GENERAL INTRODUCTION

“Knowledge is of two kinds. We know a subject ourselves, or we know where we can find information upon it.”

Samuel Johnson
STRESS

Stress is one of the buzzwords of modern life, used widely to describe a variety of situations, events and emotions (Charlton, 1992). The heterogeneous nature of stress is neatly illustrated in the excerpt from Hans Selye’s book “Stress in Health and Disease” (Selye, 1976a):

“Stress is part of our daily human experience, but it is associated with a great variety of essentially dissimilar problems, such as surgical trauma, burns, emotional arousal, mental or physical effort, fatigue, pain, fear, the need for concentration, the humiliation or frustration, the loss of blood, intoxication with drugs or environmental pollutants, or even with the kind of unexpected success that requires an individual to reformulate his lifestyle. Stress is present in the businessman under constant pressure; in the athlete straining to win a race; in the air-traffic controller who bears continuous responsibility for hundreds of lives; in the husband helplessly watching his wife’s slow, painful death from cancer; in a race horse, its jockey and the spectator who bets on them.”

Hans Selye defined stress as the non-specific response of the body to any demand. Furthermore, he regarded it as an alarm process that warns about disrupted homeostasis and helps to restore it, by inducing the “fight or flight” response (Cannon, 1929; Selye, 1936, 1956). As such, stress accounts for nearly everything disturbing the daily routine of an individual and challenging to adapt to changes (Selye, 1936). Accordingly, the stress response helps in readjusting to a new situation, by inducing multiple changes collectively named as the general adaptation syndrome (GAS). While the initial stages of the GAS are beneficial, severe and prolonged stress may lead to the stage of exhaustion, in which the acquired adaptation is lost (Selye, 1955). Currently, in biomedical sciences and clinical practice, the term stress is mainly used to describe this latter situation, when the stress response becomes maladaptive and may predispose to pathology. In this thesis, I will mainly focus on chronic stress-induced changes in the brain that may lead to psychopathology.

THE STRESS RESPONSE

Stress involves two-way communication between the brain and periphery via neural and endocrine mechanisms. Two main physiological pathways are activated in response to stress: the autonomic nervous system (ANS), particularly its sympa-
adrenomedullary division, and the hypothalamic-pituitary-adrenal (HPA) axis. Both of these systems are involved in the physiological support of behavior and respond rapidly to stressors.

Activation of the sympatho-adrenomedullary system triggers noradrenergic projections from locus coerulceus in the brain and releases noradrenaline from the sympathetic nerves and adrenaline from the adrenal medulla. Adrenaline and noradrenaline stimulate the α- and β-adrenergic receptors in the heart muscle and blood vessel walls, thereby increasing heart rate and blood pressure. This, in turn, leads to a higher blood flow to the brain and muscles, allowing a fast performance of a “fight or flight” reaction.

This immediate autonomic response is followed by delayed neuroendocrine changes, due to activation of the HPA axis (Tsigos and Chrousos, 2002). Stress results in the production of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) in the paraventricular nucleus (PVN) of the hypothalamus. Upon stimulation by a stressor, CRH and AVP are released into the hypophyseal portal system and act synergistically via specific receptors (CRH-R1 and V1B receptor, respectively) in the anterior pituitary to trigger the synthesis of adrenocorticotropic hormone (ACTH) from its precursor proopiomelanocortin (POMC). Secreted ACTH is transported by the blood stream to the adrenal glands, where it acts on the adrenal cortex via type 2 melanocortin receptors (MC2R, ACTH receptors) and induces the production of glucocorticoids (cortisol in humans and corticosterone in rodents, hereafter abbreviated as CORT). Glucocorticoids, released into the systemic circulation, promote many adaptive processes by stimulating energy mobilization, gluconeogenesis, lipolysis, and by suppressing growth, reproduction and inflammation. Such a broad variety of glucocorticoid-induced effects is due to the widespread expression of receptors for CORT throughout the body’s cells (Buckingham, 2006). Approximately 90% of CORT in the circulation is bound to a carrier protein, corticosteroid-binding globulin, and only unbound CORT readily crosses the blood-brain barrier and cell membranes. An important target organ of glucocorticoids is the brain, endowed with mineralocorticoid and glucocorticoid receptors (MRs and GRs, respectively), which have a widespread distribution and are co-expressed abundantly in the neurons of limbic structures, e.g. the hippocampus (Reul and de Kloet, 1985; Herman et al., 2003; de Kloet et al., 2005). These two receptor types differ in affinity for glucocorticoids, and thereby, in their functional role. The higher affinity MRs are predominantly occupied at lower, basal glucocorticoid levels, and are implicated in the onset of stress responses. The lower affinity GRs are activated by large amounts of glucocorticoids, and play a key role in
the termination of stress reactions, facilitation of recovery, and formation of memory related to stressful events (de Kloet et al., 2005).

Glucocorticoids, the final effectors of the HPA axis, are also responsible for neuroendocrine regulation of this system. By exerting negative feedback at several sites of the HPA axis, glucocorticoids suppress their own release, and thereby terminate the stress response. Feedback inhibition is principally mediated via the GRs. At the pituitary level, glucocorticoids suppress POMC gene transcription, and also interact with the CRH-R1 receptors, acutely inhibiting the binding of CRH to its receptors and chronically decreasing CRH-R1 numbers. At the hypothalamic level, glucocorticoids suppress CRH and AVP mRNA synthesis and their secretion. Acute and relatively rapid inhibition of CRH release is followed by chronic downregulation of CRH and AVP expression in the PVN neurons (Keller-Wood and Dallman, 1984; Papadimitriou and Priftis, 2009). Glucocorticoid negative feedback on the HPA axis is also exerted centrally, at the level of the hippocampus, amygdala and prefrontal cortex. Although these limbic structures are suggested to connect to the PVN only indirectly (Hurley et al., 1991; Herman et al., 1996; Floyd et al., 2001; Fernandes et al., 2007), a number of structural and functional studies provide evidence for their role in regulation of the HPA axis (Dedovic et al., 2009). The hippocampus exerts an inhibitory influence on hypothalamic CRH-containing neurons via a polysynaptic circuit, however, the amygdala is suggested to exert a direct excitatory influence. Other important inputs to the HPA axis structures come from ascending monoaminergic pathways. In addition, HPA axis regulation is influenced by numerous other hormones, neurotransmitters, cytokines, and growth factors.

THE STRESSED BRAIN

Since this thesis largely focuses on stress-related changes in the brain that may predispose to ill health, a brief overview of brain areas involved in the stress response will be presented.

Within the brain, a distributed neural circuitry determines what is threatening and thus potentially stressful to the individual, as well as regulates the physiological and behavioral responses which can be either adaptive or damaging (McEwen, 2007; McEwen and Gianaros, 2010). This circuitry includes three limbic system areas in particular: the hippocampus, amygdala and prefrontal cortex (PFC).

The hippocampus was the first brain region, besides the hypothalamus, to be recognized as a target of stress and glucocorticoids. It plays important roles in learning and memory, processing the contextual aspects of emotional events, and
regulating visceral functions, including the HPA axis. The hippocampus is enriched with receptors for glucocorticoids and other stress mediators that can enhance cognitive processes, affect mood and motivation, and promote excitability and neuroprotection. Yet, these same molecules can also have deleterious effects on the hippocampus under conditions of prolonged stress (McEwen, 2007). Both animal models and human studies reveal that stress and its related pathologies are associated with structural and functional changes in the hippocampal circuitry. The specific alterations will be discussed in the further sections of this thesis.

The hippocampus is closely linked to the amygdala, both anatomically and functionally (Pitkanen et al., 2000; Petrovich et al., 2001). Also, these limbic brain structures interconnect with vegetative brain areas, such as the hypothalamus and brainstem, and higher cortical areas, particularly with the PFC.

The amygdala is comprised of distinct nuclei that play important roles in detecting and responding to threats in the environment, as well as in formation of emotional memories (LeDoux, 2000; Davis and Whalen, 2001; Sah et al., 2003). In brief, sensory inputs are relayed through brainstem, thalamic and cortical-thalamic pathways to the basolateral complex of amygdalar nuclei: the lateral nucleus (LA), the basal or basolateral nucleus (BLA), and the accessory basal or basomedial nucleus. From there, sensory signals are relayed to the central nucleus of amygdala (CeA), which serves as a primary output system. The CeA has substantial projections to the hypothalamus, bed nucleus of stria terminalis (BNST), and several nuclei in the brainstem that control the autonomic system and enable the stress response. Also, both CeA and BNST project to ascending monoaminergic and cholinergic neuron groups, including noradrenergic locus coeruleus, the dopaminergic substantia nigra and ventral tegmental area, the serotonergic raphe, and the cholinergic nucleus basalis (Davis and Whalen, 2001). Importantly, the basolateral complex of the amygdala is also connected to (sub)cortical areas involved in processing of stress-related information, the hippocampus and PFC. Hence, the amygdala is in a good position to process sensory inputs, associate them to past experiences, and form memories of emotionally laden events (Sah et al., 2003).

The PFC is involved in higher cognitive functions. One of them is the top-down regulation of stress-related responding and coping processes mediated by subcortical limbic areas, including the amygdala, hippocampus and hypothalamus (McEwen and Gianaros, 2010). The PFC is organized in a topographical manner, such that regions regulating emotions are situated ventrally and medially, while regions regulating thought and action are located more dorsally and laterally (Arnsten, 2009). Thus, the emotional and stress responses are mainly modulated by the medial PFC (mPFC), and its ventral regions in particular. Yet, all the PFC regions interconnect to
orchestrate the brain’s activity for regulation of behavior, thought and emotion. The mPFC has extensive connections with subcortical structures, involved in the stress response. Under conditions of stress, the activation of the amygdala and other subcortical structures eventually leads to impaired prefrontal regulation. Animal studies on the PFC reveal stress-induced changes in neuronal structure and connectivity. For instance, the mPFC shows reduced neuronal complexity and loss of synaptic connections as a result of chronic stress (Radley et al., 2006; Holmes and Wellman, 2009). Also, repeated stress has been shown to disrupt the plastic relationship between the PFC and the hippocampus that is needed for flexible memory consolidation (Cerqueira et al., 2007).

In summary, the limbic brain system exerts diverse emotional, cognitive and mnemonic functions, involved in adaptive regulation of the stress response. These same brain regions, including the hippocampus, amygdala and PFC, show distinct changes when adaptation to stress fails.

**ALLOSTASIS AND ALLOSTATIC LOAD**

The adaptive processes that underlie the stress response have been collectively termed as allostasis (McEwen, 2003). If the stress response is inappropriate, excessive or prolonged, the costs of adaptation may become too high, leading to a condition of allostatic load. The concept of allostatic load refers to cumulative changes that result in a “wear and tear” of the body and brain (McEwen, 2003). The inability to adequately cope with stressful events, thus, may promote maladaptation and predispose to various stress-related pathologies. It is important to keep in mind, however, that since all the facets of the stress response show tremendous individual variability, only a part of the subjects (either laboratory animals or humans) exposed to stress will eventually succumb to stress-related pathology. Hence, individual vulnerability plays an important role here, and it is shaped by multiple genetic and environmental factors.
STRESS-RELATED PSYCHOPATHOLOGY

Many health problems are thought to arise, at least in part, from exposure to prolonged stress and inability to adequately cope with it. Among these stress-influenced conditions are cardiovascular diseases, diverse endocrine and metabolic disorders, and psychopathologies. The latter include, but are not limited to, depression, post-traumatic stress disorder, and burnout. In this thesis, I will mainly refer to depression, when translating preclinical research findings regarding chronic stress- and antidepressant treatment-induced changes in the brain.

DEPRESSION

Depression, also called major depression, major depressive disorder or clinical depression, is a heterogeneous disorder with a highly variable course, an inconsistent response to treatment, and no established mechanism (Belmaker and Agam, 2008). The symptoms of depression encompass mood disturbances, characterized by sadness or irritability, as well as a variety of cognitive, motoric, autonomic, endocrine, and sleep/wake abnormalities, thought to arise from the complex interaction of multiple genetic and environmental factors (Manji et al., 2001). Diagnosis of depression is based on symptomatic criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR, 2000), summarized in table 1.1. Because of a highly variable set of symptoms, depression is often viewed as a heterogeneous syndrome comprised of numerous diseases of distinct causes and pathophysiologies (Nestler et al., 2002a).

Research attempts to elucidate the mechanisms responsible for development and treatment of depression have yielded valuable insight, but its puzzling nature persists. Diverse theories have been postulated in search for the cause of depression. The most prominent hypotheses of depression are briefly reviewed in the following text, along with various predisposing factors, such as genetic and environmental changes (figure 1.1).
Table 1.1 The diagnostic criteria for major depression.

**MAJOR DEPRESSION**

**Symptoms:**
- Depressed or irritable mood
- Diminished interest in pleasurable activities (anhedonia)
- Weight loss or weight gain / decreased or increased appetite
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feeling of hopelessness, worthlessness or guilt
- Diminished ability to concentrate or think / indecisiveness
- Recurrent thoughts of death or suicide

**Diagnosis:**
Depressive episode is diagnosed when five or more of the above listed symptoms have been present during a 2-week period of time, and when they disrupt normal social and occupational functioning. Presence of either depressed mood or anhedonia is mandatory for diagnosis of depression (DSM-IV-TR, 2000).

**Genetics of depression**

The evidence for genetic influences on depression consists primarily of twin studies, where monozygotic twins show higher prevalence for depression than dizygotic twins (Uher, 2008). Epidemiological studies suggest that major depressive disorder is moderately heritable, about 40% in women and 30% in men (Sullivan et al., 2000; Kendler et al., 2006). Yet, no specific gene or a series of genes that induce depression have been reliably identified to date. Rather, certain variations in genes, called polymorphisms, may increase risk for depression (aan het Rot et al., 2009). However, most genes in quest are not depressive disorder-specific, but overlap with other psychiatric conditions and drug abuse.

A common functional length polymorphism in the serotonin transporter gene (5-HTTLPR) has been suggested to predispose to depression as well as to personality traits of anxiety and pessimism (Lesch et al., 1996; Belmaker and Agam, 2008). Yet, it turned out that this ‘short allele’ 5-HTTLPR polymorphism predicted depression
only in association with stressful life events (Caspi et al., 2003). Likewise, other depression-associated gene polymorphisms have been shown to interact with environmental factors. A single nucleotide polymorphism (SNP) in the gene encoding brain-derived neurotrophic factor (BDNF), Val66Met, has been shown to interact with the 5-HTTLPR short allele to confer sensitivity to environmental factors (Kaufman et al., 2006; Uher, 2008). A corticotropin-releasing hormone type 1 receptor (CRH-R1) also appears to interact with early life adverse experiences in the genesis of adult depression (Bradley et al., 2008). Similarly, various depression-associated glucocorticoid receptor polymorphisms seem to have a modulating, rather than causal, effect in the development of depressive disorder, as reviewed elsewhere (El Hage et al., 2009). Recently, specific polymorphisms in genes that affect dopamine transmission were also suggested to increase susceptibility to depression (Opmeer et al., 2010). Polymorphisms in several genes may combine to produce environmentally sensitive dispositions (Uher, 2008). The etiology of depressive disorder is thus believed to be under interactive influence of genetics and environmental factors (Jabbi et al., 2008). In sum, vulnerability to depression is only partly genetic, with non-genetic factors being at least as important.

**Hypotheses of depression**

Major depressive disorder is likely to have multiple causes. Here, I will briefly review existing hypotheses of depression, in order to depict a variety of mechanisms that may be involved in the etiology, pathogenesis and treatment of this complex multifactorial disorder.

**The monoamine hypothesis**

For half a century, the majority of neurobiological research and classical pharmacotherapy regimens have explained depression with the monoamine hypothesis, which proposes that low levels of brain monoamines, such as serotonin, noradrenaline and/or dopamine, are responsible for the development of depressive symptoms. In fact, many currently used antidepressant drugs, such as selective serotonin reuptake inhibitors (SSRI), noradrenaline reuptake inhibitors (NRI), serotonin and noradrenaline reuptake inhibitors (SNRI), tricyclic antidepressants and monoamine oxidase inhibitors potentiate the brain’s monoaminergic system and elevate the monoamine levels (Wong and Licinio, 2001). Furthermore, the role of dopamine deficiency in depression has been suggested by the frequency of depression
in patients with Parkinson’s disease. In line with this, some direct dopamine receptor agonists, originally developed for treatment of Parkinson’s disease, display antidepressant efficacy (Gershon et al., 2007).

The monoamine hypothesis provided first clues into chemical changes in the brain that might underlie depressive symptoms, and thus advanced the way depression was viewed and treated. However, this hypothesis has several important limitations. First, experimental monoamine depletion in humans does not decrease mood in healthy subjects and only slightly lowers mood in healthy controls with a family history of depression and in drug-free patients with remitted depression (Ruhe et al., 2007). These studies thus fail to demonstrate a causal relation between brain monoamine levels and development of depression, yet presumably clarify a vulnerability trait for depression. Similarly, acute elevation of monoamine levels is not sufficient to cure depressive symptoms. Although the above mentioned antidepressant drugs acutely potentiate brain’s monoaminergic system, however, their mood-alleviating properties take at least several weeks to become manifest (Berton and Nestler, 2006). Thus, although the monoaminergic transmitters serotonin, noradrenaline and dopamine are undoubtedly involved in the mechanism and treatment of depression, it is obvious that monoamine deficits are only part of the story and are not sufficient on their own to explain the nature of depressive disorder.

It is now thought that antidepressant-induced acute increases in synaptic monoamine levels produce secondary neuroplastic changes that last longer and involve transcriptional and translational changes, mediating molecular and cellular plasticity (Krishnan and Nestler, 2008; Pittenger and Duman, 2008). This view led to formulation of the neuroplasticity hypothesis of depression, which will be discussed separately in this text.

**Glucocorticoid (HPA axis) hypothesis**

There is considerable evidence that abnormal, excessive activation of the HPA axis is implicated in depression (Sachar and Baron, 1979; Arborelius et al., 1999; Holsboer, 2001; Nestler et al., 2002a). A subset of depressed patients exhibit increased CORT production, as shown by elevated levels of this hormone in blood plasma and urine (Burke et al., 2005). Moreover, many patients have an abnormal response to the dexamethasone-suppression test, showing a decreased ability of the potent synthetic glucocorticoid, dexamethasone, to suppress plasma levels of CORT and ACTH (Nestler et al., 2002a). On the other hand, patients with Cushing’s syndrome, who have extremely high concentrations of circulating CORT, often show depressive
Figure 1.1 Overview of the multiple players implicated in the pathophysiology of major depressive disorder. This figure is based on different hypotheses of depression, which are discussed in the main text. In brief, various internal and external factors, such as genetic background and stressful experiences interact to shape brain vulnerability to major depression. Specific neurobiological changes include altered neurotransmitter balance, decreased BDNF levels and increased HPA axis activity, followed by induction of proinflammatory cytokines. In turn, cytokines activate IDO which catabolizes tryptophan to kynurenine, and thereby reduces 5-HT levels as well as results in increased accumulation of quinolinic acid, a kynurenine metabolite, causing neurotoxicity through NMDA receptors. Decreased availability of tryptophan and 5-HT culminates into reduced melatonin production, as well as indirectly to downregulated BDNF levels. Decreased BDNF expression in the hippocampus and prefrontal cortex leads to reduced signaling through CREB,
symptoms (McEwen, 2007; Krishnan and Nestler, 2008). Also, there is direct and indirect evidence for hypersecretion of CRH in some depressed patients (Nemeroff et al., 1984; Arborelius et al., 1999; Holsboer, 2001; Kasckow et al., 2001; Merali et al., 2004). Increased levels of CRH have been found in cerebrospinal fluid (Nemeroff et al., 1984). Moreover, intravenously administered CRH has been shown to evoke blunted ACTH response, suggesting pituitary CRH receptor downregulation due to chronic CRH hypersecretion (Gold et al., 1984). In line with these observations, postmortem studies have reported upregulated CRH mRNA and protein levels in limbic brain regions, and downregulated CRH receptor numbers, perhaps as a response to elevated CRH secretion (Merali et al., 2004). In addition to these changes, elevated vasopressin levels have also been found in postmortem brains of depressed humans (Purba et al., 1996).

These correlative data are supported by longitudinal studies with repeatedly-performed neuroendocrine tests, showing that persistent HPA axis disturbances are associated with poor therapeutic response in depressed patients and may predict relapse in subjects with remitted depression (Zobel et al., 2001). Apparently, the normalization of HPA axis dysregulation is a prerequisite of successful treatment, whereas the reappearance of HPA axis disturbances is prognostically unfavorable (de Kloet et al., 2005).

Thus, HPA axis dysregulation represents a risk factor for depression and other psychopathologies, which may be predisposed by various conditions that affect this stress response system. For instance, the cortisol-awakening response, a measure of HPA axis activity, has been shown to be altered in chronically stressed humans, victims of bullying, and subjects suffering from burnout (Kudielka and Wust, 2010). Additional support for this concept comes from animal studies, showing that stress-induced behavioral and neuroendocrine disturbances resemble some of the symptoms seen in depressed patients (de Kloet et al., 2005).

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*a transcription factor, implicated in regulation of neuroplasticity, neurogenesis and cellular resilience. Cytokine-activated NF-κB is also suggested to decrease BDNF and 5-HT levels, as well as to increase CRH release and to activate the HPA axis. Together, all these players may contribute to impaired brain function in major depression.*

*Dashed arrows indicate both direct and indirect influences.*

In animal models, strong HPA axis activation and sustained elevation of glucocorticoid levels, seen under conditions of prolonged stress, have been suggested to affect hippocampal morphology and function. Chronic stress in laboratory animals has been shown to induce dendritic atrophy of hippocampal CA3 pyramidal neurons (Magarinos and McEwen, 1995a; Magarinos et al., 1996; Sapolsky, 2000), suppress neurogenesis in the dentate gyrus (Fuchs and Flugge, 1998; Gould and Tanapat, 1999) and lead to reduction in hippocampal volume (Czeh et al., 2001; van der Hart et al., 2002). Sustained glucocorticoid exposure is suggested as one of the mediators of stress responsible in causing these changes, although the precise mechanism remains incompletely understood (de Kloet et al., 2005). Regardless of the nature of the damage, it would be expected to reduce inhibitory control of the hippocampus on HPA axis, which would further increase circulating glucocorticoid levels and subsequent hippocampal damage (Nestler et al., 2002a). Thus, a sustained HPA axis hyperactivation and hypercortisolaemia may contribute to depression partly by inducing above described changes in hippocampus that might impair its function. Based on the normal functions exerted by the hippocampus, its dysfunction might lead to some of the cognitive symptoms of depression.

Moreover, in humans, there are striking parallels between some aspects of the stress response, severe depression and the effects of centrally administered CRH (Arborelius et al., 1999; Holsboer, 2001), suggesting that a hyperactive HPA axis may contribute to depression not only via hypercortisolaemia but also via enhanced CRH secretion in the hypothalamus and other brain regions that are innervated by these neurons (Nestler et al., 2002a).

Despite the compelling evidence outlined above, it is still unknown whether HPA axis abnormalities are a primary cause of depression or, instead, secondary to depressed mood. Thus, a major liability of the HPA axis hypothesis of depression is the difficulty of defining the relationship of stress to depression. There is good evidence that episodes of depression often occur in the context of some form of stress; nevertheless, stress per se is not sufficient to induce depression (Nestler et al., 2002a).

**Neuroplasticity hypothesis**

The term neuroplasticity describes the ability of the adult and differentiated brain to adapt functionally and structurally to internal and external stimuli. Altered neuroplasticity is considered as a feature of depressive illness (figure 1.1). Brain regions, such as the hippocampus, amygdala and PFC are reported to undergo structural changes in depression, and alterations in these brain areas affect
emotions, memory and cognitive functions (Sheline, 2003; McEwen et al., 2010). Such neuroplastic processes include modulation of adult neurogenesis and synaptogenesis, together with alterations in neurotrophic factor expression, governed by multiple transcriptional and translational changes.

**Neurotrophic factors**

The neuroplasticity hypothesis of depression states that a deficiency in neurotrophic support may contribute to structural and functional brain changes during development of depression, and that reversal of this deficiency by antidepressant treatments may lead to the remission of depressive symptoms (Nestler et al., 2002a). Because this theory has focused largely on the role of brain-derived neurotrophic factor (BDNF) in regulating plasticity within adult brain, it is often referred as the neurotrophic or BDNF hypothesis of depression.

Support for involvement of neurotrophins in depression and its treatment has come from preclinical studies in stressed animals as well as postmortem brain analysis of depressed humans. Acute and chronic stress in laboratory animals has been shown to reduce BDNF and its mediated signaling in the hippocampus and other brain areas (Smith et al., 1995; Duman and Monteggia, 2006; Krishnan and Nestler, 2008). This effect is suggested to be mediated partly via stress-induced glucocorticoids (Smith et al., 1995), but the precise mechanism is not well understood yet. Conversely, chronic (but not acute) treatment with various antidepressants has been shown to increase BDNF-mediated signaling in the hippocampus, and prevent the stress-induced decrease in BDNF expression (Nibuya et al., 1995; Castren et al., 2007; Martinowich et al., 2007). Similar changes have been observed in the postmortem hippocampus and PFC of depressed humans (Chen et al., 2001; Castren, 2004b; Karege et al., 2005a; Duman and Monteggia, 2006). Interestingly, several studies have found decreased blood levels of BDNF in depressed patients, and their normalization after antidepressant therapy; however, the relevance of these findings to the action of BDNF in the brain remains to be established (Shimizu et al., 2003; Karege et al., 2005b; Castren et al., 2007). Antidepressant induction of BDNF is at least partly mediated via the transcription factor cyclic adenosine monophosphate (cAMP) responsive element binding protein (CREB) (Carlezon et al., 2005). Importantly, BDNF itself might posses antidepressant-like properties as observed in the experiments where BDNF was directly infused in the hippocampus of laboratory animals (Shirayama et al., 2002). Moreover, BDNF was reported to promote hippocampal function by enhancing long-term potentiation (LTP) and other forms of synaptic plasticity (Korte et al., 1996; Patterson et al., 1996; Kang et al., 1997;
Together, these findings raised the possibility that antidepressant-induced upregulation of BDNF could help to repair stress-induced damage to hippocampal neurons and preserve hippocampal function (Nestler et al., 2002a).

However, more recent findings have necessitated a revision of this hypothesis (Krishnan and Nestler, 2008). Some studies have failed to replicate the patterns of changes associated with stress and antidepressant treatment, or have even shown opposite effects (Groves, 2007; Martinowich et al., 2007). Another important drawback of the BDNF hypothesis is that mice lacking BDNF or its receptor do not clearly and consistently show depressive-like behaviors (Zorner et al., 2003; Monteggia et al., 2007), which may however partly relate to the limitations of testing depression-like syndromes in animal models. Furthermore, the effects of BDNF are region-specific: its enhancement is antidepressant in the hippocampus, but prodepressant in the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Eisch et al., 2003; Berton et al., 2006; Krishnan et al., 2007). Finally, a previously mentioned SNP in the gene encoding BDNF, Val66Met, associated with impaired release of BDNF and decreased hippocampal volume, does not directly increase the risk of depression (Egan et al., 2003; Szeszko et al., 2005; Chen et al., 2006; Gratacos et al., 2007).

Thus, although BDNF-mediated signaling is involved in neuroplastic responses to stress and antidepressants, these effects are region-specific and function in the background of other potent genetic and environmental modifiers (Krishnan and Nestler, 2008). Nevertheless, BDNF as well as other neurotrophins play an important role in regulation of hippocampal neurogenesis (Lee et al., 2002b), also implicated in the mechanisms of stress, depression and antidepressant therapy.

**Adult neurogenesis**

The possibility of postnatal neurogenesis was claimed as early as 1890s but this concept was not widely accepted until almost a century later (Altman and Das, 1965; Kaplan and Hinds, 1977; Eriksson et al., 1998). For all these years, it was believed in the below highlighted dogma that adult brain is not capable to generate new neurons:

"Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, ended and immutable. Everything may die, nothing may be regenerated." (Ramon y Cajal, 1913).
Chapter 1

Box 1.1

Regulators of adult neurogenesis

Adult neurogenesis is an extremely dynamic process. Extensive studies have shown that both intrinsic and extrinsic factors regulate neurogenesis at different stages, including cell proliferation, fate specification, migration, integration, and survival (Ming and Song, 2005). Regulation of neurogenesis in the olfactory bulb and hippocampus overlaps to a certain extent; however, many of the regulators have differential influences on these two processes. Since the current thesis concerns hippocampal neurogenesis, here I will briefly review factors implicated in regulation of new neuron generation in the dentate gyrus.

The intrinsic genetic background influences adult neurogenesis, as significant differences in the number of newborn hippocampal cells have been found between species and strains (Kempermann and Gage, 2002). Age is another important factor in regulating neurogenesis. Hippocampal cell proliferation occurs at high rates in very young animals and rapidly declines during aging (Kuhn et al., 1996; Heine et al., 2004b). Other intrinsic regulators of adult neurogenesis include various hormones, neurotransmitters and growth factors.

Glucocorticoids are considered as the main mediators of the stress-induced suppression of hippocampal neurogenesis. This idea originates from experiments in which CORT therapy and adrenalectomy have opposing effects on hippocampal cell proliferation (Cameron and Gould, 1994; Cameron and McKay, 1999; Tanapat et al., 2001). Besides glucocorticoids, other hormones also influence adult neurogenesis. For instance, estrogen and testosterone are known to differentially regulate the levels of newborn neurons in the dentate gyrus (Galea, 2008).

Several neurotransmitter systems have been implicated in regulating adult neurogenesis. Glutamatergic signaling via N-methyl-D-aspartate (NMDA) receptors has been shown to inhibit hippocampal cell proliferation (Cameron et al., 1995). Furthermore,
activation of NMDA receptors appears to play a role in the glucocorticoid-mediated effects on progenitor proliferation. For instance, blockage of NMDA receptors rescues CORT-induced decrease in hippocampal neurogenesis (Cameron et al., 1998). Another neurotransmitter, serotonin, has been suggested as a potent activator of adult neurogenesis (Djavadian, 2004). Inhibition of serotonin synthesis by pharmacological treatment or by lesioning of the serotonergic neurons reduces cell proliferation (Brezun and Daszuta, 1999). Conversely, stimulation of serotonergic transmission by chronic treatment with antidepressants promotes the formation of new cells in the dentate gyrus (Malberg et al., 2000). Yet, the net influence of serotonin on adult neurogenesis may depend on differential activation of its numerous receptors and their possibly contradictory actions. Other neurotransmitters have also been shown to modulate hippocampal neurogenesis: γ-aminobutyric acid (GABA) (Ge et al., 2007), noradrenaline (Kulkarni et al., 2002), dopamine (Borta and Hoglinger, 2007), and acetylcholine (Cooper-Kuhn et al., 2004), to name a few examples.

Adult neurogenesis is positively influenced by a variety of growth factors, including insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and BDNF; their involvement is reviewed extensively (Anderson et al., 2002; Newton and Duman, 2004; Hagg, 2005).

Among extrinsic factors modulating hippocampal neurogenesis, environmental enrichment and physical exercise are the most potent activators of hippocampal neurogenesis (Olson et al., 2006). Training in hippocampus-dependent learning tasks has also been associated with increased neurogenesis in the dentate gyrus, although inconsistently (Gould et al., 1999b; Van der Borght et al., 2005a). Stress has been repeatedly reported to decrease hippocampal neurogenesis, however, contrasting evidence exists (Gould and Tanapat, 1999; McEwen, 1999; Thomas et al., 2006; Lucassen et al., 2010). Complex intrinsic regulatory mechanisms underlie changes in neurogenesis elicited by extrinsic factors; thus, the final effect achieved is a result of integration of multiple interactions in the regulatory pathways involved.
Currently, there is no doubt that postnatal neurogenesis occurs in at least two regions of the brain: the subventricular zone (SVZ) of the olfactory bulb and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Abrous et al., 2005; Ming and Song, 2005; Lledo et al., 2006). Adult neurogenesis is subject to complex regulation with numerous factors involved (box 1.1). Although the function of postnatal neurogenesis is not yet completely understood, it has been implicated in several neurobiological processes and its dysregulation has been associated with multiple brain disorders.

At the turn of the millennium, it was proposed that the neurogenesis process in the adult hippocampus may be oppositely regulated by depression and antidepressants (Jacobs et al., 2000). This hypothesis was based on preclinical observations that stress, an important regulator of neurogenesis and a significant risk factor for major depression, as described above, inhibits proliferative activity in the hippocampal dentate gyrus (Gould and Tanapat, 1999; McEwen, 1999). Conversely, various antidepressant treatments have been shown to increase the rate of hippocampal neurogenesis and/or prevent the effects of stress (Kempermann and Kronenberg, 2003), comprising in part their mood-improving actions (Santarelli et al., 2003). Accordingly, changes in hippocampal neurogenesis were implicated in the etiopathophysiology and treatment of major depressive disorder (Duman et al., 2000; Gage, 2000; Jacobs et al., 2000; Kempermann, 2002).

Nonetheless, although initially promising, this hypothesis has lost some of its appeal due to difficulties providing functional mechanism for the etiology of depression. So far, no causal link between a decrease in neurogenesis and depressive-like behaviors has been identified (Vaidya et al., 2007). Furthermore, artificially disrupted hippocampal neurogenesis in laboratory animals has not led to the depressive phenotype (Sapolsky, 2004; Eisch et al., 2008). With respect to antidepressant drugs, their mood-alleviating effects do not clearly depend on hippocampal neurogenesis, however, newly-born neurons may play an important role in neuronal remodeling and hippocampal function (Bessa et al., 2009).

Importantly, literature also reveals contradicting evidence concerning effects of stress and antidepressant treatment on adult neurogenesis in the hippocampus. Despite a poor documentation of these contrasting results (many of such “negative data” do not get easily published), it is becoming clear that the influence of stress/antidepressants on neurogenesis is far from unequivocal.

Yet, the notion that adult hippocampal neurogenesis may be oppositely regulated by stress and antidepressant treatment gave rise to the possibility of using it as a readout in testing the efficacy of novel treatments for major depressive
disorder. In turn, the experimental work described in the following chapters also focused on stress-induced effects on hippocampal neurogenesis in animal models, and their modulation upon treatment with the novel antidepressant, agomelatine.

**Inflammation (cytokine) hypothesis**

The brain is no longer regarded as an immunoprivileged organ, as it became clear that many interactions occur between neural and immune systems (Schiepers et al., 2005). Consequently, changes in the functional activity of the immune system have been related to several neuropsychopathologies, including major depression.

A role for inflammation in depression was first proposed in the early 1990s in the form of the macrophage theory of depression (Smith, 1991). It was based on clinical observations that depressed patients have increased blood levels of inflammatory biomarkers, including cytokines and acute-phase proteins. Later, this theory was reformulated to the cytokine hypothesis of depression, suggesting that proinflammatory cytokines play the key role in mediation of the behavioral, neuroendocrine and neurochemical features of depressive disorder (Schiepers et al., 2005). Among the most likely players are interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, that are often found to be elevated in the peripheral blood circulation and/or the cerebrospinal fluid of depressed patients (Raison et al., 2006; Miller et al., 2009). Increases in inflammatory mediators have also been correlated to depressive symptom severity (Alesci et al., 2005; Thomas et al., 2005; Von Kanel et al., 2009). Importantly, administration of cytokines or their inducers, such as lipopolysaccharide (LPS) or vaccination, in humans was shown to evoke depressive-like symptoms (Reichenberg et al., 2001; Brydon et al., 2008). Long-term exposure to cytokines may even lead to development of clinical depression, as observed in a subpopulation of patients receiving chronic interferon (IFN)-α therapy for treatment of infectious diseases or cancer (Musselman et al., 2001; Capuron et al., 2002). Moreover, proinflammatory cytokines can activate indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the catabolism of tryptophan, and implicated in the relation between chronic inflammation and depressive disorder (Oxenkrug, 2010; Raison et al., 2010) (figure 1.1). These findings in humans are consistent with a vast literature in laboratory animals showing cytokine involvement in sickness behavior, proposed as an immunological model of depression (Dantzer, 2001; Dantzer et al., 2008; Dobos et al., 2010).

Since changes in immune system are beyond the scope of this thesis, I will not discuss possible mechanisms and mediators of cytokine-induced effects. Interactions between immune and neural systems in depressive disorder are a focus of many
ongoing research studies. Thus, the future holds promise for better understanding of the etiopathogenesis of depression and development of novel treatment approaches.

**Other hypotheses of the pathophysiology of depression**

**Altered neurotransmitter balance**

The section on the monoamine hypothesis briefly described the involvement of serotonin, noradrenaline and dopamine in the pathophysiology and treatment of depression. Other neurotransmitter systems, however, have also been implicated in this disorder. Here, I will give a brief overview on glutamatergic, GABA-ergic and cholinergic changes suggested to play a role in depression.

Glutamate is the major excitatory neurotransmitter of the CNS that controls synaptic excitability and plasticity in most brain circuits, including limbic pathways (McEwen et al., 2010). The involvement of glutamatergic mechanisms has been recognized in many functions perturbed in depressed states (Paul and Skolnick, 2003; Sanacora et al., 2003; Zarate et al., 2003; Bergink et al., 2004). Current evidence suggests that the glutamatergic system is altered in mood disorders and contributes to the mechanism of antidepressant action for several treatment modalities (Kugaya and Sanacora, 2005). Elevated glutamate levels were observed in the peripheral blood of some depressed patients (Kim et al., 1982; Altamura et al., 1993; Mauri et al., 1998). Moreover, magnetic resonance spectroscopy revealed changes in concentrations of cortical glutamate (Auer et al., 2000; Mirza et al., 2004; Sanacora et al., 2004). Both increases and decreases in glutamate levels were reported; this inconsistency might be due to discrepancies among studies, which measured different brain regions in a heterogeneous population of depressed subjects (children/adults, on/off medication). So far, these observations only suggest that regulation of glutamatergic system might be altered in mood disorders, and more controlled studies are needed to elucidate the precise nature of these abnormalities. Nevertheless, preclinical studies have shown that medications affecting the glutamatergic system, e.g. NMDA receptor antagonists, may have an antidepressant effect (Muller and Schwarz, 2007; Sanacora et al., 2008).

When investigating the contributions of glutamate in the pathophysiology of depression, it is also important to consider the role of GABA, since these two systems are intricately coupled. GABA is the major inhibitory neurotransmitter of the CNS, providing balance against the cortical excitability produced by glutamate (Kugaya and Sanacora, 2005). Emerging findings reveal that mood disorders are associated
with GABA-ergic abnormalities (Tunnicliff and Malatynska, 2003; Sanacora and Saricicek, 2007). Clinical studies report decreased GABA levels in both cerebrospinal fluid and blood plasma as well as lower cortical GABA concentrations in patients with major depression (Petty, 1995; Sanacora et al., 2004). Thus, GABA-ergic activity seems to be lower in the depressed state than in the healthy brain. Future studies, however, are needed to confirm the relationship of these changes to the pathophysiology of depression.

The cholinergic changes are also suggested to contribute to depression. This notion originated from clinical reports several decades ago, showing that central cholinergic activation caused depressant inhibitory effects (Carlton, 1963; Vaillant, 1967; Domino and Olds, 1968). Based on these observations, the cholinergic hypothesis of depression was postulated (Janowsky et al., 1972; Dilsaver, 1986); however, its importance diminished over time, due to insufficient evidence linking it to the etiology of major depression. Nevertheless, recognizing the role of the cholinergic system in depression may shed light upon mechanisms involved in certain aspects of this disease. Acetylcholine is largely responsible for a number of CNS functions, including arousal, attention, learning and memory (Deutsch, 1971). Hence, cholinergic dysfunctions may account for the development of cognitive symptoms associated with depression, especially when disease is long-lasting and treatment-resistant. The implication of the cholinergic system in major depressive disorder, and especially in associated cognitive impairments, is further reviewed in chapter 6 of this thesis.

**Circadian rhythm abnormalities**

Desynchronization of internal rhythms has been hypothesized to play a role in the pathophysiology of major depressive disorder (Wehr and Wirz-Justice, 1982). Indeed, several clinical symptoms of depression, such as diurnal mood variation and early morning awakening, are characteristic of changes in circadian rhythms (Healy, 1987; Germain and Kupfer, 2008; Wirz-Justice, 2008). Clinical findings suggest that many internal rhythms can be disrupted in depression, including the 24-hour profiles of various hormones, neurotransmitters, body temperature as well as sleep architecture and timing (Souetre et al., 1989; Turek, 2007; Lam, 2008). Sleep abnormalities represent one of the diagnostic criteria of the major depressive disorder. Interestingly, manipulation of the sleep-wake cycle, e.g. by sleep deprivation, is known to acutely improve mood in depressed patients (Giedke and Schwarzler, 2002). Furthermore, a successful antidepressant treatment is able to normalize the 24-hour CORT, thyroid stimulating hormone, noradrenaline and body
temperature rhythms (Souetre et al., 1989). These observations suggest a relationship between circadian rhythmicity and affective disorders. Yet, it is not clear whether changes in circadian processes represent a symptom or a causal factor in the pathogenesis of depression.

Nevertheless, recognition that desynchronization of internal rhythms plays an important role in affective disorders has led to the reasoning that resetting normal circadian rhythmicity may have a therapeutic effect. Hence, melatonin, a major regulator of circadian processes, was identified as a target for novel antidepressant therapies.

### Melatonin

Melatonin is the principal hormone of the pineal gland, the so-called “third eye”, which transduces the external succession of light and dark into an internal hormonal message (Peirson and Foster, Neuron, 2006; Maronde and Stehle, 2006). The neural circuitry that transmits information on lighting conditions to the pineal gland is remarkably complex and has been reviewed extensively (Moore, 1996; Korf et al., 1998; Maronde and Stehle, 2007; Reiter et al., 2010). Briefly, environmental light information from the retina is carried through the retinohypothalamic tract to the circadian clock situated in the hypothalamic suprachiasmatic nucleus (SCN). The main efferent output from the SCN goes to the parvocellular autonomic component of the PVN, and from there reaches the upper thoracic intermediolateral cell column, followed by a projection to the superior cervical ganglion (Luiten et al., 1985; Moore, 1996; Reiter et al., 2010). Noradrenergic sympathetic nerve fibers originate in this ganglion and reach the pinealocytes in the pineal gland. During darkness, the SCN sends an electrical signal which causes the release of noradrenaline from postganglionic synaptic nerve endings onto the pinealocytes, which initiates and sustains, primarily via β1-adrenergic receptors, melatonin synthesis from its precursor tryptophan (Maronde and Stehle, 2007; Reiter et al., 2010). The metabolic pathway of tryptophan that culminates in melatonin production is well known and has been a topic of numerous reviews (Reiter, 1991; Ackermann and Stehle, 2006; Reiter et al., 2010).

Melatonin influences cellular physiology via membrane receptors as well as via cytosolic and nuclear binding sites. The main effects of melatonin are elicited through the cell membrane-bound receptors, which will be discussed in more detail. These two high-affinity membrane receptors, MT₁ and MT₂, are members of the G-protein-coupled receptor family, and account mainly for melatonin action. A third melatonin binding site, MT₃, shows low affinity and is seemingly identical to the
cytosolic enzyme, quinone reductase 2 (QR2) (Boutin et al., 2008); however, its actions are beyond the scope of this thesis. Although originally thought to have a rather limited distribution, both MT1 and MT2 receptors have now been uncovered in virtually every organ of the body (Dubocovich and Markowska, 2005). In the brain, they have been localized in many regions, including the SCN, hippocampus, and PFC among others (Pandi-Perumal et al., 2008).

The signaling mechanisms of melatonin via MT1 and MT2 receptors are highly complex and vary with cell type and possibly also between species (Reiter et al., 2010). Although MT1 and MT2 signaling pathways are only partly identical, they are often presented collectively, due to complementary action of these two receptor subtypes and uncertainties concerning different cell types. In brief, MT1/MT2 receptor stimulation inhibits adenylate cyclase, reduces cAMP production and modulates protein kinase A (PKA) activity (Dubocovich et al., 2003). Moreover, dependent on cell type and organ involved, exposure to melatonin leads to activation of phospholipase C (PLC), followed by elevated inositol-(1,4,5)-triphosphate (IP3) and 1,2-diacylglycerol (DAG) (Alarma-Estrany and Pintor, 2007; Hardeland, 2009). These effects are followed by activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways, and their downstream elements (Witt-Enderby et al., 2000; Hardeland, 2009). In addition, various other second messenger cascades are triggered upon binding to MT1/MT2 receptors; their complexity is reviewed comprehensively (Witt-Enderby et al., 2000; Dubocovich et al., 2003; Pandi-Perumal et al., 2008; Hardeland, 2009).

The actions of melatonin have been most thoroughly studied in the SCN, which is proposed as a mediator of melatonin-associated effects on circadian rhythms and sleep. Activation of MT1 and MT2 receptors inhibits neuronal firing and phase shifts circadian rhythms in the SCN, respectively (Liu et al., 1997). These actions of melatonin have been implicated in the regulation of sleep (Dubocovich, 2007). Accordingly, exogenous melatonin was shown to promote sleep and phase shift circadian rhythms, however, these effects depend critically on the time of administration (Arendt, 2005). Thus, melatonin has obvious therapeutic potential in treating circadian rhythm sleep disorders, such as delayed sleep phase syndrome, non-24-h sleep-wake cycle as well as sleep problems associated with jetlag and shift work (Arendt and Rajaratnam, 2008). Furthermore, since melatonin output is altered in some depressed patients, it may be beneficial in the treatment of depressive disorders, especially if rhythm abnormalities are present (Lewy et al., 2006; Pandi-Perumal et al., 2006b; Serfaty et al., 2010). Hence, the melatoninergic system represents an important target for antidepressant therapies.
CHAPTER 1

STRUCTURAL CHANGES IN DEPRESSION

A large body of data suggests that depression is accompanied by neurobiological abnormalities, which may persist even during a remission of clinical symptoms. Neuroimaging studies indicate that individuals with recurrent major depressive episodes may have relatively small hippocampi (Sheline et al., 2003; Videbech and Ravndalde, 2004). Recurring or enduring illness and lack of antidepressant treatment are thought to contribute to progressive hippocampal volume reductions in some depressed patients (aan het Rot et al., 2009). In turn, these structural changes in the hippocampus may explain cognitive as well as several other symptoms associated with depression. Patients may also present with volumetric abnormalities in other brain regions, including the amygdala, ventral striatum, anterior cingulate cortex, orbitofrontal cortex and PFC (Sheline, 2003; Campbell and MacQueen, 2006; Konarski et al., 2008).

Neuropathology in depression has been extensively examined by postmortem studies, usually in suicide victims. Such investigations have revealed multiple abnormalities, ranging from volume reductions to cellular atrophy and molecular changes (Rajkowska, 2003). Postmortem studies of brain tissue of depressed subjects have shown hippocampal shrinkage accompanied by increased density of glial and neuronal cells and decreased neuropil per cell (i.e. reduction in dendritic branching and spine complexity) (Stockmeier et al., 2004). Histopathological evidence has also revealed cellular changes in other brain areas. Decreases in cortical thickness, neuronal sizes, and neuronal and glial densities in the orbitofrontal and dorsolateral PFC were reported (Rajkowska et al., 1999). Also, a reduction in glial density in the amygdala was found in depression (Bowley et al., 2002). Thus, alterations in both glia and neuron cells appear to participate in the neuropathology of major depression (Hercher et al., 2009). Furthermore, postmortem investigations have revealed low levels of BDNF in the hippocampus and PFC of depressed subjects (Duman and Monteggia, 2006; Martinowich et al., 2007). On the other hand, increases in hippocampal BDNF immunoreactivity were observed in patients treated with antidepressants at time of death, compared with medication-free subjects (Chen et al., 2001).

Altogether, these findings have provided unique insights into the neurobiology of depressive disorder. In order to advance this knowledge even further, postmortem investigations should be preceded by and linked to longitudinal clinical studies that reveal details of the disease course and treatment. In addition to clinical evidence, animal model approach is necessary to unravel the cellular and molecular correlates underlying pathological state of depression and the working mechanisms of antidepressant therapies.
ANIMAL MODELS OF DEPRESSION

Since depression typically impairs mood and higher cognitive functions, no experimental paradigm in animals can fully mimic this disorder. Only particular behavioral aspects of depression, such as anhedonia, helplessness or sleep abnormalities, are possible to model in animals; yet, clinical implication of effects achieved in such paradigms deserves caution. For instance, a rodent which floats passively in the forced swim test may start struggling after a single antidepressant injection, however, such an experiment is a very far-fetched representation of human depression and its effective treatment. Likewise, a stressed rat or mouse which consumes less sweet solution than its non-stressed littermate does not adequately mimic a depressed individual, with respect to symptoms evoked. Thus, although literature often names these and similar paradigms as animal models of depression, one should be reluctant to use this term. In the current thesis, I will rather refer to animal models of chronic stress, when discussing prolonged stress-induced alterations in rodent brain, which may be fundamentally similar to those in affected human brain.

Here, I will briefly review several animal paradigms, used frequently to investigate development and treatment of stress-related psychopathologies. Popular experimental models of depression include despair paradigms (forced swim, tail suspension tests and learned helplessness), olfactory bulbectomy, maternal separation and exposure to prolonged stress of various types (chronic mild stress, social defeat, footshock and restraint stress).

The behavioral despair paradigms, such as forced swim and tail suspension tests, are based on observation that immobility or decreased struggling of an animal in aversive experimental conditions (“despair”) could be reversed by antidepressant drugs (Porsolt et al., 1977; Steru et al., 1985). These paradigms are widely used to detect potential antidepressant compounds and to measure their effects in animals (table 1.3). The common rationale of the above described tests is that animal immobility indicates anxiogenic freezing; however, this behavior may have many other interpretations, including those of an opposite nature (Kalueff et al., 2007). Hence, although such tests contribute to the identification of novel drugs against depression, their measured effects should be interpreted with caution. A conceptually similar paradigm of learned helplessness induces a condition, in which animal learns to behave helplessly by passively accepting aversive stimuli, even when the possibility to escape an unpleasant environment is restored (Seligman, 1972). Thereby, this experimental model mimics a perceived absence of control and
feeling of hopelessness in clinical depression. Learned helplessness paradigm is frequently used to induce depression-like behavior in animals and to test antidepressant effects therein (table 1.3).

The olfactory bulbectomy has been proposed as an experimental model of depression, based on observations that bulbectomized rats display a behavioral syndrome that reflects a disruption of the limbic-hypothalamic axis (Kelly et al., 1997). The behavioral changes following olfactory bulbectomy, e.g. motor hyperactivity and memory deficits, resemble some of the depression symptoms; moreover, they can be attenuated by treatment with antidepressants (table 1.3). The olfactory bulbi in the rat form a part of the limbic system in which the amygdala and hippocampus contribute to the emotional components of behavior. Thus, bulbectomy may cause a dysfunction of the cortical-hippocampal-amygdala circuit that underlies the behavioral, endocrine, immune and neurotransmitter changes in depression (Song and Leonard, 2005).

Maternal separation is used to induce early-life stress in rodents, suggested to shape various emotional and neuroendocrine parameters in the adulthood (Plotsky and Meaney, 1993; Pryce and Feldon, 2003). Disturbed mother-infant relationship is hypothesized to increase susceptibility of the offspring to psychopathology later in life. Nevertheless, literature demonstrates that effects of maternal separation on depression-related behaviors are rather inconsistent, and therefore, do not provide a straightforward model of early-life stress (Lehmann and Feldon, 2000; Millstein and Holmes, 2007; Hulshof et al., 2010).

Prolonged stress in the adulthood is frequently applied to laboratory animals in order to investigate the impact of stressful events on body and brain. Chronic stress can be modeled by exposing rodents to various physical and/or psychosocial stressors, e.g. immobilization, footshock, social defeat and chronic mild stress. Generally, chronic stress models differ in the degree to which they activate the HPA axis as well as in the behavioral and neural responses that they induce. Furthermore, animals show ability to habituate, at least partly, to certain stress paradigms (Dallman, 1993). Nevertheless, the above mentioned chronic stress models have revealed a wide range of stress-induced effects, from changes in gene transcription to alterations in neural plasticity, suggested to contribute to one’s vulnerability to psychopathology (Krishnan and Nestler, 2008). In addition, they have proven to unveil the effects of antidepressant drugs (table 1.3). Hence, animal models of chronic stress provide a valuable tool to investigate behavioral, endocrine and neurobiological changes underlying stress-related disorders and mechanism of antidepressant therapies (Fuchs et al., 2001; Nestler et al., 2002b). The present thesis, focused on neurobiological effects of prolonged stress and actions of the
antidepressant agomelatine, also employed animal models of chronic stress: repeated footshock (chapter 2, 3, and 5) and chronic mild stress (chapter 4). A detailed overview of both models and their induced effects will be presented in the separate chapters of the current thesis.

Recognition that inflammation and cytokines play important roles in depression gave rise to immunological models of this disorder. The LPS-induced sickness behavior in animals was shown to resemble certain symptoms of clinical depression (fatigue, reduced appetite, decreased social and physical activity, altered sleep, impaired learning and memory), which could be attenuated by antidepressant treatment (Dunn et al., 2005; Dantzer et al., 2008).

Other approaches for modeling depression include the use of selectively bred mutant or transgenic animals with altered anxiety- and depression-like phenotypes (Gonzalez et al., 1998; El Yacoubi et al., 2003; Thakker et al., 2005; Kalueff et al., 2007). Such paradigms are also valuable in detecting and testing of potential antidepressants drugs (table 1.3). In particular, gene-specific models might enable a better dissection of neurobiological mechanisms underlying development and treatment of depressive disorder. Given the importance of interaction between genetic and environmental factors in vulnerability to depression, these models would yield most information when applied in a combination with external risk factors, e.g. stress.

In addition, various anxiety paradigms are frequently employed to test behavioral traits that demonstrate marked overlap and co-occurrence with depressive disorder. Anxiety tests include exploration-based paradigms (e.g. open field, holeboard, elevated plus maze, social interaction tests) and conditioned or unconditioned threat responses (e.g. shock-probe defensive burying, ultrasonic vocalization, Vogel tests), reviewed extensively elsewhere (Rodgers, 1997; Flint, 2003; Kalueff et al., 2007).

Although researchers' confidence in the above reviewed animal models varies, they are indispensable for screening antidepressant drugs, testing neurobiological hypotheses and finding candidate genes for human depressive disorder. Nevertheless, understanding the potential benefits and weaknesses of the existing experimental models is crucial for obtaining valid animal data to parallel and/or complement the available clinical findings (Kalueff et al., 2007).
CHAPTER 1

ANTIDEPRESSANT THERAPY

The first antidepressant treatment emerged in the 1950s after a serendipitous discovery of mood-alleviating properties of the monoamine oxidase (MAO) inhibitor, iproniazid, originally developed as anti-tuberculosis drug (Lopez-Munoz and Alamo, 2009). The second generation of compounds, such as the tricyclic antidepressant (TCA), imipramine, were originally synthesized as antipsychotic drugs and their antidepressant activity was also discovered later in the clinics (Ban, 2001). Shortly after, the acute mechanisms of action of these antidepressant medications were identified: inhibition of serotonin or noradrenaline reuptake transporters by the TCA, and inhibition of MAO, a major catabolic enzyme for monoamine neurotransmitters, by MAO inhibitors (Frazer, 1997).

These discoveries led to the development of numerous more selective antidepressant drugs, such as SSRI, NRI and SNRI, which are widely used in the clinical practice today (table 1.2). These newer medications display a better side effect profile and improved tolerability; however, they have essentially the same mechanism of action as the older antidepressant agents. As a result, their efficacy is not much better as compared to the older medications. The response rates to the current antidepressant drugs range from 60% up to 80%; yet, only approximately 50% of all treated patients achieve complete remission (Mulrow et al., 2000; Nestler et al., 2002a). Moreover, existing options for antidepressant treatment are limited by their delayed onset of action and adverse outcomes (aan het Rot et al., 2009).

Thus, there is a need for novel and more efficient medications with fundamentally different mechanisms of action. Search for new targets in treatment of depression has led to the development of several novel compounds, reviewed in table 1.2. Whereas some of these drugs have already demonstrated antidepressant efficacy in clinical trials, others merely represent potential targets for the development of novel therapeutics.

Besides advances in pharmaceuticals, other approaches are also important in treatment of depression. For instance, the electroconvulsive therapy has been one of the most effective treatment methods so far. Other neurostimulation techniques, including deep brain stimulation, transcranial magnetic stimulation and vagus nerve stimulation, have been recently proposed in treatment of major depressive disorder. Last but not least, social and behavioral interventions, such as cognitive psychotherapy, social support and regular physical activity reduce the burden of depression and benefit brain and body health and resilience.
Table 1.2 Classical and novel pharmacological agents for treatment of depression. The table provides an overview of old and new antidepressant drugs as well as potential targets for pharmacotherapy.

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<th>CLASSICAL MECHANISM (MONOAMINERGIC) ANTIDEPRESSANTS:</th>
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<td><strong>Serendipitous discoveries:</strong></td>
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<td>• Monoamine oxidase inhibitors (MAOI)</td>
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<td>• Tricyclic antidepressants (TCA)</td>
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<td><strong>Optimized drugs:</strong></td>
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<td>• Serotonin selective reuptake inhibitors (SSRI)</td>
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<td>• Noradrenaline selective reuptake inhibitors (NRI)</td>
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<td>• Serotonin and noradrenaline reuptake inhibitors (SNRI)</td>
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<th>NOVEL MECHANISM ANTIDEPRESSANTS:</th>
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<td>• Agomelatine</td>
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<td>• HPA-axis targets</td>
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<td>• GR antagonants (e.g. mifepristone)</td>
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<td>• CRH-R1 antagonists</td>
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<td>• V-1B antagonists</td>
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<td>• β3-adrenoreceptor agonists</td>
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<td>• Other neuropeptide targets</td>
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<td>• Neurokinin (NK) antagonists (e.g. substance P)</td>
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<td>• Amino acid neurotransmitter modulators</td>
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<td>• NMDA antagonants</td>
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<td>• BDNF</td>
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<td>• Fibroblast growth factor (FGF)</td>
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<td>• Modulators of intracellular signaling cascades</td>
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<td>• CREB activators (phosphodiesterase (PDE4) inhibitors)</td>
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<td>• Glycogen synthase kinase-3 (GSK-3) inhibitors</td>
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<td>• Novel targets</td>
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<td>• Mitochondrial benzodiazepine receptor agonist</td>
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<td>• Neuronal nicotinic receptor antagonist</td>
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<td>• Pentapeptide analog of melanocyte-inhibiting factor (MIF-1)</td>
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<td>• Histone modifiers (histone deacetylase inhibitors)</td>
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AGOMELATINE

A part of this thesis is devoted to the experimental work on the neurobiological effects of a novel antidepressant agomelatine. Therefore, here I will briefly introduce the pharmaceutical properties of agomelatine as well as its known actions in animal and human studies.

Agomelatine (S-20098; N\[2-(7-methoxy-1-naphthyl)ethyl\] acetamide; figure 1.2A) is the first melatonergic antidepressant (Millan et al., 2003). Besides being a potent agonist of the melatonergic MT\textsubscript{1} and MT\textsubscript{2} receptors, it also acts as a serotonergic 5-HT\textsubscript{2C} receptor antagonist (Audinot et al., 2003; Millan et al., 2003). Agomelatine was initially investigated as a chronobiotic compound; however, with the discovery of the 5-HT\textsubscript{2C} antagonist activity, the emphasis shifted to its anxiolytic and antidepressant effects. In the following paragraphs, I will shortly review both preclinical and clinical evidence concerning the therapeutic properties of agomelatine as well as proposed mechanisms behind them.

Preclinical evidence: animal studies

The antidepressant activity of agomelatine has been demonstrated in several animal models, including the despair test, learned helplessness test, bulbectomy, and chronic mild stress (CMS) paradigms (Papp et al., 2003; Bourin et al., 2004; Norman et al., 2004; Barden et al., 2005; Bertaina-Anglade et al., 2006) (table 1.3). In the learned helplessness test, neither melatonin nor 5-HT\textsubscript{2C} receptor antagonists alone achieved antidepressant properties of agomelatine; moreover its effects were

![Molecular structures of agomelatine (A), melatonin (B) and serotonin (C). Agomelatine acts as a potent agonist of the melatonergic MT\textsubscript{1}/MT\textsubscript{2} receptors and as an antagonist of the serotonergic 5-HT\textsubscript{2C} receptors.](image-url)
abolished by co-administration of a MT\textsubscript{1}/MT\textsubscript{2} antagonist (Bertaina-Anglade et al., 2006). Consistent with this notion, a requirement of both melatonin and 5-HT\textsubscript{2C} receptors for therapeutic efficacy of agomelatine was observed in several other behavioral studies (Papp et al., 2003; Bourin et al., 2004; de Bodinat et al., 2010). Hence, the antidepressant action of agomelatine has been proposed to derive from the synergy between melatonin agonism and 5-HT\textsubscript{2C} antagonism (Popoli, 2009).

Nevertheless, agomelatine has similar chronobiotic action as melatonin: it is able to phase shift free-running rhythms and accelerate re-entrainment after a sudden change of the light-dark cycle. This antidepressant has been shown to resynchronize circadian rhythms in animal models of jetlag, delayed sleep-phase syndrome, blindness (free-running conditions) and aging, both in nocturnal and diurnal species (Armstrong et al., 1993; Redman et al., 1995; Martinet et al., 1996; Van Reeth et al., 1998; Weibel et al., 2000; Van Reeth et al., 2001). These resynchronizing effects of agomelatine are abolished by lesions of the SCN but not modified by pinealectomy (Redman and Francis, 1998). The ability of agomelatine to re-entrain circadian rhythms is dose- and time-dependent. The window of sensitivity to this compound is within a few hours around the light-dark transition; its maximal effectiveness coincides with the onset of the elevation in melatonin secretion and with the time of maximal melatonin receptor sensitivity (Van Reeth et al., 1997; Pandi-Perumal et al., 2006b; de Bodinat et al., 2010). Therefore, a proper timing of agomelatine administration is crucial for optimal therapeutic effects.

In addition, agomelatine also possesses anxiolytic properties, as observed in various anxiety models, including Vogel test (conflict-based anxiety test), elevated plus maze, social interaction, social defeat, and conditioned ultrasonic vocalization (Millan et al., 2005; Tuma et al., 2005; Papp et al., 2006). Anxiolytic actions of agomelatine are largely attributed to its antagonism at 5-HT\textsubscript{2C} receptors (Millan et al., 2005). Moreover, anxiolytic effects of agomelatine in the defeated animals have been shown to require an intact SCN (Tuma et al., 2005).

Unlike the classical antidepressants, agomelatine does not modify extracellular levels of serotonin (Millan et al., 2003; Millan et al., 2005). Also, chronic treatment with agomelatine does not down-regulate presynaptic and postsynaptic 5-HT\textsubscript{1A} receptors, contrary to the effects of SSRI drugs (Hanoun et al., 2004). However, this novel antidepressant was reported to increase dopamine and noradrenaline levels in the (pre)frontal cortex and hippocampus (Millan et al., 2003; Millan et al., 2005).

In an attempt to decipher the mechanisms underlying the therapeutic action of agomelatine, preclinical studies reported a variety of its mediated changes in the brain. Similarly to other antidepressant therapies, agomelatine was shown to
Table 1.3 Overview of studies demonstrating the antidepressant activity of agomelatine in animal models. Anxiety tests are not included in this table.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Agomelatine effect</th>
<th>Melatonin effect</th>
<th>5-HT_{2c} antagonist effect</th>
<th>Positive control</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forced swim test (immobility)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Imipramine</td>
<td>Bourin et al., 2004</td>
</tr>
<tr>
<td>Learned helplessness test (helplessness)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Imipramine</td>
<td>Bertaina-Anglade et al., 2006</td>
</tr>
<tr>
<td>Chronic mild stress (sucrose consumption)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(abolished by MT_{1}/MT_{2} antagonist)</td>
<td></td>
<td>(abolished by MT_{1}/MT_{2} antagonist)</td>
<td>(less effective than agomelatine)</td>
<td>Imipramine Fluoxetine</td>
<td>Papp et al., 2003; Dekeyne et al., 2008</td>
</tr>
<tr>
<td>(not abolished by MT_{1}/MT_{2} antagonist)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic GR-i mouse model (immobility in forced swim test)</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>Desipramine</td>
<td>Barden et al., 2005</td>
</tr>
<tr>
<td>Bullectomy (motor hyperactivity)</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>Imipramine</td>
<td>Norman et al., 2004</td>
</tr>
</tbody>
</table>
enhance adult hippocampal neurogenesis and to increase expression of BDNF (Banasr et al., 2006; Soumier et al., 2009). Importantly, these neurobiological effects of agomelatine may, at least partly, depend on its synergistic mechanism of action through melatonergic and 5-HT2c receptor sites. It was shown that although 5-HT2c antagonists mimic the agomelatine-induced increase in hippocampal cell proliferation, they do not reproduce its enhancement of cell survival or of levels of BDNF, which are only slightly elevated by melatonin (Soumier et al., 2009). Moreover, this antidepressant drug was reported to activate several cellular signaling proteins, i.e. extracellular signal-regulated kinase 1/2 (ERK1/2), protein kinase B (Akt), and glycogen synthase kinase 3β (GSK3β), implicated in the antidepressant action (Soumier et al., 2009).

This information considerably advanced understanding of agomelatine’s action in the brain. However, since effects of antidepressant drugs might differ in disturbed versus intact systems (Berton and Nestler, 2006), the latter findings under basal conditions cannot directly be extrapolated to the therapeutic actions of agomelatine in depression. This gap may be partially bridged by applying an animal model approach. Therefore, in the experiments described in this thesis, agomelatine was administered to chronically stressed rats, in order elucidate its effects under conditions that might predispose to mood disorders.

**Clinical evidence**

Agomelatine proved to be an effective treatment for major depressive disorder in several clinical trials (Kennedy and Rizvi, 2010). However, some of the efficacy trials against placebo failed due to an excessively high response rate in the placebo group (de Bodinat et al., 2010). Nevertheless, when compared to the SNRI venlafaxine and with the SSRI sertraline in clinical studies, agomelatine showed an efficacy at least equivalent to these established drugs. In fact, it proved superior to both venlafaxine and sertraline in improving sleep and anxiety symptoms as well as in its tolerability and safety profile (Lemoine et al., 2007; Kennedy et al., 2008; Kasper et al., 2010). Interestingly, one placebo-controlled study suggested a more rapid onset of agomelatine action as compared to classical antidepressants, showing that its therapeutic effects appeared as early as 2 weeks after treatment initiation (Loo et al., 2002). Based on the results of all clinical trials, agomelatine was granted a marketing authorization in the European Union in 2009, with the approved indication for treatment of major depressive episodes in adults.
Yet, the antidepressant efficacy of agomelatine (the response rate of 70-80%) is not much better as compared to other pharmacotherapies; hence, the advantage of this drug mainly lays in its better tolerability and ability to improve co-morbid symptoms of depressive disorder. In consequence, the discovery of agomelatine offers a benefit for initiation of treatment and long-term adherence; however, it does not abate a need for more efficient medications against major depression.

SCOPE OF THE THESIS

The overall aim of this study is to investigate the neurobiological changes associated with stress, depression and agomelatine treatment.

Chapter 1 of this thesis describes the neurobiology of stress and depression, and reviews various factors that may contribute to development of stress-related pathologies. It also provides a brief overview of antidepressant therapy in general, and introduces the properties of the novel antidepressant, agomelatine.

In Chapter 2, the effects of acute and chronic footshock stress on brain plasticity are examined, using hippocampal neurogenesis as the main readout. The aim of this experimental study is to establish the duration of the stress exposure (acute versus chronic) needed to evoke specific changes in hippocampal cell proliferation, and the persistence of the achieved effect. In addition, the effects of chronic footshock stress on HPA axis activity are investigated, as well as their relationship to changes in distinct stages of the neurogenesis process (cell proliferation, survival and neuronal differentiation).

The above described chronic footshock stress model is used in Chapter 3, in order to test the effects of the antidepressant agomelatine in the stress-compromised brain. Specifically, we examine whether chronic treatment with agomelatine influences HPA axis reactivity, and whether it induces changes in hippocampal neurogenesis (cell proliferation, survival and neuronal differentiation) and neuronal activation.

Another experimental paradigm, the chronic mild stress model, is used in Chapter 4, in order to further characterize the effects of agomelatine on hippocampal neurogenesis in the stressed brain. In particular, we investigate whether this stress paradigm induces differential changes in distinct stages of the neurogenesis process (cell proliferation, survival and neuronal differentiation), and whether agomelatine treatment reverses them.
In Chapter 5, we ask whether the action mechanism of agomelatine involves modulation of synaptic function. Specifically, the chronic footshock stress model is used in order to assess the effects of stress and agomelatine treatment on synapsin I expression and its phosphorylation pattern in several brain regions.

Chapter 6 is devoted to the review of literature regarding involvement of the cholinergic system in the pathophysiology of major depressive disorder. There, we discuss the contribution of cholinergic changes to the development of mood and cognitive impairments associated with depression. In view of this, a potential link between the cholinergic system, hippocampal neurogenesis and cognitive dysfunction in depression is outlined.

In Chapter 7, the general implication of the findings described in this thesis is discussed, together with future perspectives regarding this type of research.