Chapter 6

General discussion
Major depressive disorder (MDD) is a multifaceted disease with a broad spectrum of symptoms. Evidently, the underlying causative factors of MDD are diverse in nature. Environmental factors exert a major influence on the initiation of MDD; a common factor causing the disease is stressful life events. Stress impacts on the individual thereby affecting mood- and cognition-related functioning. In particular, this disease-triggering factor can be modeled in animals, thereby providing the opportunity to investigate the molecular and cellular changes in distinct brain areas.

In this thesis, research focused on the impact of a natural occurring type of stressor, social defeat stress, on the functioning of the hippocampus in terms of learning and memory and the potential alleviating role of antidepressant and behavioral therapy. This thesis yielded several novel findings concerning the molecular and cellular correlates of MDD that originated from animal models of depression, and will be discussed below.

**Major conclusions**
First, we identified a mechanism by which ketamine may exert a direct and lasting antidepressant effect. We found that ketamine probably exerts its long-term antidepressant effects by increasing hippocampal synaptic membrane α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor function. This sheds new light on using ketamine in the treatment of depression.

Second, we found that impaired hippocampal function observed both short- and long-term after social defeat stress originated from different synaptic mechanisms. In particular, we noticed alterations in the glutamate receptor system only at the short-term, whereas on both time points hippocampal spatial memory performance was clearly affected.

Third, we showed that long after social defeat stress, hippocampal plasticity was severely affected. This translates into concomitant behavioral dysfunction. The long-term impairment of the hippocampal functioning can be alleviated by imipramine treatment and, much to our surprise, to the same extent with behavioral therapy.

Fourth, we found that neurogenesis in the hippocampal dentate gyrus was affected in a lasting manner after social defeat by a decrease in a distinct neuronal population of more mature newborn cells. Here, imipramine was able to rescue the neurogenic deficit.

Fifth, we were able to identify synaptic mechanisms that are regulated long-term after social defeat stress. A proteomics analysis turned out to be a useful approach to identify potentially new targets for MDD research.
Overall, in this thesis, observations are made concerning the molecular and cellular changes in the hippocampus related to depressive-like phenotypes. In the next sections I will discuss these findings in the context of ongoing MDD research.

Animal models of depression

Model validity
To appreciate the improvement of using animal models for depression based on long-term effects of stress over acute stress, it is important to compare how they meet criteria for a valid animal model.

At the very least, animal models must resemble the human condition in several respects\(^{220}\), including (a) similarity in the symptom profile presented (face validity), (b) amelioration or attenuation by treatments effective in treating the human condition (predictive validity), (c) provocation by events thought to be important in eliciting the human disorder (etiologic validity), and (d) involvement of similar neurochemical processes (construct validity) (Box 1).

An acute stress model to study antidepressant action
In this thesis several stress-induced animal models for depression have been used. The most basic and straightforward is the forced swim test (FST). This model has been proven efficient in predicting the clinical efficacy of antidepressants in a cost efficient way and therefore has good predictive validity\(^{80}\). However, this model has low etiologic validity as acute swim stress is used to induce a depression-like phenotype. Also, its face validity is limited as only behavioral despair is accessed as readout parameter.

Using the predictive validity of this model, we showed in chapter 2 that ketamine, an N-methyl-D-aspartic acid (NMDA) receptor antagonist with acute and lasting clinically antidepressant effects, also showed acute and lasting efficacy in the FST and we revealed aspects of the contributing synaptic mechanisms. However, considering the limited validity of the test, care should be taken when interpreting the clinical relevance of these findings.

Using chronic stress to gain insight into mechanisms of depression
To gain insight in the molecular and cellular neurobiology of depression, animal models with the highest attainable validity should be used. In rodents, the social defeat paradigm is a good candidate. Since a natural type of social defeat stress is used, this model has good...
Box 1. Validation criteria for evaluating animal models for psychiatric disorders

<table>
<thead>
<tr>
<th>Category of validation criteria</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face validity</td>
<td>The phenomenological similarity between the behavior exhibited by the animal model and the specific symptoms of the human condition.</td>
</tr>
<tr>
<td>Predictive validity</td>
<td>The ability to predict changes in the human subject based upon changes in the model. This requires constant reality checking with clinical measures to make sure that the changes in the model correspond to those in the human. In terms of drug development, the special condition of predictive validity is usually determined through pharmacological validation that refers to clinically effective drugs showing activity in the test or model (pharmacological isomorphism).</td>
</tr>
<tr>
<td>Etiologic validity</td>
<td>The concept of etiological validity is closely related to the causes of the disorder in humans. When etiology can be established, the model becomes very useful. Unfortunately, the causes of behavioral disorders are often diverse. Therefore, this validity is limited to hypothesis regarding possible etiology.</td>
</tr>
<tr>
<td>Construct validity</td>
<td>Construct validity is closely related to the pathology and symptomatology of the disorder, and the accuracy with which changes in the model organism reflects that in the human. For example, close correspondence of changes in neurochemical or endocrinological parameters in depressed subjects and in the model systems used study depression endow the model with increasing construct validity.</td>
</tr>
</tbody>
</table>

Etiologic validity, especially since chronic psychosocial stress is particularly effective in predicting human depression. Moreover, social defeat has been shown to induce behavior relevant to depressive symptoms both acute and long-term after defeat stress. These symptoms include anhedonia (reduced sucrose preference or anticipation towards sucrose), behavioral despair, and reduced social interest, and therefore the model meets the criterium of face validity. Moreover, these symptoms were counteracted by chronic, but not acute antidepressant treatment thereby establishing appropriate predictive validity. Finally, rodents subjected to the social defeat paradigm show neurobiological changes also observed in postmortem human studies, such as increased BDNF expression in the nucleus accumbens and reduced hippocampal volumes indicating that the paradigm has at least to some extent construct validity.

Here we adopted a social defeat paradigm, in which rats were subjected to severe social defeat stress once every day for five days. In chapter 3, we showed that this results in an overactive hypothalamic-pituitary-adrenal (HPA) axis response 24 hour after the last social defeat encounter, expressed by an increase in plasma levels of the stress hormone corticosterone. This further supports the construct validity of social defeat stress, as dysregulation in HPA axis responses are also observed in MDD patients. As further shown in this chapter, this social defeat stress reduced performance in a hippocampus dependent spatial memory task, a phenotype relevant to the cognitive symptoms of depression.
Long-term effects of chronic stress

In humans, depressive episodes are still apparent long after the cessation of the stress exposure and depression is often considered as a chronic disease. Moreover, depression is a highly recurrent disorder; more than 75% of depressed patients have more than one depressive episode, often relapsing within two years of recovery from a depressive episode\textsuperscript{225}. Indeed, between one-half and two-thirds of the people, who have ever been clinically depressed, will be in a relapse episode in any given year over the remainder of their lives\textsuperscript{226}. Therefore, interest is in understanding the underlying mechanisms that determine particularly longitudinal aspects of the disorder and its maintenance\textsuperscript{182}.

To model the maintenance phase of depression rather than its induction phase, thereby increasing validity of the social defeat model, we adopted a social defeat paradigm in which five days of social defeat stress was followed by individual housing for 12 weeks\textsuperscript{33}. In chapter 4, we confirmed face validity by showing that this model induces depressive-like behavior by invoking anhedonic behavior, as indicated by reduced anticipation towards a palatable 5% sucrose solution, and by depression-associated cognitive impairments. Moreover, predictive validity was confirmed by showing that both behavioral and antidepressant therapy recovered both symptoms.

Novel neurobiological mechanisms of depression

Synaptic plasticity processes

Throughout this thesis, several novel mechanisms have been characterized concerning stress-induced depressive-like phenotypes and antidepressant action. These mostly concern synaptic plasticity processes. In chapter 2 of this thesis, we showed that ketamine exerts its antidepressant effects by interfering with glutamatergic signaling in hippocampal synapses. Ketamine is a non-competitive (NMDA) receptor antagonist that has been shown to have immediate antidepressant effects in treatment-resistant patients when administered at a subanesthetic dose\textsuperscript{31,85}. Its primary mechanism of action is blocking the NMDA receptor at the phencyclidine site, i.e. in the pore of the ion channel. In addition, ketamine induces rapid increases in presynaptic release of glutamate, a process hypothesized to be mediated by NMDA autoreceptors, and/or mediated by activated GABAergic neurons\textsuperscript{89}. We show that the long-term antidepressant effects of ketamine in the FST were paralleled by increased Ser-845 phosphorylation of GluA1 in hippocampal synapses. Moreover, a molecular blockade of regulated AMPAR endocytosis, using a TAT-Glu2\textsubscript{3Y} peptide in the CA1 region of the dorsal
hippocampus, mimicked this antidepressant effect. This substantiates the hypothesis that the antidepressant effects of ketamine are initiated by an increased AMPAR turnover and functionality. These results argue that ketamine exerts its antidepressant effects through glutamatergic signaling mechanisms. The role of glutamate signaling in MDD is supported by growing evidence showing that antidepressants ultimately converge to regulate AMPA and NMDA receptor-mediated synaptic plasticity\textsuperscript{94}, via a cascade of time-dependent signaling.

Using the social defeat paradigm, we found that after severe social stress synaptic plasticity mechanisms are affected. In chapter 3, we showed that reduced spatial memory after social defeat is associated with reduced hippocampal synaptic expression of the glutamatergic receptor subunits GluN1, GluN2B and GluA2. In particular, the regulated synaptic expression of NMDA receptor subunits provides a dynamic and potentially powerful mechanism for the regulation of synaptic efficacy and remodeling. Indeed, it has been shown that alterations in NMDAR number and/or subunit composition contribute to the expression mechanisms of LTP (NMDAR-LTP)\textsuperscript{150} and LTD (NMDAR-LTD)\textsuperscript{151}. This is another example in which synaptic glutamate signaling plays a role in generating a depressive phenotype. The reduced expression of NMDA receptor subunits is of particular interest since it might represent a mechanism of metaplasticity in the glutamatergic system\textsuperscript{37}, and most likely underlies the cognitive impairments in a hippocampus-dependent memory task.

Long after social defeat, synaptic changes are also apparent (chapter 4). Brain slice recordings of the hippocampus revealed a typical depressed state of the synapse. This reduced LTP in CA1 subfields probably affected hippocampus-dependent cognitive performance. We then used a proteomics analysis to identify synaptic alterations at the molecular level. The cognitive impairments and reduced LTP were associated with an increased hippocampal synaptic expression of the Na,K ATPase subunit AT1B2. Na,K ATPases are membrane localized proteins responsible for active transport of Na\textsuperscript{+} and K\textsuperscript{+} ions across the plasma membrane, thereby generating a gradient responsible for cell polarization and repolarization\textsuperscript{173}. Increased expression and increased transporter activity could cause a faster clearance of K\textsuperscript{+} from the synaptic cleft, and of Na\textsuperscript{+} from the intrasynaptic compartment. This would result in a faster re-polarization after firing of an action potential, and therefore less glutamate release and calcium influx. Together, this might cause less plasticity in hippocampal synapses and underlie impaired LTP. Moreover, this Na,K ATPase dependent mechanism is a possible route through which stress can alter plasticity mechanisms, thereby inducing depressive symptoms.
Different mechanisms short and long-term after stress

Stress has well-established effects on spatial memory performance in rodents by affecting hippocampal LTP\textsuperscript{40,41,43}. Since hippocampal LTP is dependent on subunit specific regulation of AMPA receptors, it is not surprising that synaptic expression of GluA2 is affected after social defeat stress. In line with this, the stress hormone glucocorticoid enhances AMPA receptor mobility in hippocampal synapses\textsuperscript{135}. Our observation that also synaptic localization of two NMDA receptor subunits was affected provides another mechanism through which plasticity mechanisms are regulated acutely after stress.

Also, long after social defeat spatial memory performance and LTP in the CA1 subfields were reduced hinting towards altered glutamatergic signaling. However, in contrast to 24 hour after social defeat, none of the glutamate receptor subunits were differentially expressed in synaptic membrane fractions. Instead, we observed an increase in synaptic expression of AT1B2. The fact that this Na,K ATPase subunit was not differentially expressed 24 hours after social defeat (data not shown), further confirms that on the long-term different synaptic plasticity mechanisms are involved in causing reduced hippocampal LTP and memory. This argues that direct changes in levels of glutamate receptor subunits are involved in the installment of cognitive impairments immediately after stress exposure, while different synaptic mechanisms, e.g., changing the membrane potential, account for the maintenance of these symptoms.

Depressive-like phenotypes after normalization of corticosterone levels

The hypothalamic-pituitary-adrenal (HPA) axis has been found abnormal in depressed patients\textsuperscript{224}. For example, a significant percentage of depressed patients have increased levels of cortisol in the saliva, plasma and urine, and increased size (as well as activity) of the pituitary and adrenal glands\textsuperscript{227}. It is hypothesized that impaired hippocampal signal processing, due to damage by increased glucocorticoid levels, interferes with feedback inhibition of the HPA axis\textsuperscript{35}. The resulting hyperactive HPA axis would then lead to an inappropriate stress response and, thereby, to the installment of depressive symptoms. However, here we show that despite hippocampal signaling being affected long after social defeat stress, corticosterone levels were normal. This argues that impaired hippocampal processing can induce depressive-like phenotypes, independent of HPA axis dysfunction. This supports the hypothesis that HPA axis hyperactivity is not a simple consequence or an epiphenomenon of depressive phenotypes, but on the contrary, that it is a risk factor predisposing to the development of depression\textsuperscript{228}.
Social defeat stress affects hippocampal neurogenesis

In chapter 5, we showed that after three months of individual housing, following a 5-day period of repeated social defeat, hippocampal neurogenesis is reduced indicated by a decrease in doublecortin positive (DCX+) cells. This was most profound for older DCX+ cells with long apical dendrites, whereas younger cells remained unaffected. Chronic imipramine treatment subsequently increased both cell populations. Whether there is a causal relation between depressive phenotypes, cognitive impairments and a decrease in neurogenesis is still an active field of investigation. However both neurogenesis-dependent and neurogenesis-independent mechanisms are likely to contribute to the reversal of depressive-like behaviors by antidepressants. This is in agreement with the network hypothesis of depression stating that depressive-like behavior reflects problems in information processing within particular neural networks in the brain and that antidepressant drugs and other treatments that alleviate depression function by gradually improving information processing within these networks.

Behavioral therapy

Another interesting novel finding is the efficacy of behavioral therapy consisting of housing in an enriched environment for one hour daily. This type of therapy resembles activation and physical exercise therapy and aspects of behavioral therapy for depressed patients. Behavioral therapy was found equally effective in treating anhedonic symptoms, depression-associated cognitive impairments, as well as their underlying molecular and cellular correlates when compared with chronic imipramine treatment. Enriched environments produce functional and anatomical changes in neural networks that are reflected in the gradual improvement of natural behavior. In analogy, behavioral psychotherapy might also have therapeutic effects on mood disorders through use-dependent neuronal plasticity. Therefore, behavioral and pharmacological therapies, might all lead to improved information processing and mood recovery through mechanisms that stimulate similar processes of plasticity. In this scenario, a combination of drug treatment and psychotherapy would be expected to be more beneficial than either treatment alone, and there is evidence that this might be the case.

Depression and neuroplasticity

Increasing evidence demonstrates that neuroplasticity, a fundamental mechanism of neuronal adaptation, is disrupted in mood disorders and in animal models of depression. Chronic stress, which can cause depression, also disrupts neuroplasticity, whereas...
several forms of antidepressant treatment induce opposing effects. As discussed in the previous section, throughout this thesis, we made several novel observations supporting the neuroplasticity theory of depression. These findings hold throughout three different levels: structural plasticity, functional plasticity, and molecular mechanisms accompanying such changes. At the structural level we found that neurogenesis was affected after social defeat stress and imipramine treatment was able to restore the neurogenic process at this late time point, long after stress experience. This indicates that antidepressants might facilitate optimization of neuronal connectivity by increasing the choice of neurons available for selection through activity-dependent mechanisms. This process is expected to take time to develop and mature, which is consistent with the delayed appearance of the clinical effects of antidepressants.

Chronic stress impairs hippocampal LTP. Short after social defeat stress, we found that hippocampal memory impairments were associated with decreased synaptic expression of glutamate receptor subunits. These are possibly caused by synaptic signaling pathways affected by social defeat stress. For example, several forms of chronic stress have been observed to increase the phosphorylation of MAPK. The activation of MAPK appears to be critical for the effects of behavioral stress on hippocampal LTP. Also, increased stress hormone levels and chronic stress can impair CREB activity.

The increased expression of AT1B2 that is associated with a decrease in LTP long after social defeat stress is a novel mechanism by which hippocampal plasticity is affected, and supports the neuroplasticity hypothesis of depression. However, care should be taken when projecting these findings to other brain regions. For example, in the amygdala and nucleus accumbens stress has opposing effects when compared with our data and increases neuroplasticity. Thus, our findings are brain region- and circuit-dependent and relate to the specific role of the hippocampus in MDD pathophysiology. The observation that both behavioral therapy and imipramine treatment (both effective treatments for MDD) have similar effects on plasticity and reverse of depressive-like phenotypes at the affective and cognitive domain argues that these changes are relevant for the disease phenotype of MDD.

Clinical relevance

Depression and cognition
The observed changes in neuroplasticity in our depression model hint to aberrant information processing. Indeed, we found reduced cognitive hippocampal performance
CHAPTER 6

associated with plasticity changes both short- and long-term after social stress exposure. By employing the social defeat model, we were capable of modeling aspects of cognitive impairments after a severe stress experience. Socially stressed rats showed decreased spatial memory, a symptom also observed in MDD patients\textsuperscript{130}. Our finding that increased plasticity induced by both pharmacological treatment and behavioral therapy were able to relieve symptoms in both the affective and cognitive domain argues that improved information processing contribute to their antidepressant effects.

There is a long history of research investigating the interaction of cognition and emotion in MDD. Clinicians and researchers alike have focused on cognitive processes and on the content of depressive cognition in trying to gain a more comprehensive understanding of MDD. These studies postulate that “associative networks” lead to cognitive biases on negative emotions in depressed individuals. Biases in cognitive processes, such as attention and memory, may not only be correlates of depressive episodes; they may also play a critical role in increasing the individuals’ vulnerability for the first onset and recurrence of depression\textsuperscript{236}. Most cognitive theories propose vulnerability-stress hypotheses that posit that the onset of this disorder is due to the interaction of a psychological vulnerability (e.g., certain cognitions or particular ways of processing information) and a precipitating stressor. Importantly, one of the most effective interventions for depression, cognitive-behavioral therapy, focuses on modifying biased interpretations and dysfunctional automatic thoughts\textsuperscript{237}.

Depressed people experience difficulties involving concentration and memory (Burt et al. 1995). A general accepted attempt to integrate these findings with cognitive biased processes is the resource-allocation hypothesis. This postulates that because cognitive capacity is reduced, depressed individuals have deficits in remembering and in engaging in other effortful cognitive processes\textsuperscript{238}. Additionally, the amount of resources available for cognitive operations is limited and depression either occupies or functionally reduces these resources, for example, because resources are used by task-irrelevant emotional processing. Thus, deficits should become evident in effortful, resource-demanding components of memory tasks.

Given these still unsubstantiated views, the question remains how cognitive deficits are related to the hallmark feature of depression, the sustained negative affect. Therefore this interaction should be a focus of future depression research.
**Implications for treatment strategies**

Throughout this thesis, several observations were made with important implications for treatment strategies for MDD. Our social defeat paradigm models symptoms in the affective and cognitive domains. In this paradigm, depressive-like behavior is induced by social stress experience in adult rats and, therefore, models adult onset stress-induced depression with cognitive impairments seen in depressed patients\(^{239}\). Here, we found that behavioral therapy was just as effective as imipramine treatment in rescuing depressive phenotypes and their underlying cellular and molecular correlates. This argues that behavioral therapy should be explored further as treatment option for depressed patients with cognitive impairments, that do not need, do not respond to or have adverse effects from pharmacological treatment.

Furthermore, antidepressant effects of imipramine and behavioral therapy went hand in hand with an increase in hippocampal plasticity in glutamatergic systems as expressed by an increase in LTP after social stress. Also the acute and lasting antidepressant effects of ketamine were associated with a possible increase in AMPA receptor function in the hippocampus. This underscores that glutamatergic signaling pathways should be explored for potential therapeutic targets of MDD with faster therapeutic effects\(^{54}\).

Finally, we showed that during the maintenance phase, likely different cellular and synaptic mechanisms are involved in stress-induced depressive phenotypes when compared with synaptic changes involved in the establishment of these symptoms directly after stress exposure. Since MDD patients are mostly treated (long) after cessation of the stress period, i.e., when depressive symptoms are evidently manifest, data originating from paradigms that model the maintenance phase of depression might be more relevant for implementation of treatment strategies. Therefore I argue for a shift in pre-clinical depression research, in which studies should focus increasingly on the maintenance phase of the disease rather than the induction phase of depression.