, Nissl and Bodian staining. The patterns of reactive astrogliosis (glial fibrillary acidic protein), synaptic density (synaptophysin), synaptic reorganization (growth-associated protein B-50), and early signs of Alzheimer’s disease (AD, Alz-50) were examined immunocytochemically. Subsequently these patients were investigated using in situ end-labeling for DNA fragmentation and apoptosis, and heat-shock protein 70 and nuclear transcription factor kappaB immunocytochemistry for damage-related responses. No indications for AD changes or obvious massive cell loss could be observed. The absence of any major pyramidal cell loss and the very rare occurrence of apoptosis, notably absent from areas at risk for glucocorticoid damage like CA3, indicate that apoptosis has probably contributed to only a very minor extent to the volume changes in these conditions and that other mechanisms must have been involved (Lucassen et al., 2001; Muller et al., 2001). Using Alz-50, a marker for neuritic plaques and tangles, they did not find in those studies any evidence that patients with a lifelong history of depression would develop more Alzheimer neuropathology in the hippocampus, as claimed by others (Rapp et al., 2006).

Other independent postmortem studies on depressed patients also concluded there were not more Alzheimer-type lesions in the entorhinal cortex, subiculum, and hippocampus in depression (Damazic et al., 2002) and that the liability for some patients to develop cognitive impairment during a depressive episode was not related to an increase in AD or vascular neuropathology (O’Brien et al., 2001).

Concluding, many structural differences have been reported in mood disorders (Kempston et al., 2008; Price and Drevets, 2010). The macro- and microscopic brain differences described are sometimes especially localized in gray and sometimes in white matter; they are often lateralized and are dependent on brain region and cell type, on the type of mood disorder, and on the presence of a familial or a sporadic disorder. The reported alterations are not specific for mood disorders, and cannot be used to confirm the clinical diagnosis neuropathologically. However, they support the idea that these disorders have a neurodevelopmental underpinning. Although these structural and functional differences are neither relevant for diagnostic purposes nor provide information about the molecular processes involved, they may, interestingly, be useful predictors of clinical outcome. Patients with a greater than median gray-matter volume in the ACC, insula, and right temporo-parietal cortex had faster rates of improvement and significantly lower residual symptom scores after 8 weeks of treatment with fluoxetine. Faster improvement was also predicted by greater functional activation of the cingulate cortex by emotional faces (Chen et al., 2007).

The rest of this chapter is dedicated to the characteristic molecular neuropathology of mood disorders, starting with the central position of the HPA axis in the network changes, and subsequently dealing with alterations in monoamines, L-glutamic acid (glutamate), GABA, and neuropeptides.

The central position of the HPA axis

The first time the HPA axis became the focus of depression studies was with the correlation found by Hans Selye in the 1930s between the dysregulation of the
stress response and mood disorders, especially depression (Selye, 1998). It has since become well accepted that, although the stress response is necessary to maintain homeostasis, long-term activation of the stress system brings hazardous or even lethal effects, and increases the risk of depression (Selye, 1998). Abnormalities in the “stress system” have been documented in at least one subset of depressed individuals (Young et al., 1991, 2003; Holboer, 2001). The neural network that encodes and evaluates the stressful event comprises the HPA axis, the arginine vasopressinergic (AVP) systems, and the noradrenergic system, which are the very same brain circuit that, when it is hyperactive due to a combination of a genetic background, developmental sequelae, and life events, underlies negative emotions and moods (Swaab, 2004; Akil, 2005). MDD thus arises from the interaction of vulnerability genes and developmental and environmental factors (Swaab et al., 2005; see Chapter 14). Prenatal environmental stressors such as placental insufficiency, food shortage, or nicotine exposure due to smoking of the pregnant mother may sensitize the child to developing depression in later life (Clark, 1998; Swaab, 2004). Psychosocial stress such as early maternal separation, child abuse, or neglect may also program the HPA axis permanently into a higher activity, while death or loss of a spouse, or personal injury or illness form risk factors that may trigger early episodes of depression (Swaab et al., 2005; Bao et al., 2008; see Chapter 14). Brain areas that are closely involved in a stress response include the brainstem, which is the first relay station for many physiological stressors, the amygdala, which processes fear and anxiety responses, the hippocampus, which mediates learning and memory and encodes the importance of a stimulus to the organism, and the PFC, which not only holds cognitive and executive functions but also regulates the stress axis (Swaab et al., 2000; Drevets et al., 2008). The stress-induced neural activation converges on the hypothalamus, and the HPA axis is regarded as the final common pathway in mediation of the stress response (Swaab, 2004; Bao et al., 2008).

When activated, the HPA axis stimulates the synthesis and release of cortisol, which normally has broad biological effects throughout the body that are adaptive but can become damaging when chronically elevated. Cortisol exerts negative feedback at the pituitary and the hypothalamic level via two types of receptor, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), to shut down the stress responses after the threat has passed. The termination of the stress response is as important as its initiation, since superfluous cortisol can cause broad endocrine dysregulation (Swaab et al., 2005). Moreover, the high proportion of depressed patients in cases of Cushing’s disease and during treatment with high dosage of synthetic corticosteroids points to a possible causal role of glucocorticoids in a subset of mood disorders (Bao et al., 2008). Furthermore, it should be noted here that only a proportion of the corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN) project to the portal blood vessels of the median eminence and regulate adrenocorticotropic hormone (ACTH) production in the pituitary. Other CRH neurons that are also activated in the stress response and depressive disorders, which may induce at least part of the symptoms (see below), project to different brain areas. Activity changes in this subgroup of centrally projecting CRH neurons cannot be directly monitored by measuring hormonal alterations in the periphery, but might be reflected in CRH cerebrospinal fluid (CSF) levels.

Concluding, during development the HPA axis may be permanently activated by genetic or environmental factors. The activated HPA axis may affect mood by central projections of CRH and/or enhanced circulating levels of corticosteroids.

**Intense interaction between the HPA axis and monoamines, glutamate, GABA, and neuropeptides: some basic aspects**

**Monoamines**

The HPA axis is innervated and regulated by a large number of neurotransmitters and neuropeptides that are altered in mood disorders. Over the past four decades, the focus on the brain monoaminergic systems, which contain serotonin (5-hydroxytryptamine, 5-HT), noradrenaline, and dopamine (DA), has contributed significantly to our knowledge of the pathophysiology and treatment of depression (Belmaker, 2008). In addition, the focus on amines has contributed to the development of a growing number of antidepressants, including the tricyclic antidepressants, monoamine oxidase (MAO) inhibitors, selective serotonin reuptake inhibitors (SSRIs), and monoamine receptor antagonists (Hirschfeld, 2000). It should be noted, however, that antidepressants aiming at the amimergic systems appear to be effective in only 40–60% of patients. Moreover, there is a huge discrepancy between the pharmacological/biochemical function of antidepressants (a few minutes) and their clinical mood-altering responses (10–15 days or longer) (Leonard, 2007), which is a serious problem in understanding what mechanisms antidepressants modify in their effects to relieve MDD. It is now also clear that there is no simple direct relationship between changes in the amimergic systems and the occurrence of depression (Ruhe et al., 2007; see Chapter 14).

Stress activates not only the HPA axis, but also 5-HT neuronal activity (Grahn et al., 1999; Greenwood et al.,...
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2003), and increases extracellular 5-HT levels in the dorsal raphe nucleus (Maswood et al., 1998). In addition, glucocorticoids were found to exert a permissive effect on the stimulation of tryptophan hydroxylase (TPH) activity in rat cortex and midbrain in response to acute stress (Singh et al., 1990). Also, a large body of evidence shows a remarkable effect of an altered 5-HT system on the activity of the HPA axis. In particular, acute administration of 5-HT receptor ligands increased the plasma levels of ACTH and cortisol in both animals and humans (Wetzler et al., 1996; Klaassen et al., 2002). A light and electron microscopic immunocytochemical study in the rat brain demonstrated that serotonin-containing terminals formed axodendritic and axosomatic synapses with corticotrophin-releasing factor (CRF = CRH) immunoreactive neurons in the hypothalamic PVN, which indicates that the central serotonergic system can influence the function of the HPA axis via a direct action upon the CRH-synthesizing neurons (Liposits et al., 1987). Indeed, 5-HT1A and 5-HT2A receptors with a high degree of colocalization in the CRH neurons were found in the PVN, while microinjection of 5-HT1A agonist (8-OH-DPAT) into the PVN triggered ACTH secretion through local 5-HT1A receptor activation (Zhang et al., 2004; Osei-Owusu et al., 2005).

Communication between norepinephrine and the HPA axis has also been extensively reported during stress responses. Electrical stimulation of the ventral noradrenergic ascending bundle, the principal pathway of norepinephrine fibers to the PVN, was found to increase CRH release to the portal system, an effect that could be blocked by an α1-adrenergic antagonist (Plotkin, 1987). Norepinephrine microinjection into the PVN of conscious rats induces a rapid increase of CRH heteronuclear RNA expression in the parvocellular division of the PVN (Ito et al., 1999). In addition, immobilization stress has been observed not only to enhance norepinephrine release, reuptake, metabolism, and synthesis in the PVN (Fracak et al., 1992) and the medial PFC (Shimizu et al., 1994), but also to trigger transcriptional activation of the genes encoding catecholamine biosynthetic enzymes in the locus ceruleus (LC), a target for CRH neurons (Serova et al., 1999). Moreover, local CRH infusion showed that CRH serves as an excitatory neurotransmitter in the LC, and suggests that its actions on LC neurons are translated into enhanced norepinephrine release, which has an impact on cortical targets (Smagin et al., 1995; Curtis et al., 1997). Furthermore, norepinephrine receptor sensitivity is altered by the circulating level of glucocorticoids (McEwen, 2005).

DA, too, plays a role in stress response. Stress exposure and elevated levels of glucocorticoids enhance DA release (Thierry et al., 1976; Dunn, 1988; Piazza and Le Moal, 1996; Rouge-Pont et al., 1998), and influence the activity or synthesis of tyrosine hydroxylase in the nucleus accumbens (Rastogi and Singhal, 1978; Trembleau and Bloom, 1998). At the anatomical level, a large number of DA receptor D-1 subtype positive neurons exist in both magnocellular and parvocellular parts of the hypothalamic PVN (Czyzak et al., 2000), and there is evidence that corticosterone may regulate the expression of D-1 while DA via D-1 receptors may regulate the amount of circulating corticosterone (Eaton et al., 1996; Czyrak et al., 2000). Human brain imaging studies have provided further evidence that stress-related increases in cortisol are associated with DA accumulation in the ventral striatum (Pruessner et al., 2004; Oswald et al., 2005). It seems that not only glucocorticoids, acting through GRs that are widely distributed throughout the brain, may regulate the function of the dopaminergic systems, but that DA, especially via D-1 receptor, found in the hypothalamic PVN, supraoptic nucleus (SON), and suprachiasmatic nucleus (SCN) (Fremeau et al., 1991), may participate in a wide variety of hypothalamic functions involved in the integration of endocrine, autonomic, and behavioral processes. Expression of D-1 receptors in the SCN, the biological clock of the hypothalamus, suggests that D-1 receptors may participate in the regulation of circadian rhythm.

Concluding, the HPA axis is innervated by the serotonergic, norepinephrinergic, and dopaminergic systems, while during the stress response the interplay between the HPA axis and these aminergic systems forms a concerted action.

GLUTAMATE AND GABA

Amino acid neurotransmitters are also presumed to play an important role in the pathogenesis of depression. Glutamate and GABA are, respectively, the principal excitatory and inhibitory neurotransmitters in the central nervous system (Petroff, 2002). Unlike the monoamine transmitters that occupy about 5% of the total synapses in the brain, glutamate and GABA are thought to account for at least 50% of the synapses (Leonard, 2007). In addition, exocytotic glutamatergic and inhibitory GABAergic synaptic inputs have been identified on hypothalamic CRH-expressing neurons (Boudaba et al., 1996, 1997). Glutamate administration in the PVN of rat causes a rise in ACTH and corticosterone serum levels by promoting CRH secretion (Feldman and Weidenfeld, 1997; Herman et al., 2004), while reduced GABAergic tone on the parvocellular neurons in the PVN was observed during chronic stress. This implies a diminished inhibition to CRH-producing cells that may lead to an activation of the HPA axis (Verkuyl et al., 2004). In fact, it is well accepted now that two major inhibitory mechanisms serve to constrain the basal
and stress-induced activity of the HPA axis: (1) the corticosteroid feedback and (2) the inhibition of the PVN by the neurotransmitter GABA, the two of which work together and influence each other (Bartanusz et al., 2004; Kovacs et al., 2004). These data offer a basis for close interactions between the HPA axis and these amino acid neurotransmitters in relation to depression.

**NEUROPEPTIDES**

There is strong evidence implying that neuropeptides, e.g., CRH and AVP and oxytocin (OXT), not only play an important role in the integration of endocrine, autonomic, and higher brain functions, but also contribute to the signs and symptoms of depression (Bao et al., 2008). CRH secreted by the parvocellular neurons of the hypothalamic PVN is the central driving force of the HPA axis. A proportion of the CRH neurons are projecting to other brain areas. It is central CRH that contributes to the symptoms of depression. Intracerebral injection of CRH in rodents induces behavioral changes like decreased food intake, decreased sexual activity, disturbed sleep and motor behavior, and increased anxiety (Holsboer, 2001). Some of these parvocellular neurons secrete both CRH and AVP as neurohormones in the median eminence into the portal capillaries that transport them to the anterior lobe of the pituitary. AVP strongly potentiates the ACTH-releasing activity and, eventually, the production of corticosteroids from the adrenal gland (Engelmann et al., 2004). Thus, there is a close interaction between CRH, AVP, and HPA axis activity.

In contrast to AVP, OXT was found to inhibit basal HPA axis activity and to attenuate the stress-induced activity of the HPA axis in various species, including humans (Legros, 2001). Activation of OXT neurons has also been found in depression and was presumed to be related to decreased appetite and weight loss in this disease, due to the central effects of this neuropeptide as a satiety hormone (Purba et al., 1996; Gimpl and Fahrenholz, 2001; Meynen et al., 2007a). Enhanced OXT production in the PVN was indeed found in postmortem material of melancholic depressed patients (Meynen et al., 2007a). In contrast, decreased peripheral serum OXT levels were observed in depressed patients both pre- and posttreatment, at least in females (Ozsoy et al., 2009). This illustrates that neuropeptides such as OXT, AVP, and CRH can independently be secreted into the circulation and to brain areas, and that by measuring circulating hormonal alterations in the periphery we do not necessarily get the right information on the central release.

Another neuropeptide orexin (hypocretin) is produced in the lateral hypothalamus and also has reciprocal connections with the aminergic system, the cholinergic system, and the CRH neurons. CSF levels of orexin are significantly lower in suicidal patients with MDD (Brundin et al., 2007, 2009), and in depressive disorder patients, from which those with a high suicidal risk were excluded (Salomon et al., 2003). CSF orexin levels are significantly diminished in narcolepsy as well, which is frequently associated with secondary depression (Salomon et al., 2003).

In summary, based upon the observations mentioned above, one can prudentely support the concept that the HPA axis holds a prominent position, i.e., as a final common pathway, in a network of stress- (and reward-) related neurotransmitter and neuromodulator systems, all of which are also crucially involved in depression.

**THE MONOAMINE HYPOTHESIS OF DEPRESSION**

MDD is still unfortunately often simplified in the lay and scientific media as the result of a shortage of serotonin (Damasio, 1994; Cowen, 2008). This simplification of monoaminergic deficits as an explanation for MDD is useful for clinicians to explain the mechanism of most antidepressants like SSRIs or TCAs, but does not logically provide an argument for low serotonin activity as the cause of depression and is, moreover, incorrect. Depressed mood, one of the core symptoms of MDD, might be induced by experimentally lowering the availability of serotonin only in specific subgroups of vulnerable human subjects (Ruhe et al., 2007; Cowen, 2008). The conclusion of a systematic review and meta-analysis of 90 monoamine depletion studies in humans by one of the authors (Ruhe et al., 2007) is summarized below.

**Monoamine depletion paradigms**

After the discovery of monoaminergic neurotransmitters, the essential steps in their production were identified and methods of interference with monoaminergic formation developed (Young et al., 1985). The best evidence about the possible contribution of serotonin in mood disorders comes from tryptophan depletion studies (Cowen, 2008). Serotonin depletion as an interference can be achieved by rapidly lowering the levels of the essential amino acid tryptophan, which cannot be synthesized by the body and must thus be ingested to enable serotonin formation. To achieve depletion, a tryptophan-free amino acid mixture is administered (acute tryptophan depletion (ATD)). Because other available amino acids compete with uptake of tryptophan by the brain, additional depletion of tryptophan is achieved by the amino acid mixture.

Depletion of norepinephrine and DA uses the same concept (acute depletion of the essential amino acids phenylalanine/tyrosine (APTD)), but unfortunately
intervention in the formation of DA from norepinephrine cannot be achieved (Mojjo et al., 1996). As an alternative to inducing a state of depletion, enzyme-blocking agents decrease the production of the monoamines. Para-chlorophenylalanine (PCPA) blocks serotonin synthesis (Shopsin et al., 1975; Gratton, 1982), and alpha-methyl-para-tyrosine (AMPT) blocks norepinephrine and DA synthesis (Delgado et al., 1993). PCPA appeared, however, to be toxic in humans, and therefore only two studies were published. AMPT is not toxic. For PCPA and AMPT, Ruhe et al. (2007) found insufficient studies to pool in their meta-analysis.

Monoamine depletion has often been studied in small groups of patients who undergo repeated testing in cross-over designs with true and placebo depletion (for ATD: amino acid mixture without and with tryptophan) separated by at least 1 week (within-subjects design). Alternatively, randomization of participants between these two conditions was used (between-subjects design). To monitor the success of depletion, plasma (or free) levels of tryptophan, tyrosine, or relevant levels of monoamine metabolites before and after intervention and control condition are usually measured 5–6 hours after ingestion of the amino acid mixture, when depletion is at its maximum. After ATD, normally a 70–90% reduction of tryptophan levels is observed. For AMPT, the reduction in phenylalanine/tyrosine might be somewhat lower (Booij et al., 2003). To measure changes in mood, the Profile of Mood States is the questionnaire most commonly used, followed by the Multiple Affect Adjective Checklist, Visual Analogue (Mood) Scales, Hamilton Depression Rating Scale, and Montgomery-Asberg Depression Rating Scale. Several overviews previously described the techniques of monoamine precursor depletion and enzyme-blocking methods (Booij et al., 2003; Hood et al., 2005), including the methodology of depletion tests (Van der Does, 2001).

Meta-analysis

Manuscripts were identified by a comprehensive search strategy in PubMed and EMBASE (until October 1, 2006). For references to the original studies, we refer to the original publication (Ruhe et al., 2007). Data from identified studies were extracted on abstract forms, and the validity of each study was assessed. The overall methodological quality of the identified studies was judged as good, with appropriate application of the ATD, AMPT, or AMPT depletion. Three clinically heterogeneous study populations were acknowledged a priori: (1) healthy controls (with or without a positive family history of MDD); (2) patients with a previous MDD currently in remission (currently either using antidepressants or not); and (3) patients with a current episode of MDD (with or without antidepressants), which will be considered separately. If appropriate and possible, the results found in these studies populations were pooled with conservative random-effects models (n = 52 studies) (DerSimonian and Laird, 1986). In order to pool continuous effect estimates from different mood scales, a standardization was applied (providing Hedges’ g, which can be interpreted as a standardized effect size) (Ruhe et al., 2007). Effect estimates and the corresponding standard error (st) were entered in the inverse-variance statistics for pooling in Review Manager v4.2 for Windows (Cochrane Collaboration). In order to investigate effect modification of results by family history for MDD, gender, duration of remission, or the use and type of antidepressants, stratified analyses were used for these variables, and calculated stratified pooled Hedges’ g, with 95% confidence intervals (CI). Differences between strata were tested by subtracting the chi-squared heterogeneity statistic per stratum from the total chi-squared heterogeneity statistic. This residual (Q residual) has a chi-squared distribution, with the total number of strata-1 degrees of freedom.

Acute Tryptophan Depletion

Healthy controls

In healthy controls, a small decrease in mood was observed (pooled Hedges’ g with 95% CI −0.27 (−0.45 to −0.09)). When these healthy controls were stratified for family history of MDD, healthy controls with a negative family history had significantly less decrease in mood after ATD (pooled Hedges’ g −0.19 (−0.43 to 0.05)) than controls with a positive family history (pooled Hedges’ g −0.56 (−1.00 to −0.13) (P = 0.01; Fig. 8.2). Furthermore, in healthy controls with a negative family history, females especially had significantly more decrease in mood after ATD than males (pooled Hedges’ g −0.44 (−0.81 to −0.06) versus 0.23 (−0.10 to 0.57) respectively; P < 0.001). In controls with a positive family history for MDD, males experienced a larger decrease in mood after ATD (studied in one study; P < 0.001) when compared with males with a negative family history, while for females a positive family history only slightly reduced the mood effects of ATD, compared with females with a negative family history (P = 0.43). In conclusion, ATD had the most profound, but still moderate, mood effects in female healthy controls, and controls with a positive family history of MDD (possibly attributable to deterioration of mood in males with a positive family history).

Recovered MDD patients

In the remitted patients without current antidepressants, two studies investigated patients who had been in remission for 3–6 months and found much larger
efforts of ATD (pooled Hedges’ g = -4.35 (−7.39 to −1.31)) compared with three studies of patients who had been in remission for at least 6 months (pooled Hedges’ g = −0.60 (−1.38 to 0.18); P < 0.0001).

In remitted patients currently using antidepressants, ATD caused a decrease in mood (pooled Hedges’ g = −0.49 (−0.89 to −0.10). Hedges’ g varied slightly but nonsignificantly, depending on the type of antidepressant (P = 0.82). In remitted patients using antidepressants, decreased mood after ATD was especially seen in the first 5 months after the achievement of remission (pooled Hedges’ g = −0.55 (−0.90 to −0.21); P < 0.0001).

Relapse rates in remitted patients with AD were increased after ATD compared with control depletion (pooled difference in relapse rate 47% (28–66%); Fig. 8.3). Increased relapse rates were especially seen in patients using SSRIs (47% (27–67%)) or SNRIs (35% (14–56%)). However, the noradrenergetic drug desipramine showed no significant difference in relapse rate (7% (−6% to 19%)) after ATD. This effect modification of relapse rates by drug was statistically significant (P < 0.001).

Depressed MDD patients

In patients who were depressed at the time of ATD we found two studies for meta-analysis. These studies included patients who either used or did not use antidepressants. The effects of ATD were opposed: Hedges’ g was 0.32 (−0.22 to 0.86) for patients using different types of antidepressants, and −0.12 (−0.45 to 0.21) for patients without antidepressants.

ACUTE PHENYLALANINE TYROSINE DEPLETION

Healthy controls

APTD did not decrease mood in healthy controls (pooled Hedges’ g 0.10 (−0.23 to 0.43) and 0.12 (−0.43 to 0.68) in within-subjects and between-subjects studies,

Fig. 8.2. Acute tryptophan depletion (ATD) in healthy controls studied in a within-subjects design, stratified by status of family history for depression (negative or positive for major depressive disorder). Please note different subgroups per study handled as separate studies with appropriate pooling weights. FH−, family history negative; FH+, family history positive; N/A, not reported. (Reproduced from Kühne et al., 2007, with permission.)
Fig. 8.3. Acute tryptophan depletion (ATD) in former depressed patients in remission. Differences of relapse rates versus control-depletion studied in a within-subjects design in remitted patients stratified by current medication use. SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin–norepinephrine reuptake inhibitor. (Reproduced from Ruhe et al., 2007, with permission.)

respectively). However, in one study with healthy controls with a positive family history for MDD, a moderate but nonsignificant effect on mood was found (Hedges’ g –0.49 (−1.17 to 0.19)).

Recovered MDD patients
In patients with MDD in remission without antidepressants, no effect of APTD was observed in two studies (pooled Hedges’ g –0.02 (−0.50 to 0.47)).

Depressed MDD patients
No studies were identified.

Conclusions and discussion of the depletion of monoamine systems
The above meta-analysis led to the conclusion that depletion of monoamine systems (both 5-HT and norepinephrine/DA) does not decrease mood in healthy controls. However, in healthy controls with a family history of MDD the results suggest that mood is slightly decreased, by both ATD and APTD. Additionally, healthy females are more affected by ATD than healthy males, especially in controls without a family history of MDD. In patients who were previously depressed but in remission without antidepressants, APTD moderately decreases mood, while ATD does not significantly decrease mood. The site of action of these antidepressants (5-HT or norepinephrine/DA) predicts the occurrence of a lowering of mood or a short relapse in MDD after depletion of the corresponding monoamine. These findings are in line with the summaries in previous reviews (Reilly et al., 1997; Moore et al., 2000; Bell et al., 2001; Van der Does, 2001; Booj et al., 2003). However, this meta-analysis was the first summary that pooled results over individual studies.

The absence of robust mood effects in healthy controls indicates that mood is not a correlate of 5-HT or norepinephrine levels in the brain. The only healthy controls who are modestly affected by monoamine depletion studies are healthy controls with a positive family history for MDD. This might be indicative of a biological vulnerability, which is revealed by depletion studies. Of interest are the studies that combined ATD with neuroimaging or genetic sampling (Brenner et al., 1997, 2003; Smith et al., 1999; Yatham et al., 2001; Neumeister et al., 2002, 2004, 2006; Praschak-Rieder et al., 2004, 2005; Cools et al., 2005; Evers et al., 2005, 2006a, b; Rubia et al., 2005; Allen et al., 2006; Talbot and Cooper, 2006; van der Veen et al., 2007), reviewed by Fusar-Poli et al. (2006). The general conclusion of these findings was that the observed metabolic changes do not directly relate to brain 5-HT or norepinephrine levels, but reflect postsynaptic effects of lowered 5-HT and/or norepinephrine neurotransmission in vulnerable brain regions. Functional polymorphisms of serotonin transporter gene promoter (the 5-HT transporter gene-linked polymorphic region, 5-HTTLPR) influenced the mood effects of ATD in healthy female controls (Neumeister et al., 2002). Furthermore, differences in regulation of regional cerebral glucose metabolism in corticobulbar circuits of remitted depressed patients after ATD
were associated with functional polymorphisms of 5-HTTLPR (Neumeister et al., 2006). Indeed, 5-HT transporter gene and MAO-A polymorphisms are known risk factors for MDD, BD, and suicide (Anguelova et al., 2003; Lotrich and Pollock, 2004; Christiansen et al., 2007; Doornbos et al., 2009; Wray et al., 2009). Therefore, these combined studies suggest that monoamine depletion discloses a trait vulnerability rather than a pure state-dependent change due to depletion.

Only when the target of the depletion (serotonin, norepinephrine) coincides with the working mechanism of the antidepressant used does a depressive relapse occur after monoamine depletion in remitted patients who use antidepressants. This emphasizes that antidepressants indeed specifically affect their supposed target systems. However, from these studies, it can only be concluded that an undepleted 5-HT system is required for serotonergic antidepressants. The same holds for the norepinephrine system and norepinephrinergic antidepressants. An alternative explanation for the decrease in mood after monoamine depletion in patients is that depletion of 5-HT may give the same effect as abrupt discontinuation of SSRIs (Delgado, 2006). Rapid discontinuation is also associated with mood effects, which are considered to be different from a depressive relapse (Rosenbaum et al., 1998).

What certainly cannot be concluded is that MDD is caused by low levels of 5-HT and/or norepinephrine/DA. This simplification, which is often used to promote the use of antidepressants specifically affecting 5-HT or norepinephrine or both systems, ignores the notion — emphasized by the above neuroimaging findings — that serotonergic and norepinephrinergic antidepressants presumably act via a network of circuits involved in stress regulation on a final common pathway, in which changes in levels of neuropeptides such as CRH are supposed to be responsible for the remission of MDD (Charney, 1998; Belmaker and Agam, 2008). The fact that a diminished activity of the serotonergic system is not central in the pathogenesis of mood disorders, as is often presumed, is also apparent from postmortem studies. In the raphe nuclei no loss of neurons was reported in one study (Hendricksen et al., 2004), while a loss of neurons was found in another (Baumann et al., 2002), and even higher numbers of neurons and levels of TPH mRNA were reported in suicide victims (Underwood et al., 1999; Bach-Mizrachi et al., 2008).

From the findings of the meta-analysis one could also question the validity of monoamine depletion as a model to study the pathogenesis of MDD. However, monoamine depletion by ATD, APTD, and AMPT remains a useful model to manipulate safely and directly 5-HT, norepinephrine, and DA function in living humans, and to study the behavioral consequences of this manipulation (Reilly et al., 1997; Bovij et al., 2003; Hood et al., 2005).

THE AMINO ACID HYPOTHESIS OF DEPRESSION

The glutamatergic system in depression

A number of proton magnetic resonance spectroscopy (1H MRS) brain-imaging studies showed changes in the glutamatergic system in patients with depression. Elevated levels of glutamate/glutamine have been reported in the frontal lobe, basal ganglia, and occipital cortex of depressed patients (Castillo et al., 2000; Michael et al., 2003b; Sanacora et al., 2004). In the anterior cingulate, increased glutamate and glutamine contents were reported in nonmelancholic BD patients (Frye et al., 2007), whereas reduced glutamate/glutamine levels were found in patients with unipolar depression (Auer et al., 2000; Pfeiferer et al., 2003; Mirza et al., 2004; Rosenberg et al., 2005). Recently, a study using a combined functional magnetic resonance imaging and magnetic resonance spectroscopic approach has found that patients with highly anhedonic MDD showed decreased glutamine but normal glutamate and GABA concentrations in the pregenual anterior cingulate cortex (pACC) compared to controls, and that glutamate and N-acetylaspartate concentrations in the pACC correlated with negative blood oxygenation level-dependent responses induced by emotional stimulation in MDD (Wallace et al., 2009). It thus seems that BD and unipolar depression patients have different glutamate brain levels. However, treatment may affect these amine levels. A recent study demonstrated that short-term treatment with citalopram, an SSRI, increases occipital concentrations of glutamate and glutamine levels in healthy subjects (Taylor et al., 2008), while electroconvulsive shock may cause significantly increased glutamate/glutamine levels in the amygdala, the dorsolateral PFC, and the left anterior cingulum (Michael et al., 2003a, b; Pfeiferer et al., 2003).

In patients with MDD, glutamine synthetase (GS) transcripts were downregulated in the ACC, dorsolateral PFC, and amygdala (Choudary et al., 2005). The reduction of GS may increase residual glutamate concentrations and trigger feedback inhibition of glutamate transport synthesis and/or allow a rise in extracellular glutamate with a potential for excitotoxic effects. GS transcript regulation may, however, be dependent on the type of depressive disorders and on brain region, since Toro et al. (2006) did not find any significant changes in GS in dorsolateral and orbitofrontal cortex in patients with MDD and BD. It should be noted, though, that the majority of the patients from the latter
study had an additional history of substance abuse and high alcohol use, which may have confounded the results.

The expression of glutamate transporters, the excitatory amino acid transporters (EAAT), EAAT1 and EAAT2, are reduced in two frontal brain regions in post-mortem brain samples of MDD patients (Choudary et al., 2005). In addition, decreased levels of EAAT3 and EAAT4 mRNA expression are present in the striatum of subjects with mood disorders (McCullumsmith and Meador-Woodruff, 2002). Because EAAT1 and EAAT2 are expressed in astroglia and EAAT3 and EAAT4 are mainly located in neurons, decreased EAAT1 and EAAT2 mRNA expression suggests an astroglia-specific glutamatergic abnormality in the PFC, in contrast to the abnormalities in neuronal glutamate transporters (EAAT3 and EAAT4) in the striatum in mood disorders. Deficits in these molecules may result in increased levels of glutamate and its metabolites in the synapse. Accumulations of extracellular glutamate may not only perturb the ratio of excitatory–inhibitory neurotransmitter levels (Cryan and Kaufmann, 2005), but also potentially cause cytotoxic damage to neurons and glial cells (Petroff, 2002; Miller, 2005b).

Although the binding of [3H]dizocilpine maleate (MK-801) to NMDA receptors did not differ between suicides and controls (Holemans et al., 1993), Nowak et al. (1995, 2003) observed that the proportion of glycine displaceable [3H]CGP-39653 binding to glutamate receptors is reduced in the PFC of suicide victims, which is consistent with findings of decreased glutamate receptor subunit NR1 expression in the hippocampus and superior temporal cortex of MDD and BD patients (Law and Deakin, 2001; Scarr et al., 2003; Toro and Deakin, 2005; McCullumsmith et al., 2007). In addition, reduced transcript expression of glutamate receptor subunits GluR5, GluR1, GluR3, NR2B, NR1, and NR2A was reported in the perirhinal cortex in patients with mood disorder (Beneyto et al., 2007), while the amount of NR1 protein was found to be normal in both the LC and cerebellum in depression (Karolewicz et al., 2005).

In conclusion, the glutamatergic pathway shows alterations in mood disorder patients. In addition, glutamate is known to stimulate the HPA axis (Feldman and Weidenfeld, 1997; Herman et al., 2004). It is therefore of interest that an increasing number of reports suggest the potential antidepressant effects of anti-glutamatergic agents such as ketamine (Berman et al., 2000; Kudoh et al., 2002; Zarate et al., 2006), amantadine (Stryer et al., 2003), and riluzole (Cadic et al., 2003; Zarate et al., 2004) in depression. The glutamatergic system may thus be a promising target for the development of new antidepressants.

The GABAergic system in depression

In 1999, Sanacora et al. revealed, for the first time, using HMRS, significantly reduced GABA levels in the occipital cortex of medication-free depressed inpatients. In a follow-up study combining data from this inpatient sample with a larger sample of outpatients, GABA reductions were found to be most pronounced in patients exhibiting melancholic features (Sanacora et al., 2004). This finding is not unprecedented: in 1991, Roy et al. reported a significant reduction of CSF GABA concentrations in B3 melancholic patients. In addition, occipital levels of GABA were significantly lower in patients with a history of treatment resistance and in recovered unmedicated subjects with a history of mood disorder, than in healthy controls (Bhagwagar et al., 2007; Price et al., 2009). Reduced brain GABA content was also found in the anterior cingulate cortex and dorsal medial/dorsal anterior PFC of MDD patients (Hasler et al., 2007; Bhagwagar et al., 2008).

Diminished GABAergic neurotransmission may be explained by a diminished activity of glutamic acid dehydrogenase (GAD), the rate-limiting enzyme responsible for conversion of glutamate to GABA. In several postmortem studies of GAD mRNA in BD patients, GAD protein, the density of GAD mRNA-containing neurons, and the density of GAD terminals in the anterior cingulate, PFC, cerebellum, and hippocampus were reduced (Benes et al., 2000; Guidotti et al., 2000; Heckers et al., 2002; Woo et al., 2004; Fatemi et al., 2005). Significant reductions in cerebral and cerebellar GAD have also been found in MDD patients (Perry et al., 1977; Fatemi et al., 2005). However, increased density of GAD-expressing neurons in the orbitofrontal and entorhinal cortex has also been reported in BD and MDD patients (Biels et al., 2007). A possible explanation for this discrepancy might be that there exists a decreased transport — and, as a consequence, an accumulation of GAD — in spite of a diminished GAD production in depression, a phenomenon that has also been observed for AVP expression in the suprachiasmatic nucleus in depression (Zhou et al., 2001). In addition, higher neuronal densities of GAD were found in MDD patients, but not in BD patients, in the dorsolateral PFC, superior temporal cortex, and hippocampus (Biels et al., 2007). Moreover, a remarkable increase in the relative density in the staining of GAD-containing nerve fibers was reported in the hippocampal formation, specific for suicidal patients, while a significant decrease of GAD-staining fibers was found in the dorsolateral PFC that was specific for nonsuicidal depressed patients (Gos et al., 2009). Therefore, antidepressant use, GAD transport, and subtype of depression should be taken into consideration in future studies.
Although GABA transporters (GATs) play an important regulatory role in the function of GABAergic systems, limited data are available concerning GAT changes in the postmortem human brain. The binding of the presynaptic GABA ligand to GAT-1 was found to be unchanged in depressed suicide victims compared with controls (Sundman et al., 1997; Sundman-Eriksson and Allard, 2002). However, the selective GABA reuptake inhibitor tiagabine, which targets GAT-1, was shown to be effective in treating anxiety-related behaviors in mouse and human (Gorman, 2003). In addition, GAT-1<sup>−/−</sup> mice showed a lower level of depression and anxiety-like behaviors in comparison to wild-type mice (Liu et al., 2007). Both reports suggest that the lack of GAT-1 would affect mental status through the enhancement of the GABAergic system. Thus, medication acting at GAT-1 might provide novel approaches to modify mood disorders such as depression and anxiety.

If GABA neurotransmission is deficient in depression, any resulting adaptive change might be found in the density of GABA receptors. Elevated densities of benzodiazepine receptors, a part of GABA-A receptors, have indeed been reported in the PFC of depressed suicide victims (Cheetham et al., 1988; Pandey et al., 1997). Consistent upregulation of GABA-A-α1 and GABA-A-β3 mRNA was found in the anterior cingulate cortex of suicide victims affected with MDD or BD (Choudary et al., 2005). However, there are also studies that have not demonstrated significant alterations of GABA receptor binding between depressed suicide victims and nonpsychiatric controls. For example, GABA-A receptor binding measured with [<sup>3</sup>H]flunitrazepam was not altered in the LC (Zhu et al., 2006), the frontal cortex, temporal cortex, and hippocampus. Furthermore, no change was found for the number of GABA-B binding sites in depressed suicide victims (Cross et al., 1988; Arranz et al., 1992). In addition, in the amygdala and hippocampus, the number and affinity of benzodiazepine binding sites of suicides and controls did not differ significantly (Stocks et al., 1990). Moreover, Merali et al. (2004) reported low levels of mRNA encoding several alpha subunits of the GABA-A receptor in the frontal cortex of suicide victims. It seems that different subtypes of the GABA receptor and different types of depression and brain areas should be considered in the interpretation of the above-mentioned studies on GABA receptor changes.

In brief, the majority of studies revealed lower GABA levels in the brain of depressed patients, suggesting a hypoactive GABAergic neurotransmission. This is also of interest since GABA is known to inhibit the HPA axis (Bartanusz et al., 2004; Kovacs et al., 2004). In addition, GABAergic drugs, particularly those that enhance GABAergic functions, have proven to be highly effective in sustaining a positive response in the treatment of depression. For example, progabide and fengabine are two GABA-mimetic compounds that reached clinical trials as antidepressant agents (Sanacora and Saricicek, 2007). Tiagabine, a selective GABA reuptake inhibitor, significantly improved depression and anxiety (Carpenter et al., 2006).

THE NEUROPEPTIDE HYPOTHESIS OF DEPRESSION

CRH in depression

CRH-expressing neurons in the hypothalamic PVN are the central driving force in regulating the activity of the HPA axis. CRH mediates the stress-induced behaviors and plays a central role in the pathogenesis of depression. The number of CRH neurons, the number of CRH neurons coexpressing AVP, and the amount of CRH mRNA in the PVN are significantly increased in deceased depressed subjects with a long history of depressive disorder, independently of whether they died during a depressive state or not (Raadsheer et al., 1994a, 1995; Bao et al., 2005; Wang et al., 2008).

That hyperactive CRH neurons are involved in the etiology of depression is demonstrated not only by activation of the HPA axis, which results in hypersecretion of glucocorticoids, but also by the central CRH effects, including cardiovascular regulation, respiration, appetite control, stress-related behavior and mood, cerebral blood flow regulation, and stress-induced analgesia (Swaab, 2003). Similar symptoms, such as decreased food intake, decreased sexual activity, disturbed sleep and motor behavior, and increased anxiety, can all be induced in experimental animals by intracerebroventricular injection of CRH (Holsboer, 2001). During postnatal development of the stress system, CRH controls the activity of the HPA axis and mediates the effects of early disturbances such as maternal deprivation through the CRH receptor CRH-R1 (Schmidt et al., 2006). Both basic and clinical studies suggest that disrupting the CRH signaling through CRH-R1 can ameliorate stress-related clinical conditions. It was found in transgenic mouse models that CRH overexpression in the entire central nervous system resulted in stress-induced hypersecretion of stress hormones and increased active stress coping behavior. These changes were related to acute effects of overexpressed CRH as they were normalized by CRH-R1 antagonist treatment (Lu et al., 2008a, b). In addition, single nucleotide polymorphisms (SNPs) in the CRH-R1 gene were found to be associated with increased susceptibility to MDD, indicating a possible primary role for CRH-R1 in some cases of depression (Liu et al., 2006b). It is also of interest to notice that mice lacking the CRH gene exhibit normal stress-induced behavior that is specifically blocked by a CRH-R1 antagonist. Another mammalian ligand for CRH receptors is
urocortin. However, in mice urocortin mRNA is absent from regions known to mediate stress-related behaviors. Consequently an unidentified alternative CRH-like molecule other than CRH or urocortin was proposed to act through the CRH receptors in brain regions to mediate stress-induced behaviors, either alone or in concert with CRH (Weninger et al., 1999). This possibility remains to be investigated.

GR mRNA concentration and glucocorticoid-binding activity were increased in brain tissues of animals chronically treated with antidepressants. The time course of the antidepressant effects on GRs coincides with their long-term actions on the HPA system activity and follows closely that of clinical improvement of depression (Barden, 1996). The clinical observation that patients with treatment-resistant depression noticed a significant improvement in mood after receiving dexamethasone while remaining on their antidepressant (sertraline or fluoxetine) further supports the concept that hyperactive CRH neurons play a causal role in the symptomatology of depression (Dinan et al., 1997). Moreover, the CRH concentrations in CSF in healthy volunteers and depressed patients decrease due to prolonged treatment with antidepressant drugs (Heuser et al., 1998), although it should be noted that CSF CRH is also derived from other brain areas, such as the thalamus (Hsu et al., 2001; Bao et al., 2005).

Lastly, in depressed patients significantly increased CRH mRNA levels in the PVN were found to be accompanied by a significantly increased expression of genes involved in the activation of CRH neurons, such as CRH-R1, MR, estrogen receptor-alpha (ERα), and AVPR1a, and with a significantly decreased expression of genes involved in the inhibition of CRH neurons, such as the androgen receptor (AR)-mRNA (Wang et al., 2008). These findings, obtained by laser microdissection of the PVN (Fig. 8.4), further raise the possibility that a disturbed receptor balance in the PVN may contribute to activation of the HPA axis in depression. Together, the arguments mentioned above have led to the CRH hypothesis of depression: hyperactivity of CRH neurons, and thus of the HPA axis, is of crucial importance for induction of the symptoms of depression, at least in a subgroup of patients. Some CRH neurons project to brain areas other than the median eminence. Centrally

![Fig. 8.4. Sections for laser microdissection (LMD). Sections of the paraventricular nucleus (PVN: A–C) and supraoptic nucleus (SON: D–F) on the right side of the hypothalamus as seen under the PALM laser dissection microscope. A thionin-stained section of the PVN for orientation is shown in panel A. An unstained section adjacent to panel A is represented in panel B before LMD, in which the PVN area is outlined under the microscope. Section B is represented in panel C after laser dissection. The sections of the right SON under the PALM laser dissection microscope with the same LMD procedure as for the PVN are represented in panels D–F. Bar = 300 µm. The arrows show the orientation: V, ventral; D, dorsal; M, medial; L, lateral; OT, optic tract; III, third ventricle. (Reproduced from Wang et al., 2008, with permission.)](image-url)
released CRH may be crucial to the symptoms of depression, since intracerebroventricular injection of CRH induces symptoms of depression (Holsboer, 2001). This also means that, even in cases when no enhanced activity of the HPA axis is shown in the periphery by hormone assays, the centrally projecting CRH neurons might be hyperactive and contribute to the symptoms in depressed patients. Therefore, more attention should thus be paid to CSF levels of CRH in such cases.

**Sex difference in depression: relationship with HPA axis activity**

The sex difference found in depression might provide additional support for the CRH hypothesis of depression. Since MDD is twice as prevalent in women of reproductive age as in men, organizing and/or activating effects of sex hormones on the HPA axis are proposed as risk factors for depression. The possible importance of fluctuating levels of sex hormones as a risk factor for depression is underlined by the higher prevalence of premenstrual depression, antepartum or postpartum depression, and depression during the transition to menopause (Bao et al., 2004, 2008). Studies in rodents have found that the female brain’s innate strategy to handle stress differs from that of the male brain (Ter Horst et al., 2009). In addition, organizing effects of estrogen-like compounds during fetal life may also be responsible for a higher prevalence of mood disorders, as appeared in children exposed in utero to diethylstilbestrol (Meyer-Bahlburg and Ehrhardt, 1987). Furthermore, age-related sex differences have been found in CRH-expressing neurons in the human hypothalamic PVN, which illustrates a relationship between sex hormones and CRH. First, from the age of 24 years onward, men had significantly more CRH-expressing neurons than women, while in the second place, there was a significant age-related increase of CRH-expressing neurons in men, but not in women (Fig. 8.5). It is of interest to note here that AVP neurons in the hypothalamic SON, which is the major source for circulating AVP, showed the opposite pattern. These neurons were only activated during the course of aging in women, and not in men (Ishunina et al., 1999). Moreover, an abnormal hormone status, induced by castration, ovariec-tomy or a sex hormone-producing tumor, was accompanied by changes in the number of CRH-expressing neurons (Bao and Swaab, 2007).

It should be noted that sex hormones can also be synthesized locally in the brain as neurosteroids, which may interact with circulating sex hormones by as yet unknown mechanisms. Sex hormones act by binding to sex hormone receptors that are expressed in CRH neurons of the hypothalamus and in corticotropes of the adenohypophysis (Stefaneanu et al., 1994; Pereira-Lima et al., 2004; Bao et al., 2005, 2006; Scheithauer et al., 2008). We have found colocalization of CRH and sex hormone receptors, indicating a direct effect of sex hormones on CRH neurons. Both nuclear ERα (Fig. 8.6) (Bao et al., 2005) and nuclear AR (Fig. 8.7) (Bao et al., 2006) were present in CRH-expressing neurons in the human hypothalamic PVN. In addition, there was a significantly positive correlation between the increased number of CRH-expressing neurons containing nuclear ERα and the increased number of CRH neurons in mood disorders, in both males and females (Bao et al., 2005). The human CRH gene promoter contains five perfect, half-palindromic estrogen-responsive elements (Vamvakopoulos and Chrousos, 1993), and animal studies have shown that estrogens stimulate CRH expression (Lund et al., 2004). We have also identified an androgen-responsive element in the CRH gene promoter region, but that appeared to initiate a repressing effect of AR on CRH transcription (Bao et al., 2006), which is in agreement with an animal study showing that androgens inhibit CRH production (Lund et al., 2004).
Thus, human postmortem brain, animal, and cell line studies confirm a key stimulating role of estrogens on CRH production while androgens diminish CRH production. This opposite effect of estrogens and androgens on CRH neurons may be the basis for the sex difference in the prevalence of depression.

**AVP and depression**

Even after decades of intense research, new data are still emerging in relation to the classic antidiuretic nonapeptide, AVP. Besides their role in the regulation of osmolality, blood pressure, temperature, and corticosteroid secretion, the central vasopressinergic fibers are also involved in the stress response, cognition, paternal behavior and social attachment, aggression, and emotionality. Intranasal AVP differentially affects social communication in men and women (Thompson et al., 2006); polymorphisms in the AVP receptor subunit AVPR1a were found to be related to the autism spectrum (Yirmiya et al., 2006) and to pair-bonding behavior in men and thus in the evolutionary development of the social brain (Walum et al., 2008).
behaviors, while AVP deficit, in turn, may cause signs of diabetes insipidus, hypoanxiety (Inder et al., 1997; Landgraf et al., 2007; Mlynarik et al., 2007), and disturbed rhythmicity (Zhou et al., 2001).

**CHRONIC STRESS, AVP, AND DEPRESSION**

As mentioned above, a multiple imbalance of receptor genes involved in the regulation of the HPA axis activity has been proposed in depression (Wang et al., 2008), in which the increased AVPR1a suggests a role of enhanced somatodendritic AVP release in the PVN (Surget and Belzung, 2008). Increased co-storage of CRH and AVP in the CRH terminals was found after immobilization or social defeat stressors (de Goede et al., 1991; Bartanusz et al., 1993; Keeney et al., 2006). When released together into the portal capillaries, AVP strongly potentiates the ACTH-releasing activity of CRH (Gillies et al., 1982; Rivier and Vale, 1983). In addition, circulating AVP from the SON may induce ACTH release from the pituitary (Gispen-de Wied et al., 1992). Transient activation of the HPA axis following a single exposure to a stressor may induce delayed and long-lasting hyperproduction, hyperstorage, and hypersecretion of AVP, e.g., from hypothalamic CRH neurons, which result in hyperresponsiveness of the HPA axis to subsequent stimuli (Schmidt et al., 1995, 1996). The sensitization of neuronal processes is proposed to be an important feature in promoting depressive-like states. It was suggested that with each stressor experience and with each successive episode of depression, neuronal sensitization becomes more pronounced, and hence the stressor severity necessary to elicit the neurochemical changes (and thus to induce a depressive episode) becomes progressively smaller (Post, 1992). This is in line with clinical observations that the relation between life events and recurrences of depressive episodes is less clear in those patients who have highly recurrent MDD. It is also important to note that, whereas in acute stress CRH is the main cause of increased ACTH release, animal experiments show that in chronic stress there is a switch from CRH to AVP stimulation of ACTH release (Scott and Dinan, 1998). Since depression is a chronic disorder, the AVP-driven HPA axis hyperactivity in depression is receiving more and more attention (Meynen et al., 2006). In case of chronic depression, AVP is proposed to be persistently increased within CRH neurons, so that even minor day-to-day annoyances might trigger excessive CRH/AVP release. This, in turn, would favor the presentation of dysphoric symptoms, and might even be a factor responsible for triggering MDD and dysthymia (Griffiths et al., 2000). The number of CRH and AVP-colocalizing neurons is indeed increased in the human PVN in depression and during the

**DIFFERENT AVP SYSTEMS**

There are at least four different vasopressinergic systems intimately involved in the signs and symptoms of depression (for reviews, see Swaab, 2003, 2004). First, AVP is produced as a neurohormone by the large magnocellular neurons of the hypothalamic SON and PVN, whose axons run to the neurohypophysis where they release AVP and OXT into the general circulation. Circulating AVP has an influence on the anterior pituitary and high circulating levels also affect mood. In the second place, small parvocellular neurons of the PVN secrete CRH and AVP also as neurohormones from their axons in the median eminence into the portal capillaries that transport them to the anterior lobe of the pituitary. AVP strongly potentiates ACTH-releasing activity (Engelmann et al., 2004). Third, additional vasopressinergic fibers are found to project from the hypothalamus to subregions of the hippocampus, septum, amygdala, and brainstem areas, where AVP serves as a neurotransmitter/neuromodulator via AVPR1a and AVPR1b receptors that are widely distributed (Surget and Belzung, 2008). Moreover, particularly the magnocellular hypothalamic neurons release AVP from their dendrites and somata, subsequently diffusing through the brain's extracellular fluid to act as neuromodulators on receptors at some distance from their site of release (Ludwig et al., 2005). Fourth, AVP is also released into the brain with a circadian rhythm by neurons of the SCN, which show significant changes in depression. Once overexpressed and overreleased, AVP may contribute to hyperanxiety and depression-like

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**Fig. 8.7.** Frontal section of the paraventricular nucleus in subject (00182) stained for corticotropin-releasing hormone (CRH) (red) and androgen receptor (AR) (blue). III, the third ventricle. The upper-right corner represents a higher magnification of the framed field and shows cytoplasmic CRH (red) - AR (blue) nuclear double-staining neurons. The arrow points to AR single-staining cells. Bar in the upper-right corner = 16 μm; in the lower right corner = 100 μm. (Reproduced from Bao et al., 2006, with permission.)
course of aging, at least in males (Raadsheer et al., 1993; 1994b; Bao and Swaab, 2007).

CRH and AVP mediate ACTH release via different second-messenger systems. CRH activates G protein-linked adenylate cyclase, leading to cAMP formation and protein kinase A activation. AVP works through a specific AVP receptor subtype termed AVPR1b, which is strongly expressed by pituitary corticotropes and activates only phospholipase C. The AVPR1b receptor is required for a normal pituitary and adrenal response to acute stressful stimuli and is necessary for a normal ACTH response during chronic stress (Lolait et al., 2007). AVPR1b receptor mRNA levels and coupling of the receptor to phospholipase C are stimulated by glucocorticoids, while AVP facilitates corticotrope responsiveness in spite of elevated levels of plasma glucocorticoids during chronic stress or depression (Rabadjan-Diehl and Aguilara, 1998). In this respect it is interesting that following a challenge with desmopressin (an analog of vasopressin that stimulates the AVPR1b receptor), the ACTH and cortisol responses were appreciably greater in MDD patients than in controls, suggesting that the AVPR1b receptor was more reactive in MDD patients than in controls, supporting the possibility of a direct involvement of AVP in the pathogenesis of depression, at least in some subjects. A recent study on AVP-deficient Brattleboro rats has confirmed that AVP can be involved in the development of depression-like behavior, in particular of the passive coping style and anhedonia. In addition, behavioral and endocrine responses were found to be dissociated, which suggested that brain vasopressinergic circuits that are distinct from those regulating the HPA axis are involved in generating depression-like behavior (Mlynarik et al., 2007). The identification of polymorphisms in AVP-related genes underlying a special subtype of depression may help to characterize potential targets for therapeutic interventions (Frank and Landgraf, 2008).

Feedback of corticosteroids takes place on the PVN, SON, and SCN. Following different types of corticosteroid treatment in different disorders or during the presence of high levels of endogenous corticosteroids produced by a tumor, we found in postmortem tissue not only that CRH-expressing neurons are strongly downregulated, but also that AVP expression in the SON and PVN is strongly decreased. On the other hand, OXT neurons were not affected. Therefore, in the human brain, the negative feedback of corticosteroids is acting selectively on CRH cells and cells that (co-)express AVP but not on OXT cells. The glucocorticoid-induced suppression of AVP synthesis has been proposed to occur at the posttranscriptional level (Erkut et al., 1998, 2002). In the human SCN we found a diminished AVP mRNA following administration of corticosteroids (Liu et al., 2006a), which may be one explanation for the disturbed circadian rhythms in depression.

**AVP in the SON and PVN in Depression**

In the PVN of MDD patients, the number of AVP- and OXT-expressing neurons is increased (Purba et al., 1996). Using radioactive in situ hybridization, our group determined the amount of AVP mRNA in the PVN and SON in formalin-fixed, paraffin-embedded archival postmortem brain tissues of depressed subjects and their controls (Fig. 8.8). In the SON, a 60% increase of AVP mRNA expression was found in depressed subjects (Fig. 8.9a) but AVP mRNA expression was significantly increased in both the SON and the PVN only in the melancholic subgroup (Fig. 8.9b) (Meynen et al., 2006). Enhanced AVP mRNA production in the SON of depressed patients (Meynen et al., 2006) leads to increased plasma levels of AVP (van Londen et al., 1997, 1998b, 2001) that are also reported to be related to an enhanced suicide risk in depression (Inder et al., 1997), as well as to an anxious-retarded type of depression (de Winter et al., 2003; Goeke et al., 2006). It has also been proposed that AVP may be important not only in mediating psychomotor retardation but also in affecting memory processes in depressed patients, possibly by altering arousal and attention (van Londen et al., 1998a, b). The possibility that circulating AVP may also induce

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**Fig. 8.8.** Radioactive *in situ* hybridization vasopressin mRNA signal on film of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of a representative patient. Note the intense signal in both nuclei. III, Third ventricle. (Reproduced from Meynen et al., 2006, with permission.)
symptoms of depression is supported by the case history of a man displaying chronically elevated plasma AVP levels due to AVP secretion by an olfactory neuroblastoma, which induced his first episode of MDD. Depressive symptoms improved markedly after surgical resection of the tumor and subsequent normalization of plasma AVP levels. In this patient the HPA axis was suppressed (Müller et al., 2000), indicating a primary role of AVP in the pathogenesis of depression.

In addition, in depression and suicide, alterations in centrally released AVP from the PVN may play a role, since immunoreactive AVP was elevated in the dorsomedial PFC and reduced in the dorsal vagal complex (Merati et al., 2006).

**AVP in the Circadian System in Depression**

The hypothalamic SCN, the biological clock, is of interest in relation to depression for various reasons. Light is directly influencing the activity of the SCN, and light therapy is effective in depression. In addition, seasonal affective disorder (SAD) is more prevalent in the northern states of the USA than in southern states (Miller, 2005a). Moreover, the symptoms of depression fluctuate over the day, and also the stress response is strongly influenced by the time of day via the SCN. The human SCN shows not only circadian but also circannual variations in neuronal activity (Hofman and Swaab, 1992, 1993), which are supposed to be related to circadian and circannual fluctuations in mood and to sleeping disturbances in depression (van Londen et al., 2001). Polymorphisms in the clock genes are associated with dysfunctional circadian rhythm and susceptibility for mood disorders, in particular in SAD and BD (Johansson et al., 2003; Shi et al., 2008; Kishi et al., 2009), indicating that the SCN may also play a causal role in depression at least in a subgroup of patients.

A disorder of SCN function, characterized by an increased number of AVP-expressing neurons, a decreased amount of AVP mRNA in this nucleus, and diminished circadian fluctuation of AVP mRNA, has been found in the postmortem brain of depressed patients (Fig. 8.10) (Zhou et al., 2001). Decreased activity of the SCN in depression is presumed to be at least partly due to the increased circulating plasma cortisol levels, since corticosteroids were found to inhibit SCN function (Liu et al., 2006a). Light therapy is effective not only in SAD but also for MDD, while as an adjuvant to conventional antidepressants in unipolar patients, or lithium in bipolar patients, morning light hastens and potentiates the antidepressant response (Wirz-Justice et al., 2005). This poses the question how light therapy may work. Animal data have shown that AVP neurons of the SCN exert an inhibitory influence on CRH neurons in the PVN (Kalsbeek et al., 1992). Depressed patients have a deficient SCN (Zhou et al., 2001), which may subsequently fail to inhibit sufficiently the CRH neurons in the PVN of depressed patients. Such an impaired negative-feedback mechanism may lead to a further increase in the activity of the HPA axis in depression. Both the resulting higher CRH and cortisol levels may contribute to the symptoms of depression. Light therapy may activate the SCN, directly inducing an increased synthesis and release of AVP that will inhibit the CRH neurons (see Fig. 8.1). However, it should be noted that human beings are diurnal creatures and might hold a different mode of interaction between SCN AVP neurons and the PVN CRH neurons to that of the nocturnal
rat, especially because of the fact that opposite actions of hypothalamic AVP have been observed on the circadian corticosterone rhythm in nocturnal versus diurnal species (Kalsbeck et al., 2008). The exact mechanism of the action of light in depressed patients thus deserves further study.

DEPRESSION IN ALZHEIMER'S DISEASE

There is a very high prevalence of depression in AD, affecting up to 50% of patients. We observed a positive relationship between the depressive state in Alzheimer patients and the presence of neuritic plaques in the neocortex (Meynen et al., 2009), which may be an explanation for the high prevalence of mood disorders in AD. The question is whether the same neurobiological systems are involved in depressed AD patients as in MDD patients. Indeed, AD is accompanied by an activated HPA axis. CRH mRNA levels in the PVN of AD patients were markedly higher than those of comparison subjects, which may make them at risk for depressed mood, as they are lower than in depressed patients (Fig. 8.11) (Raadsheer et al., 1995). In the group of AD patients the mean postmortem CSF total cortisol level was 83% higher than that in the controls, especially in presenile AD patients (<65 years) (Swaab et al., 1994). CRH neurons in the PVN of AD patients showed, however, similar age-dependent increases in AVP colocalization to those of the control subjects (Raadsheer et al., 1994b).

We found a positive correlation between the Cornell Scale for Depression in Dementia and the number of CRH neurons in the PVN, suggesting that MDD and depression in AD share their pathogenesis, at least partly (Fig. 8.12) (Meynen et al., 2007b). The SCN is also clearly affected in AD (Swaab et al., 1985). A diminished AVP mRNA content was already present in the SCN from the very first preclinical AD stages onward, explaining the disruption of circadian rhythms and nightly restlessness in AD (Wu et al., 2006). However, no difference was found between the AVP mRNA levels in the SCN of depressed versus non-depressed AD patients (Liu et al., 2000). Our long-term, double-blind, placebo-controlled trial showed, however, that a whole day of bright (±1000 lux) versus dim (±300 lux) light not only improved circadian rhythmicity and attenuated cognitive deterioration on the Mini-Mental State Examination Scale, but also alleviated depressive symptoms on the Cornell Scale for Depression in Dementia (Riemersma-van der Lek et al., 2008).

There are, however, also differences between MDD and depression in AD. Although CSF cortisol levels in AD patients were found to be more than twice those of controls, no significant difference was found between depressed and nondepressed AD patients (Hoogendijk et al., 2006). Moreover, AD patients did not differ from controls with respect to the amount of AVP or OXT mRNA in the PVN or SON (Meynen et al., 2009), which indicates that there are also differences in neurobiological systems in depressed AD patients as compared to the depressive disorders.

In conclusion, there are both similarities and differences between neurobiological systems that are involved in depressed AD patients and in MDD patients.
CONCLUSIONS

Many macro- and microscopic differences have been reported in mood disorders. The brain differences described are sometimes especially localized in gray and sometimes in white matter; they are often lateralized, and are dependent on brain region and cell type, on the type of mood disorders, and on the presence of a familiar or a sporadic disorder. The reported structural alterations are, however, not specific for mood disorders, and can not be used to confirm the clinical diagnosis neuropathologically. However, they support the idea that these disorders have a neurodevelopmental underpinning.

This chapter further focused on the molecular neuropathology of mood disorders and elucidated the role of the HPA axis, monoamines, glutamate and GABA, neuropeptides and their interplay in the pathogenesis of mood disorders. Besides alterations in these factors, the development of mood disorders is linked with sex, age, developmental history, and environmental stressors. Developmental risk factors include genetic background, placental function, smoking and alcohol use, and the medicines taken by the pregnant mother, while environmental factors during development are the availability of food, chemicals, and child neglect and abuse. All these factors make different parts of the stress-related brain system more vulnerable to react to stressful life events or other psychological stresses, causing alterations in the network of neurotransmitters such as monoamines and amino acids, and neuromodulators such as CRH, AVP, OXT, and orexin, which finally make individuals in different ways at risk for depressive disorders. There are differences in the alterations in the neurotransmitters and neuropeptides in mood disorders according to brain area, type of depression, and the presence or absence of suicidal ideation or suicide. Furthermore, there are both similarities and differences in neurobiological systems that are involved in depressed patients with AD, compared to other patients with depression. Based on the molecular neuropathological data reviewed, the HPA axis and AVP seem to have a central position in the development of depressive symptoms.

There are many genetic polymorphisms and individual developmental differences giving rise to functional changes in the network of neurotransmitters and...
neuropeptides in depression. One may hope that in future these data will allow a better prediction for the vulnerable neurobiological system in an individual depressed patient, and that this will lead to an optimal tailor-made antidepressive therapy.

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REFERENCES


Dempster EL, Burcescu I, Wigg K et al. (2007). Evidence of an association between the vasopressin V1b receptor gene (AVPR1B) and childhood-onset mood disorders. Arch Gen Psychiatry 64: 1189–1195.


Dinan TG, O’Brien S, Lavelle E et al. (2004). Further neuroendocrine evidence of enhanced vasopressin V3 receptor


Erkut ZA, Gabreels BA, Eijkelenboom J et al. (2002). Glucocorticoid treatment is associated with decreased expression of processed AVP but not of proAVP, neurophysin or oxytocin in the human hypothalamus: are PC1 and PC2 involved? Neuro Endocrinol Lett 23: 33–44.


Fatemi SH, Stacy JM, Earle JA et al. (2005). GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 72: 109–122.


Guidotti A, Auta J, Davis JM et al. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 57: 1061–1069.


Kovacs KJ, Miklos IH, Bali B (2004). GABAergic mechanisms constraining the activity of the hypothalamo-


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Singh VB, Corley KC, Phan TH et al. (1990). Increases in the activity of tryptophan hydroxylase from rat cortex and midbrain in response to acute or repeated sound stress are blocked by adrenalectomy and restored by dexamethasone treatment. Brain Res 516: 66–76.


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