“The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them”

William Lawrence Bragg
ABSTRACT

The previous chapters of this thesis provided experimental evidence for the existence of neurobiological changes associated with stress, depression and the novel antidepressant agomelatine. The aim of this final chapter is to give an overall interpretation and integration of the results. At first, the caveats concerning stress research are discussed. Second, chronic stress models used in this thesis are reviewed, in an attempt to place the present data within the greater framework of our knowledge regarding the pathophysiology of stress and related disorders. Also, potential neurobiological changes underlying the mechanism of action of the antidepressant agomelatine are discussed. At last, the future perspectives concerning this research field are highlighted.
WHAT IS STRESS?

“Everybody knows what stress is and nobody knows what it is” (Selye, 1973).

The concept of stress, reviewed in chapter 1, has been a subject of scientific debate ever since its introduction. In particular, the definition of stress in terms of threat to homeostasis has been criticized as too broad and almost meaningless since virtually all actions of an organism are involved in maintaining a homeostatic equilibrium (Day, 2005; Koolhaas et al., 2010). Although the complexity of this issue has been addressed, yet, there is no universal definition of stress with operationally defined terms that can be applied equally to rodents and humans (Kim and Diamond, 2002). Not surprisingly, this hampers the interpretation of results coming from stress research.

Stress is relative

The term stress is often generalized implying that exposure to any kind of stressor evokes similar changes in physiology and behavior. In addition, it is intuitively believed that the stressor with a higher intensity has more severe effects. However, this is not entirely true. First, the “non-specific” stress response, as postulated by Selye, is followed by changes specific to each stressor (Selye, 1955). Although most stressors lead to similar neurochemical alterations, it is important to note that not all stressors in general have the same effects (Anisman and Matheson, 2005). Second, the intensity of a stressor is not so much related to the physical nature of the stimulus, but rather to the degree to which it challenges adaptive defense mechanisms (Koolhaas et al., 2006). Thus, a seemingly very aversive stressor (e.g. footshock) may have rather mild effects, as discussed in the following sections. Moreover, it is uncertain what role should be addressed to anticipatory processes in chronic stress paradigms since it is unknown whether anticipating an adverse event is stressful for rodents as it is for most humans (Anisman and Matheson, 2005). Finally, stress occurrence is determined by how an organism perceives and reacts to the stimulus (Kim and Diamond, 2002). Hence, stress pathology occurs due to psychological appraisal of the stressor, not due to its physical characteristics (Koolhaas et al., 2006).

Another caveat in stress research is the absence of unique physiological measures that are stress specific. Although the elevation of glucocorticoid levels
often serves as a measure of a stress response, it is not a definitive indicator of a stress state (Koolhaas et al., 2010). In fact, increased glucocorticoid levels are also associated with pleasurable activities, such as exercise, feeding and sex (Bronson and Desjardins, 1982; Rosmond et al., 2000; Kanaley et al., 2001; Kim and Diamond, 2002).

Acute versus chronic stress

The acute stress response is initiated immediately after the exposure to a challenging stimulus and terminated when the stressful experience is over. In case of chronic stress, however, this response is commenced repeatedly and may persist if exposure to a stressful situation endures. Yet, the effects of chronic stress are not simply an extrapolation of the effects of acute stress, as complex adaptive and maladaptive phenomena must be taken into account in the long run.

In chapter 2 of the present thesis, this notion is elaborated by investigating whether repeated footshock stress leads to changes that are not seen after exposure to a single footshock session. Hippocampal neurogenesis, and cell proliferation in particular, served as the main readout in these experiments. Although both acute and repeated exposure to footshock stress led to strong activation of the HPA axis, only the latter caused suppression in hippocampal cell proliferation. This study thus demonstrated that repeated stress is necessary for the induction of this specific change in the brain. Since the effect of chronic stress on new cell generation was temporary (the rate of cell proliferation recovered within 24 hours), the present study also emphasized that timing of animal sacrifice is an important consideration in stress research (Dagyte et al., 2009).

CHRONIC STRESS PARADIGMS AS PUTATIVE ANIMAL MODELS OF STRESS-RELATED DISORDERS

“No perfect animal model exists for any aspects of any CNS disorder, as implied by the term modeling” (Mogil, 2009).

In this thesis, animal models of chronic stress were used in order to elucidate neurobiological alterations that may be fundamentally similar to those in human
brain prone to psychopathology. Induction of a stress-vulnerable state in the brain was also a prerequisite in the studies aimed at unraveling the working mechanism of the antidepressant agomelatine. As stated in the introductory chapter, however, no experimental paradigm in animals can fully mimic depressive disorder in humans. With this in mind, I will review the chronic footshock stress model which was employed in the majority of experiments described in the present thesis (chapter 2, 3, and 5).

The chronic footshock stress model: review

Footshock is a frequently used stimulus in stress and learning paradigms. The focus of this section, however, is only on the chronic footshock stress model, established to investigate effects of prolonged stress and their implication for psychopathology (Trentani et al., 2002; Kuipers et al., 2003; Trentani et al., 2003). Previous studies using this stress model illustrated a variety of gender-related dimorphisms, emphasizing different responses to stress in male and female individuals. Although undoubtedly interesting and important, these gender differences will not be discussed in more detail, since experiments described in the current thesis involved only male rats.

An important methodological difference of the present thesis as compared to the above mentioned studies is the timing of animal sacrifice in relation to the last footshock exposure. Whereas the earlier studies investigated changes 2 hours post-stress exposure, the current thesis first examined the time-dependency of chronic footshock-associated effects (2 / 24 hours post-stress; chapter 2) and employed the 24 hours post-stress sacrifice schedule in the following experiments (chapter 3 and 5). This was done with the intention to avoid confounding effects of the acute stress response as well as with an attempt to focus on lasting effects of chronic stress.

Effects on physiological and endocrine measures

The chronic footshock stress model has been associated with changes at multiple levels. At the systemic level, this stress paradigm was shown to affect several physiological and endocrine measurements. Exposure to repeated footshock reduced food intake (this thesis) and decreased body weight gain (this thesis and previous studies) (Trentani et al., 2002; Kuipers et al., 2003; Westenbroek et al., 2003a). Moreover, this type of stress enhanced plasma adrenaline levels (previous studies) and induced a robust HPA axis response as reflected by elevated ACTH and CORT.
concentrations (this thesis and previous studies) (Trentani et al., 2002). These changes in stress hormone levels were observed after exposure to repeated footshock; yet, they were measured 2 hours after the last stress session at the latest, and may therefore rather represent the acute stress response than long-lasting alterations in endocrine regulation. In agreement, the data described in this thesis revealed no differences in baseline levels of ACTH and CORT, measured after 3 weeks of daily footshock exposure (chapter 2). Nevertheless, the previous studies reported an increase in adrenal weight following this chronic stress paradigm (Trentani et al., 2002; Westenbroek et al., 2005), although these effects were not always significant (Westenbroek et al., 2003a), like in the present thesis (unpublished data).

**Effects on behavior**

At the behavioral level, exposure to footshock stress induced massive ultrasonic vocalization (this thesis). Surprisingly, however, this chronic stress model was associated with rather mild changes in various other behavioral tests, or was even devoid of effects therein. Westenbroek and coworkers using this stress paradigm reported significantly increased locomotor activity in the open field test following exposure to repeated footshock (Westenbroek et al., 2003b); however, this finding was not confirmed in the current thesis (figure 7.1A). Differences in experimental design (reversed light-dark cycle and repeated testing in the study of Westenbroek and coworkers) might explain this discrepancy. The present thesis attempted to further characterize behavioral correlates of this stress model. In turn, several experiments were performed where chronically stressed rats were tested in various behavioral paradigms, including elevated plus maze and sucrose preference tests. Notably, none of these behavioral paradigms detected significant changes following chronic footshock stress (figure 7.1B and 7.1C). One explanation for the lack of effects could be the timing of the stress exposure in relation to behavioral testing. In the studies described in this thesis, rats were generally tested about 20 hours after the previous footshock session, right before the stress session of that day, in order to assess the chronic effects of stress on behavior. One possibility might thus be that chronic footshock did induce short-lasting changes in behavior that went unnoticed in these experiments. Studying the time-dependency of stress effects on behavior might clarify this issue; however, it was beyond the scope of this thesis. Another possibility is that despite a wide array of behavioral paradigms used, these were not sensitive enough to detect chronic footshock-induced changes. Moreover, behavioral parameters are associated with high individual variation that may hamper obtaining significant results, unless high numbers of animals per group are used. Additional in-depth behavioral testing is therefore necessary to fully understand chronic foot-
shock stress-evoked effects on behavior. Furthermore, rats might be able to cope with daily stress of this type, despite the lack of adaptation in terms of ultrasonic vocalization and the peak HPA axis response. Faster recovery of the CORT response at the end of the chronic footshock period (chapter 2) suggests that animals partly habituate to this type of stress at the endocrine level. In turn, they might also adapt to chronic footshock stress at the behavioral level.
Neurobiological effects

A variety of chronic footshock effects have been described in the brain at the cellular and molecular levels. Figure 7.2 attempts an integration of these findings, discussed in detail in the following text.

Effects on neuronal activity

An in-depth analysis of neuronal activity was carried out using c-Fos as a marker (Trentani et al., 2002; Kuipers et al., 2003; Westenbroek et al., 2003a). These studies revealed that 2 hours after the last stress exposure, when c-Fos expression is at peak levels, there were pronounced region-specific changes in neuronal activity. In particular, rats showed increased c-Fos immunoreactivity in stress-related brain areas, including medial prefrontal cortex (mPFC), amygdala, paraventricular nucleus (PVN) of the hypothalamus, and raphe nuclei, demonstrating enhanced activity in this neural circuit after repeated footshock (Trentani et al., 2003). Interestingly, however, reduced c-Fos expression was found in the hippocampal dentate gyrus, suggesting that chronic stress decreased neuronal activity in this limbic region (Trentani et al., 2003). The latter observation was further investigated in the current thesis which largely focused on stress-induced changes in the hippocampus. As described in chapter 3, repeated footshock stress also downregulated basal c-Fos expression (24 hours after the last stress exposure) in the dentate gyrus and showed a similar tendency towards decrease in the CA1 and CA3 areas of the hippocampus (Dagyte et al., 2010b). Interestingly, however, chronic stress did not alter basal c-Fos immunoreactivity in the PVN (unpublished results). These data suggest that repeated footshock stress leads to region-specific changes in basal c-Fos expression. The induction of c-Fos reflects the activity of the neurons (Kovacs, 1998). Thus, decrease in c-Fos expression in the dentate gyrus and CA areas may indicate reduced neural activity and suppressed hippocampal function in response to chronic stress.
Figure 7.2 Overview of the neurobiological effects observed in the chronic footshock stress model. This figure is based on several studies, including the present thesis, which are discussed in the main text.

Effects on hippocampal neurogenesis

In this thesis, adult hippocampal neurogenesis was used as one of the main readouts of stress effects on the brain. Impact of chronic footshock stress on different stages of the neurogenesis process was investigated in chapter 2. These experiments revealed that exposure to chronic footshock transiently suppressed hippocampal cell proliferation: whereas the number of dividing progenitors decreased 2 hours after the last stress exposure, it returned to control levels within the next 24 hours. Similar dynamics of stress effects on new cell production was reported in a social defeat model in mice (Lagace et al., 2010). As described above, the present study also assessed changes in cell proliferation after acute footshock, but no significant effects were found. Thus, negligible impact of a single footshock session became evident after repeated stress. Such gradually developing changes in the production of new cells in the course of chronic stress might have consequences in the long run. The present study showed that chronic footshock stress decreased the number of newly-born immature neurons in the dentate gyrus. Although several processes might account for changes in this neuronal population, the transient reduction in the generation of new cells by the end of the chronic stress period is a likely cause since fewer cells born after stress may yield fewer young neurons (Dagyte et al., 2009).

Repeated footshock had no effect on survival of hippocampal cells that were born several days before the start of the chronic stress protocol (a single BrdU labeling on day −4) (Dagyte et al., 2009). Notably, a previous report using the same stress model showed that repeated footshock decreased survival of cells born early during the chronic stress period (daily BrdU injections on days 4–8) (Westenbroek et al., 2004). However, when proliferating cells were labeled in the second half of the chronic footshock stress period (daily BrdU injections on days 13–16), only a non-significant trend towards a decrease in cell survival was found (Kuipers, 2004). Given such BrdU labeling schedules in the latter two studies, they were unable to isolate effects of stress on cell proliferation per se from the mere impact on cell survival that occurs later. Nevertheless, these data together suggest that hippocampal cell survival is regulated dynamically by chronic footshock stress. Thus, the final outcome of each study might very much depend on the timing of BrdU labeling as well as on other factors known to influence adult neurogenesis. Literature suggests that similar dynamics of stress impact on hippocampal cell survival also occurs in response to other forms of stress. For instance, a recent study reported that chronic restraint stress increased the survival of 14-day old cells, but did not affect survival of 21-day old cells (Snyder et al., 2009). In sum, this shows that subtle changes in the study design may explain some of the variation reported in the literature.
The overall impact of chronic stress on hippocampal neurogenesis in this stress model was rather mild. Yet, exposure to footshock repeatedly and strongly activated the HPA axis. Together, these findings point to a limited role of glucocorticoids in regulating neurogenesis. Although an increase in CORT levels has been suggested as a prerequisite for negative effects of stress on neurogenesis, it alone is, however, not sufficient and does not strictly correlate with the effect on newly-generated cells as discussed in detail in chapter 2 of this thesis.

**Effects on neuronal plasticity**

Previously, multiple changes in cellular signaling cascades regulating neuronal plasticity were reported using the chronic footshock stress model. Repeated footshock was shown to target the ERK1/2–CREB cascade; specifically, it increased ERK1/2 phosphorylation in dendrites of the mPFC and reduced CREB phosphorylation in neurons of various cortical regions (mPFC, cingulate and perirhinal cortex) as well as subcortical areas (hippocampal dentate gyrus, lateral and basolateral amygdala, and paraventricular thalamic nucleus) (Kuipers et al., 2003; Trentani et al., 2003; Kuipers, 2004). In addition, chronic footshock was reported to decrease the expression of calcineurin (PP2B) in the cingulate cortex and hippocampus (Kuipers et al., 2003; Kuipers, 2004).

**Effects on synaptic plasticity**

The chronic footshock stress model was also associated with changes in the expression of several synaptic proteins. Specifically, repeated stress was shown to increase mRNA levels of synaptotagmin, synaptophysin and synapsin in both the mPFC and hippocampus (Kuipers, 2004). This thesis further investigated chronic footshock-induced effects on synapsin I (SynI) expression and its phosphorylation pattern in several stress-related brain regions. These immunohistochemical findings are described in detail in chapter 5. In brief, chronic stress induced region-specific changes in total and phosphorylated SynI. Repeated footshock increased expression of total SynI protein but decreased the fraction of its phosphorylated form in the mPFC. Yet, chronic stress did not affect total SynI in the hippocampal subregions but selectively decreased the fraction of phosphorylated SynI in the outer and middle molecular layers of the hippocampal dentate gyrus. Given the function of SynI in synaptic transmission and plasticity, these data suggest alterations in synaptic regulation following chronic stress.
Validity of the model

The previous paragraphs described a variety of effects reported in the chronic footshock stress model. Yet, it is important to ask to what extent these findings represent changes in the human situation.

Animal models for any human disorder are assessed by their construct validity (theoretical rationale of the model), face validity (phenomenological similarities between the model and the disorder), and predictive validity (correspondence of drug action in the model to that in the clinic). The construct validity of the chronic footshock stress model is based on the notion that stress, especially when severe and prolonged, might predispose to affective disorders, including major depression. However, this model has shortcomings with respect to face validity. Minor behavioral changes observed so far are difficult to compare to the clinical symptoms of patients suffering from major depressive disorder. Although loss of appetite and body weight are often associated with major depression, their occurrence alone is not sufficient to diagnose this disorder. A strong induction of the HPA axis in response to footshock confirms the aversive nature of this stressor. Nonetheless, this model does not seem to produce long-term endocrine changes that are often observed in depressed patients. Chronic footshock stress induces a variety of effects on neuronal and synaptic plasticity, which are proposed to underlie the pathophysiology of major depressive disorder. At present, it is not clear whether these changes are cause or consequence of depression, as clinical research on this topic is hampered by technical limitations. Yet, since chronic stress-induced alterations are, at least partly, reversed by antidepressant drugs, this model shows predictive validity.

In conclusion, albeit limited in effects at the behavioral level, the chronic footshock stress model is associated with changes at the cellular and molecular level. Although these alterations are rather mild, yet, they may indicate impaired brain plasticity and increased vulnerability to stress-related pathology.

The chronic mild stress model: effects on hippocampal neurogenesis

Another stress model used in this thesis is the chronic mild stress (CMS) paradigm (chapter 4). This model has been reviewed extensively (Willner, 1997, Willner, 2005); therefore, the current text will focus on specific effects obtained in the present study. The primary goal of this experiment was to further characterize the actions of the antidepressant agomelatine in the stress-compromised brain. Nevertheless, here I will briefly discuss stress-induced effects on hippocampal neurogenesis in this model.
Although this experiment was not designed to study differences between two stress paradigms (chronic footshock and CMS), their neurobiological effects will be indirectly compared.

As described in chapter 4, exposure to a five-week CMS paradigm did not change the rate of hippocampal cell proliferation. Nevertheless, it decreased the survival of hippocampal cells, born before the stress period. Moreover, CMS was associated with a reduction in the expression of DCX, a protein present mainly in the newborn immature neurons. These data suggest that distinct aspects of the neurogenesis process might be differentially regulated by stressful stimuli. Chapter 4 provides an overview of other studies supporting this notion as well as discusses possible mechanisms underlying such distinct effects. Here, I will focus on the lack of changes in hippocampal cell proliferation following CMS.

As indicated previously, timing is an important issue in this type of research. The temporal dynamics of footshock stress-associated changes in cell proliferation is described in chapter 2. In brief, chronic footshock stress had a short-term effect on new cell production, which was detected 2 hours post-stress but gone 24 hours later. The sacrifice of animals in the CMS experiment was carried out 4–6 hours after the last stress session. At present, this looks like a methodological flaw, when viewed from the perspective of above described results. However, the results of chapter 2 were not yet completely analyzed when the CMS experiment was designed. Anyhow, I do not expect that CMS would have inhibited cell proliferation, even if rats were sacrificed 2 hours after the last stress session. The present data do not indicate even a slightest tendency towards a decrease in new cell production. Therefore, it is rather hard to comprehend that the rate of cell proliferation recovered completely within a couple of hours. Hence, there must be an alternative explanation for the lack of effect on newborn cell generation. The last stressor in the current CMS paradigm was exposure to predator sounds. Being a rarely used stressor, its effects are unknown. Therefore, another possibility is that animals were not sufficiently stressed right before they were sacrificed. Hippocampal neurogenesis, and cell proliferation in particular, is a highly dynamic process, heterogeneously regulated by various factors that act in concert to shape the final overall effect. Thus, it might be that other procedures performed in this experiment, e.g. behavioral testing, also influenced the rate of cell proliferation, and thereby masked the stress effect. Of course, it is also feasible that the current CMS paradigm simply did not inhibit cell proliferation.
Different stress models: distinct effects on hippocampal neurogenesis

Interestingly, the present findings in the CMS paradigm partly contrast with those observed in the chronic footshock stress model. Whereas both paradigms induced changes in DCX-positive hippocampal neuron population, yet, they had distinct effects on cell proliferation and survival. The different outcomes obtained in two stress models cannot be compared directly. For instance, it is not clear whether the CMS-induced decrease in cell survival is a result of specific stressors used or a longer duration of an overall stress period, or some other factors. Notably, newborn cells were labeled before the stress period in both experiments (BrdU injection on day –4); however, their survival was assessed at different time points after chronic stress (five weeks versus three weeks). Thus, this may again reflect the importance of timing in studying effects of stress on the dynamic process of hippocampal neurogenesis.

TARGETING NEURAL AND SYNAPTIC PLASTICITY BY AGOMELATINE

The action of agomelatine in the brain has been shown to involve changes in hippocampal neurogenesis and neuroplasticity in general (chapter 1). This information was obtained from experiments using unchallenged laboratory animals. However, antidepressant drugs are meant to treat pathological conditions and their effects might differ in disturbed versus intact systems (Berton and Nestler, 2006). Therefore, the present thesis investigated actions of agomelatine in the brain exposed to chronic stress as a risk factor for mood disorders.

Agomelatine-induced changes in the stress-compromised brain were the focus of the experiments described in chapter 3, 4, and 5. As mentioned previously, two stress models, namely chronic footshock and CMS, were used in order to characterize the actions of agomelatine under conditions of prolonged stress. Despite differences in stress paradigms and their evoked effects on the brain, here I will attempt an overall synthesis of the findings on agomelatine. Hence, I will refer to “the action of agomelatine in the stressed brain”, and will not specify the type of stress involved.

The present data suggest that agomelatine acts by normalizing stress-affected hippocampal neuronal activity (chapter 3). Moreover, this antidepressant enhances adult hippocampal cell proliferation and survival under conditions of chronic stress.
and/or reverses stress-induced changes therein (chapter 3 and 4). Furthermore, agomelatine counteracts, at least partly, the stress-associated decline of DCX expression in the hippocampal dentate gyrus. Interestingly, in control conditions agomelatine itself reduces the expression of DCX, which may depend on its ability to speed up the maturation of newborn cells (Soumier et al., 2009) and shortening in the time window of the expression of this marker (Banasr et al., 2006). Finally, agomelatine treatment is associated with region-specific changes in SynI, a presynaptic protein involved in the regulation of synaptic transmission and plasticity (chapter 5). Importantly, this antidepressant prevents some of the stress effects on SynI in the mPFC and reverses stress-induced alterations in the hippocampal dentate gyrus. Altogether, this indicates that modulation of neural and synaptic plasticity underlies the action mechanism of agomelatine (figure 7.3).

**Figure 7.3** The figure depicts the multiple targets by which agomelatine may increase brain plasticity and cellular resilience. This model is based on several studies, including the present thesis, which are discussed in the main text. The targets that have been shown to participate in the action mechanism of agomelatine are framed; the others represent putative players. 

As described in chapter 1, agomelatine is an agonist of the melatonergic MT₁/MT₂ receptors, and also a serotonergic 5-HT₂C receptor antagonist. Neurobiological effects of agomelatine may, at least in part, depend on its synergistic action through these receptor sites (Soumier et al., 2009; de Bodinat et al., 2010; Tardito et al., 2010). The downstream signaling of agomelatine, depicted in figure 7.3, is proposed to involve multiple molecules implicated in brain plasticity and cellular resilience. Treatment with agomelatine was associated with increased BDNF expression in the hippocampus; this effect involved synergy between melatonergic and 5-HT₂C receptor-dependent pathways (Soumier et al., 2009). Moreover, agomelatine was reported to increase the phosphorylation of ERK1/2, Akt and GSK3β, intracellular signals known to convey the effects of antidepressants and mood stabilizers on hippocampal neurogenesis and cell survival (Jiang et al., 2005; Silva et al., 2008; Wexler et al., 2008). Importantly, inhibition of GSK3β by phosphorylation was suggested to exert antiapoptotic effects (Jope and Bijur, 2002) as well as to produce antidepressant-like behavioral changes in rodents (Gould et al., 2004). Phosphorylation of ERK1/2 activates the transcription factor, CREB, implicated in the regulation of neuroplasticity, neurogenesis and survival through the downstream gene, e.g. BDNF, transcription. ERK1/2 also induces phosphorylation of SynI, a synaptic vesicle-associated protein involved in neurotransmitter release. In turn, modulation of synaptic transmission by SynI might contribute to cellular resilience. Furthermore, treatment with agomelatine was shown to enhance noradrenergic and dopaminergic transmission; this effect was achieved through 5-HT₂C receptor blockage (Millan et al., 2003; Millan et al., 2005). Increase in noradrenaline and dopamine might also ultimately lead to activation of CREB and thereby contribute to its associated effects on brain plasticity and cellular resilience.

Overall, the cascades targeted by agomelatine are similar to those involved in the mechanism of action of other antidepressants and mood stabilizers (Malberg and Blendy, 2005; Tardito et al., 2006; Schloesser et al., 2008). This notion thus highlights the possibility that drugs with diverse pharmacological profiles ultimately converge on the same signaling cascades. The following neuronal and synaptic changes might be required to attain an adaptive response of the brain underlying clinical recovery and/or prevention of psychopathological syndromes. If the neuroplasticity hypothesis of depression holds true, then such convergence in cellular signaling governing brain plasticity may also provide an explanation for a rather similar clinical efficacy of various antidepressant drugs. Diversity in their targeted receptor profile, however, may account for differences in tolerability and side effects associated with antidepressant therapy.
The innovation of agomelatine thus lays in its actions achieved through melatonergic receptors, in combination with the blockage of 5-HT$_{2C}$ receptors. Importantly, treatment with agomelatine does not modify extracellular levels of serotonin and does not down-regulate presynaptic and postsynaptic 5-HT$_{1A}$ receptors, contrary to the effects of the classical antidepressants, SSRIs.

These data on agomelatine, however, has been gathered in the experiments using male animals only. Given the higher prevalence of depressive disorders in women and the existence of gender dimorphisms in the mechanisms underlying associated neuronal dysfunctions, the actions of agomelatine should also be evaluated in the female counterpart.

**TOWARDS AN INTEGRATED VIEW OF DEPRESSION**

Depression has been identified since antiquity but it is still conceptualized as a disorder without firmly established mechanism. As stated in chapter 1, many different theories have been postulated in an attempt to elucidate its etiology and pathophysiology. To this date, however, none of the proposed hypotheses alone could adequately explain the mechanisms underlying development and treatment of depression. Nonetheless, each and every theory has yielded valuable insights into specific aspects of this illness.

An intriguing possibility holds that previously highlighted multiple hypotheses may act in concert to explain neuronal defects underlying major depressive disorder. For instance, changes in the HPA axis activity, neurotransmitter systems, neuroplasticity, proinflammatory cytokines and other factors implicated in depression together with individual genetic make-up may comprise vulnerable phenotypes prone to the development of this disorder (see figure 1.1 in the introductory chapter). Hence, an attempt to integrate actions of these diverse players may help to formulate a unifying hypothesis of depression. A true understanding of the pathophysiology of depressive disorder must, therefore, also encompass changes at different levels at which the disease manifests: molecular, cellular, systemic, and behavioral (figure 7.4).

The multiple hypotheses of depression yield a variety of changes associated with this complex disorder. Despite conceptual differences, these theories do not exclude each other but rather complement one another or even overlap. For instance, the serotonergic system influences the HPA axis regulation and its feedback mecha-
Figure 7.4 The figure illustrates multiple factors that encompass the pathophysiology of major depressive disorder at different levels. These levels are indicated in the central part of the figure. On the left, the findings of the current thesis are outlined. On the right, the literature data are summarized. Adapted from Schloesser et al., 2008.


nisms (Chung et al., 2000; Hemrick-Luecke and Evans, 2002); vice versa, corticosteroids modulate 5-HT synthesis and turnover (Azmitia et al., 1993; Clark and Russo, 1997). Furthermore, the monoamine systems as well as glucocorticoids have been implicated in regulation of neuronal plasticity and hippocampal neurogenesis (Brezun and Daszuta, 1999; Gould, 1999; Huang and Herbert, 2005). Interestingly, whereas these processes are enhanced by antidepressants with different modes of action, they are oppositely regulated by stress (Dranovsky and
Hen, 2006; Pittenger and Duman, 2008). Although the exact mechanisms underlying antidepressant- and stress-evoked changes in neuroplasticity are not yet fully understood, the current evidence suggests the convergence of their effects on common molecular pathways (Pittenger and Duman, 2008). Thus, depressive disorder may involve alterations in neuronal and synaptic plasticity, triggered by various intrinsic and extrinsic factors predisposing to psychoneuropathology. Chapter 6 of the present thesis provides yet another example of how several theories of depression may be integrated in an attempt to explain cognitive impairments associated with this illness.

Hence, approaching depression from a broader perspective and acknowledging the role of different systems in its etiology and pathophysiology may yield a more complete picture on the nature of this disorder (see figure 1.1 in the introductory chapter). Naturally, the degree of influence conveyed by multiple factors will differ in individual cases of depression, thereby clarifying its symptom heterogeneity, highly variable course and differences in response to treatment.

**FUTURE VIEWS AND CONSIDERATIONS**

In view of future research, it would be of interest from a clinical perspective to further pursue the neurobiological substrates and neurochemical profiles underlying the states of chronic stress and depression in the human brain. Advances in the imaging techniques might one day allow us to directly assess the aforementioned processes of neuroplasticity in the living human brain, and to trace time-dependent changes in the course of disease and its treatment. The ongoing clinical investigations of the role of inflammation in depression hold promise for better understanding of the etiopathogenesis of this disorder. Further genetic analyses are necessary to reliably determine the impact of genetic make-up in depression, as the findings of previous association studies have often been inconsistent and difficult to replicate (Lopez-Leon et al., 2008; Bosker et al., 2010). Altogether, research on depression would benefit from longitudinal clinical studies followed up by postmortem investigation of the brain. Despite the emerging prospects for examining the disease mechanisms in humans, scientific advances in this research field will also depend on preclinical investigations using animal models.

Several issues should be considered when translating the preclinical findings in laboratory animals to the human situation. First, as already discussed in the present thesis, no experimental paradigm in animals can fully mimic depressive disorder in
humans. This is unfortunate because complex illnesses, such as depression, are at present unlikely to be fully unraveled without animal models; yet, a good experimental paradigm requires thorough knowledge of the mechanisms of the disease, thereby creating a catch-22 situation. Second, depression occurs more than twice as often in women than in men. Yet, most preclinical studies, including the current thesis, focus on changes in males. Since findings associated with one gender cannot be automatically extrapolated to the other, future research should pursue the neurobiological substrates underlying development and treatment of depression in both genders. Third, the rodent and primate brain differ structurally and functionally; therefore, effects of stress in rat and/or mouse brain might not adequately correspond to depression-associated changes in human brain, especially in its highly developed cortical areas. This could be illustrated by an example of the PFC, which is not only subdivided differently in rodent and primate brain but also partly contrasts in the functional characteristics of its subregions (box 7.1). Literature suggests that the infralimbic cortex (IL) of the rat is homologous to the ventromedial PFC (VMPFC) in humans, and that the rat prelimbic cortex (PL) corresponds to the dorsolateral sector (DLPFC) of the human PFC (Vertes, 2006). Functionally, both the IL and VMPFC are involved in visceromotor control of emotion, although VMPFC is also ascribed “affective” functions. The dorsal sector of the PFC, mainly implicated in “affective” and “cognitive” activities in rat brain (PL), however, is primarily associated with “cognitive” or “executive” functions in human brain (DLPFC) (Vertes, 2006; Koenigs and Grafman, 2009). This illustrates that comparisons between certain PFC regions in rodent and primate brain should be exercised with caution. Interestingly, the majority of studies on depression in humans report alterations in the DLPFC, whereas preclinical research using rodents usually investigates changes in the entire mPFC and only occasionally mentions its subregions. Given the disparate functionality of the mPFC areas, the work described in this thesis intentionally focused on the changes in the PL, proposed to most closely relate to the human DLPFC.

Besides the PFC, multiple other brain regions show functional impairments in major depressive disorder. Nevertheless, recent research has mainly focused on the hippocampal formation. Although this brain region is important in regulating the HPA axis and orchestrating other limbic structures involved in affection, yet, the main function of the hippocampus remains contextual learning and memory. In view of this, the specific structural and functional alterations in the hippocampus may underlie the cognitive symptoms of depression. This issue is covered in chapter 6 of the present thesis. The hippocampal formation is subdivided into dorsal and ventral parts, and the latter is suggested to play a role in the affective symptoms of depression (Bannerman et al., 2004). Other limbic brain regions, including amygda-
CHAPTER 7

Box 7.1
Subregions of the PFC: nomenclature and function

The PFC in both humans and rodents is organized in a topographical manner, such that regions regulating emotions are situated ventrally and medially, and regions regulating thought and action are located more dorsally and laterally (Arnsten, 2009). In humans, the PFC is generally divided into the ventromedial (VMPFC) and dorsolateral (DLPFC) sectors (Koenigs and Grafman, 2009). The VMPFC includes the ventral portion of the medial PFC and medial portion of the orbitofrontal cortex. The main function of the VMPFC is regulation of emotional responses, e.g. processing of fear and modulation of the visceral autonomic activity associated with emotion. The DLPFC includes portions of the middle and superior frontal gyri on the lateral surface of the frontal lobes, and plays a key role in regulating attention, thought and action (Arnsten, 2009; Koenigs and Grafman, 2009). The PFC in rats is divided into the orbital, medial and lateral sectors, however, a generally accepted nomenclature for the PFC subregions is still lacking (Uylings et al., 2003). According to the rat brain atlas consulted in the current thesis (Paxinos and Watson, 2007), the PFC is divided into the medial orbital (MO), infralimbic (IL), prelimbic (PL), anterior cingulate (AC or Cg1), and secondary motor (M2) areas. The AC, PL and IL together comprise the medial PFC (mPFC), suggested to functionally correspond to the primate PFC (Vertes, 2006). Whereas AC has been linked to various motor behaviors, ventral regions of the mPFC (IL and PL) have been associated with diverse emotional, cognitive, and mnemonic processes (Heidbreder and Groenewegen, 2003). More specifically, the IL of rats primarily controls visceromotor functions, and the PL is involved in limbic and cognitive activities (Vertes, 2006)
lar nuclei and the aforementioned subregions of the PFC, closely interact with the ventral hippocampus as well as with one another in regulating mood and emotions. Their function implies a more straightforward role in stress-related pathologies, and therefore urges future research to move beyond the hippocampus and pay more attention to other brain structures.

The biased focus on the hippocampus in stress research could be explained by the discovery of dentate neurogenesis in adulthood and its linkage to depression. The majority of the experiments described in the present thesis also used hippocampal neurogenesis as the readout of stress and antidepressant effects on the brain. In order to understand the functional implications of these findings, it is crucial, however, to functionally decipher the process of adult neurogenesis. The present debate on the function of hippocampal neurogenesis has suggested its involvement in the processes of learning and memory as well as in the regulation of mood and emotion (Sahay and Hen, 2007; Deng et al., 2010). Consequently, suppressed dentate neurogenesis has been proposed as a final common pathway in many brain disorders associated with mood and cognitive dysfunction, such as geriatric depression and the depression-mild cognitive impairment-dementia complex (Maes, 2009). Nevertheless, the precise function of adult-born dentate granule cells in these processes remains elusive. There is no direct evidence that hippocampal neurogenesis is causally implicated in the development of depressive disorder, although it might mediate the behavioral effects of antidepressant drugs under certain (Santarelli et al., 2003; Surget et al., 2008; David et al., 2009) but not all conditions (Bessa et al., 2009; David et al., 2009). Therefore, the aim of future research on this topic is to elucidate the function of adult neurogenesis, in order to adequately interpret addition and removal of new neurons to the hippocampal circuit. From a broader perspective, it would be helpful to better understand the role of the dentate gyrus in hippocampal functioning as it might explain how changes in information processing by this circuitry relate to depression.

CONCLUDING REMARKS

Stress and its related disorders pose a considerable threat to health in modern societies. Much attention in research has been devoted to deciphering mechanisms underlying maladaptation to stress and development of associated psychopathologies, including major depression. Although many drugs have been developed to treat this pathological condition, their therapeutic effects are suboptimal and the specific mechanisms of action remain obscure. A better understanding of the patho-
physiology of stress, a risk factor of depressive disorder, may therefore clarify fundamental changes in the brain predisposing to psychopathology, and enable the development of novel therapeutic strategies. These and related issues were at the core of the present thesis, which largely focused on investigation of the neurobiological effects associated with chronic stress and antidepressant treatment in adult rats.

Taken together, the work presented in this thesis supports the notion that prolonged stress is accompanied by neuronal and synaptic changes in the brain. Nonetheless, the extent of these effects is highly dependent on the timing of their investigation as well as on the type and duration of stress procedure. The current thesis also suggests that the processes of neuronal and synaptic plasticity may underlie the action mechanism of agomelatine, the antidepressant with an innovative pharmacological profile. Further characterization of cellular signaling cascades targeted by agomelatine is necessary to more thoroughly understand the molecular pathways governing its therapeutic actions. This information might also yield more general insight into mechanisms underlying the recovery from affective disorders, and thereby, may open up novel prospects for their treatment and prevention.