Chapter 1

General introduction
1. Major depressive disorder

1.1 Clinical diagnosis of major depressive disorder

Major depressive disorder (MDD) is among the most common psychiatric disorders with a lifetime prevalence estimated at 16.2% in the United States\(^1\) (see www.hcp.med.harvard.edu/ncs/ for up to date and comprehensive statistics) and 17.5% in The Netherlands\(^2\), with females at higher risk than males. It is estimated that in the year 2020 MDD will be the second leading cause of disability, only to be surpassed by ischaemic heart disease\(^3\). The consequences of MDD are of compound nature and characterized by the fact that MDD has a high tendency towards relapse, recurrence and chronicity\(^4\).

Diagnosis of MDD is based on symptomatic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The diagnostic criteria for MDD require persistence of either depressed mood or loss of interest and pleasure (anhedonia), in association with at least four out of the following symptoms: inattention, fatigue, self-depreciating or suicidal thoughts, and disturbances of psychomotor activity, sleep, appetite and weight. Symptoms need to be present consistently for at least two weeks. The variety in clinical symptoms of MDD underscores the complexity of the disease and, therefore, MDD is regarded as a heterogeneous disorder comprising of many different syndromes rather than a single disease. In particular, the clinical course of MDD is pleiomorphic and varies from subthreshold syndromes, single episodes (short or long), multiple recurrent episodes with or without inter-episodic recovery, residual symptoms after an episode, to chronicity\(^5\).

1.2 The pathogenesis of depression

An open question in MDD is which factors underlie a person’s vulnerability (or resilience) to the disease. Various putative risk factors have been suggested arising from epidemiological studies but difficulties in differentiating between association and causation have left many inconsistencies. However to date, it is generally accepted that both genetic and non-genetic factors, and the interaction between those, comprise disease liability\(^6,7\).

Genetic epidemiological studies have shown depression to be a familial disorder with increased risk odd ratios (ratio of risks of first-degree relatives of MDD probands vs. the general population) of ~2.8\(^7\). Familial studies can, however, not distinguish between genetic and shared environmental influences. Therefore, twin studies using mono- and dizygotic twins have been performed. These confirmed that genetic influences for the most part contribute to this familial aggregation, and heritability of liability is estimated to be ~37%\(^7\). In
GENERAL INTRODUCTION

terms of genetic contribution in psychiatric disorders this makes MDD at the lower end of the scale. Of the environmental factors, the most consistent and dominant one is exposure to stressful live events. Twin studies have shown a causal relation between MDD and events related to ‘bad luck’ or stressful experiences related to a person’s own behavior (65 – 75%), with odd ratios – the ratio of the risk of disease to individuals experiencing an event compared with persons without the stressful event – of 2.33 and 5.64, respectively. Furthermore, the occurrence of stressors are 2.5 times more likely in depressed patients compared with controls, and in community samples, 80% of the depressed cases have experienced major life events prior to the onset. In particular chronic forms of stress, often psychosocial in nature, may predict precipitation of depression.

1.3 Brain structures involved in MDD

Despite the prevalence of depression and its considerable impact, knowledge about the pathophysiology of MDD is limited. This is caused by the fact that MDD is heterogeneous in terms of disease and underlying causes. Additionally, the impossibility to take a brain biopsy from depressed individuals hampers investigation of the affected brain tissue.

Since currently available antidepressants adjust monoaminergic signaling (Box 1), early studies focused largely on monomaminergic nuclei such as the dorsal raphe nucleus and the locus coeruleus. From these nuclei, serotonergic and noradrenergic neurons innervate most other brain regions, respectively.

However, the development of structural and functional neuroimaging technologies has permitted in vivo characterization of the anatomical correlates of mood disorders. Among these are brain regions and circuits that are known to regulate emotion, reward and executive function. Dysfunction of these highly interconnected ‘limbic’ regions has been implicated in depression and antidepressant action. In particular, these regions include the amygdala (anxiety and emotional memory), hippocampus (cognition), mediodorsal and midline thalamic nuclei (emotional expression), hypothalamus (vegetative symptoms and hormonal regulation), subgenual anterior cingulate cortex (negative mood states), and the ventral striatum (anhedonia). However, published findings are not consistent and are often complicated by comorbid diagnoses and medication history, and there has been little success in demonstrating any clear cause–effect relationships of pathological changes and MDD.

Among the most consistent findings are a reduced grey-matter volume in the hippocampus and prefrontal cortex. These pathological changes have been linked to
the cognitive aspects of depression, potentially underlying a ‘diminished ability to think or concentrate’. Cognitive difficulties in major depression fall into at least two domains. First, impairment of concentration and attention is likely to relate to the well-documented abnormalities of dorsolateral prefrontal cortex (DLPFC)-function in MDD subjects. Second, MDD patients also exhibit prominent deficits in explicit memory, a cognitive capacity well-known to depend on the function of the hippocampus and the medial temporal lobe. Apart from structural changes, hippocampal atrophy has been repeatedly documented in MDD. Whereas the total number of neurons and glia has not been found altered, neurons are reduced in size and the volume of the neuropil is reduced. Disruption of hippocampal function, including the capacity for neuroplasticity, might contribute to several cognitive aspects of severe forms of MDD.

In addition to its role in declarative memory, the hippocampus is a key regulator of prefrontal cortical function; the hippocampus and DLPFC act concertedly to regulate explicit memory. Disruption of hippocampal function in MDD might therefore contribute to the observed deficits in concentration, described above. The hippocampus is also a critical activity regulator of both nucleus accumbens and ventral tegmental area (VTA). It has been hypothesized that an indirect excitatory projection from hippocampus to VTA is important for coordinating the firing of VTA cells in response to novelty. Impairment of this hippocampal function might lead to reduced dopaminergic tone and contribute to anhedonia. Finally, the hippocampus provides an important source of negative modulation of the hypothalamus-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brand</th>
<th>Class</th>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>Sertraline</td>
<td>Zoloft</td>
<td>SSRI</td>
<td>Inhibits serotonin transporter, and partly dopamine reuptake</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>Lexapro</td>
<td>SSRI</td>
<td>Inhibits serotonin transporter</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Prozac</td>
<td>SSRI</td>
<td>Inhibits serotonin transporter</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Wellbutrin</td>
<td>NDRI</td>
<td>Inhibits norepinephrine and dopamine transporters</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Tofranil</td>
<td>TCA</td>
<td>Inhibits serotonin, norepinephrine, and dopamine transporters. Is an antagonist of acetylcholine and histamine receptors. And is a dopamine receptor agonist</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>Efexor</td>
<td>SNRI</td>
<td>Inhibits serotonin and norepinephrine transporters</td>
</tr>
<tr>
<td>Citalopram</td>
<td>Cipramil</td>
<td>SSRI</td>
<td>Inhibits serotonin transporter</td>
</tr>
<tr>
<td>Trazodone</td>
<td>Trazolan</td>
<td>SARI</td>
<td>Inhibits serotonin transporter and antagonizes several serotonin and noradrenalin receptors</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Tryptizol</td>
<td>TCA</td>
<td>Inhibits serotonin, and norepinephrine, transporters. Is an antagonist of serotonin and adrenergic receptors</td>
</tr>
</tbody>
</table>

SSRI: specific serotonin reuptake inhibitor; NDRI: norepinephrine-dopamine reuptake inhibitor; TCA: tricyclic antidepressant; SNRI: Serotonin-norepinephrine reuptake inhibitor; SARI: Serotonin antagonist and reuptake inhibitors.
pituatory–adrenal (HPA) stress hormone axis through its projections to the hypothalamus; hippocampal dysfunction therefore may contribute to dysregulation of the stress response\textsuperscript{21} that is seen in major depression.

1.4 Treatment of MDD

There are several effective treatments for depression. The large majority (80\%) of people with MDD show improvement during treatment with antidepressants or with electroconvulsive therapy (ECT). In particular ECT is still being considered the most effective\textsuperscript{22,23} treatment for patients with melancholic or psychotic depression. In addition, several forms of psychotherapy – in particular, cognitive and interpersonal psychotherapy – can be effective for patients with mild to moderate symptoms.

The first antidepressants were discovered by chance almost 50 years ago, when iproniazid, a drug registered for the treatment of tuberculosis, was found to elevate mood in MDD patients\textsuperscript{24}. Simultaneously and independently, imipramine, an experimental antihistamine with a tricyclic structure, was found to have antidepressant effects\textsuperscript{24}. Soon after this, drugs with antidepressant activity were shown to increase the extracellular concentrations of two important monoamine neurotransmitters in the brain, serotonin (5-hydroxytryptamine or 5-HT) and noradrenaline, by inhibiting their catabolism or reuptake into nerve endings. These findings were the basis for the monoamine hypothesis of depression, which proposes that mood disorders are caused by a deficiency in serotonin or noradrenaline at functionally important receptor sites in the brain\textsuperscript{24,25}. Over the last few decades, the view that depression is produced by a chemical imbalance in the brain has become widely accepted among scientists, clinicians and the public, despite the lack of evidence for a direct role of 5-HT in this.

It soon became evident that the monoamine hypothesis in its original form did not explain all of the antidepressant effects\textsuperscript{26}. In particular, available antidepressants immediately increase monoaminergic availability, while it takes up to several weeks for the clinical antidepressant response to occur\textsuperscript{23}. Therefore, the focus of research was re-directed towards the receptors and intracellular signal transduction molecules that are regulated by antidepressant treatment\textsuperscript{27}, thereby generating new theories of the pathophysiology of MDD, the action of antidepressant medications, and identifying potential targets for novel antidepressant therapies\textsuperscript{28}. A striking observation, as these downstream molecular events have been elucidated, is the degree of overlap between the molecular and cellular changes induced by antidepressant treatment and the molecular mechanisms of neuroplasticity, especially synaptic plasticity\textsuperscript{29}. 

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In line with this, accumulating evidence suggests that the glutamatergic system and its plasticity play an important role in the neurobiology and treatment of depression. For example, the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine has consistently shown antidepressant effects within a few hours of its administration\textsuperscript{30,31}. Also, it is hypothesized that increased alpha-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid receptor (AMPAR) throughput may represent a convergent mechanism for the rapid antidepressant actions of ketamine\textsuperscript{30,32}. This raises the possibility that AMPA receptor potentiators might be useful in the treatment of MDD\textsuperscript{30}.

2. Stress, depression and neuroplasticity

As discussed above, there is a clear relationship between psychosocial stressors and MDD. Also, in animal models of depression, stress induces depressive symptom-like behaviors\textsuperscript{33,34}, and can lead to atrophy of the hippocampus similar to that seen in depression\textsuperscript{35}. Increasing evidence shows that exogenously applied chronic stress has detrimental effects on neuroplasticity\textsuperscript{18,36,37}.

As outlined below, in these animal models an overlap between antidepressant-induced changes and mechanisms of neuroplasticity is observed. For example, antidepressant action in the hippocampus is found to be dependent on cAMP response element-binding (CREB) protein\textsuperscript{38} that has a well-established role in synaptic plasticity and learning and memory.

These findings have resulted in the hypothesis that chronic stress, which can precipitate or exacerbate depression, disrupts neuroplasticity, whereas antidepressant treatment produces opposing effects and can enhance neuroplasticity (Fig 1). Experimental evidence for this hypothesis is discussed below, and originated for a large part from animal models on stress-related research (Box 2).

2.1 Stress and neuroplasticity

Memory, plasticity and cell survival

In animal models, transient mild stress has been shown to increase hippocampus-dependent memory performance\textsuperscript{39}. However, more severe or chronic stressors induce hippocampus-dependent spatial memory impairments\textsuperscript{40,41}. Similar results were obtained by treatment with glucocorticoid stress hormones\textsuperscript{40}. Specific impairments of hippocampus-
dependent explicit memory were also observed after treatment of human subjects with glucocorticoids\textsuperscript{42}.

Hippocampus-dependent memory formation is known to be dependent on long-term potentiation (LTP) and long-term depression (LTD), the basic cellular mechanisms of synaptic plasticity within the hippocampus\textsuperscript{29}. Interestingly, both stress and increased glucocorticoid levels inhibit LTP in the rodent hippocampus\textsuperscript{43}. Conversely, paradigms inducing stress in rodents enhance hippocampal LTD\textsuperscript{43}.

Sustained levels of stress or glucocorticoids could damage hippocampal cells. Stress leads to atrophy and retraction of the apical dendrites of hippocampal pyramidal cells\textsuperscript{44}. In addition, different forms of acute and chronic stress have been shown to reduce adult hippocampal neurogenesis in rodents\textsuperscript{18}. It has been established that new neurons are generated in the dentate gyrus region of the hippocampal formation of the adult mammalian brain\textsuperscript{45}. Neurogenesis appears to be required for the behavioral response to antidepressants in rodents\textsuperscript{46} and impaired neurogenesis has been hypothesized to represent a core pathophysiological feature of MDD\textsuperscript{18,47}. Thus, neurogenesis might mediate the effects of stress on mechanisms of neuroplasticity and may contribute to the development of MDD.

Similar effects of stress have been obtained in the PFC, such as reduced synaptic plasticity in projections coming from the amygdala\textsuperscript{48}, regression of the apical dendrites of pyramidal cells\textsuperscript{49}, and a reduction in the number of glial cells\textsuperscript{50}. In contrast, in the amygdala, stress enhanced synaptic plasticity and the function of amygdala neurons\textsuperscript{51}. Stress could also enhance amygdala-dependent learning\textsuperscript{52}, and the size and activity of the amygdala were increased in depressed patients\textsuperscript{53}. This contrast in stress-related response of different brain areas makes clear that the well-documented effects of stress on hippocampal morphology and function are not the mere manifestations of a universal effect of stress hormones, or other aspects of stress, on neuronal integrity. Rather, the effects of stress on brain morphology and function are region- and circuit-dependent.

\textit{Stress and molecular plasticity}

Accumulating evidence suggests a role for glutamate in response to stress that might subsequently yield depression\textsuperscript{54}. Glucocorticoid excess increases glutamate release in the CA1 region of the hippocampus\textsuperscript{55} and chronic behavioral stress increases extracellular levels of glutamate in the CA3 region\textsuperscript{56}. This excess glutamate likely contributes to cell damage in these regions and possibly even cell death\textsuperscript{35,57}. Chronic stress increases glutamate levels, which activates extrasynaptic NMDA receptors\textsuperscript{58}. Extrasynaptic NMDA receptors have been found to inhibit LTP\textsuperscript{58}. 
Figure 1. The neuroplasticity hypothesis for depression. In healthy humans or animals (left), stress can disrupt neuroplasticity in specific neuronal networks, i.e. the hippocampus or PFC (right). Antidepressant drugs, electroconvulsive treatment, or behavioral therapy can all enhance neuroplasticity, thereby bringing plastic processes back to normal levels and relieving (cognitive) depressive symptoms.

Stress can also alter downstream molecular signaling at the synapse in several ways. For example, both acute and chronic stress alter the activity of mitogen-activated protein kinase (MAPK) and calcium-calmodulin-dependent kinase II (CaMKII), two proteins that become activated by sufficient synaptic activity and therefore are involved in both early and late phase LTP. In addition, stress leads to reductions in hippocampal brain-derived neurotrophic factor (BDNF) mRNA levels, suggesting an impairment of some of the mechanisms of neuroplasticity. BDNF is known to be induced in activity-dependent LTP and it has a critical role in stabilizing synaptic change.

2.2 Antidepressants and neuroplasticity

Memory, plasticity and cell survival
As stress reduces neuroplasticity, and MDD is associated with a depressed synaptic state, it is likely that antidepressant treatment has the opposite effect. Indeed, accumulating evidence shows that antidepressants influence plasticity in a contrasting, but not exactly opposite way. In this respect, studies in healthy individuals / non-stressed animals are sparse and sometimes conflicting, but antidepressants consistently restore decreased plasticity.

Some lines of research show that, in healthy humans, antidepressant treatment increases memory and acts on other cognitive domains, although evidence is sparse.
Also in naïve animals, there is not a lot known of the effects of antidepressants. Some, although not all, antidepressants increase performance in the Morris water maze, a spatial learning and memory model. At the synaptic level, antidepressant treatment predominantly increases plasticity. In naïve animals, several studies show increased LTP in dentate gyrus and CA1 synapses, although results have not been consistent and were dependent on the type of antidepressant. More importantly, antidepressant treatment has consistently been found to rescue stress-induced reductions in LTP and increases in LTD. Furthermore, chronic antidepressant treatment blocks the stress-induced changes in dendritic morphology and neurogenesis, and some classes of antidepressants also increase neurogenesis in naïve animals.

**Molecular changes induced by antidepressants**

Several lines of evidence suggest that antidepressants can directly modulate glutamate neurotransmission. For example, both riluzole and lamotrigine, which have antidepressant properties, increase the surface expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits GluR1 and GluR2, and riluzole reversibly attenuates AMPAR-mediated synaptic currents in cultured cells. More typical antidepressants, such as fluoxetine, can regulate the phosphorylation state and thereby the function of AMPARs in frontal brain areas. These effects of antidepressants, targeting other molecules and pathways, have led to the proposal that direct modulators of AMPAR function, such as AMPAkines, can act as antidepressants.

Furthermore, in rodents, chronic antidepressant administration increases cAMP levels, and activates cAMP-dependent protein kinase (PKA) and CREB. Since this cAMP-PKA-CREB pathway is important in the maintenance of LTP, it is hypothesized that activation of this pathway accounts for the antidepressant induced increase in LTP. However, this suggested direct link needs validation in future studies. Another line of evidence supporting the hypothesis that antidepressant treatment leads to altered neuroplasticity, is the regulation of neurotrophic factors by antidepressants. Chronic antidepressant administration increases the expression of BDNF in the hippocampus and PFC. Roles for BDNF in depression and its antidepressant action are supported by studies showing that levels of BDNF were decreased in the brains of MDD patients and were increased in patients receiving antidepressant treatment at the time of death. Also, BDNF infusions produced an antidepressant response in animal models.
Non-pharmacological treatment
Apart from pharmacological treatment, also ECT and behavioral therapy have profound
effects on neuroplasticity. Both these forms of treatment have been found to increase
plasticity in frontal brain areas, i.e., reverse the stress-induced decrease in neuroplasticity in
animal models of depression. Both treatments increase hippocampal LTP\(^78\) and prevent
stress-induced alterations in cellular morphology and neurogenesis\(^20\).

2.3 Neuroplasticity and the relevance for depression
The effects of stress and antidepressants suggest that decreased neuroplasticity is a core
pathophysiological feature of MDD. However, these data are best applicable to the
hippocampus and PFC, whereas in other brain areas, such as the amygdala and the
nucleus accumbens, increased plasticity after stress is related to depressive-like phenotypes
in animal models\(^34,51\). For example, expression of CREB in the nucleus accumbens
increased behavioral despair and helplessness in the forced swim and learned helplessness
paradigms, and CREB inhibition has an antidepressant effect\(^79\). Therefore it is likely that
decreased plasticity contributes to the installment of a depressive state only in certain brain
areas.

Most of the behaviors used to assay antidepressant effects in rodents, e.g., learned
helplessness, the forced swim test, and the tail suspension test (see below and Box 2), are
models of behavioral despair and coping. Animals are placed in an adverse environment
from which it is difficult or impossible to escape, and, after a period of struggle, they enter a
behavioral state of passivity. It might be that enhancement of neuroplasticity, and the
concomitant increased capacity to adapt and learn, lead to an enhanced potential repertoire
of behaviors or capacities to explore new escape options in adverse circumstances, and
thus reduces the tendency to enter a state of behavioral despair. This interpretation predicts
that enhanced neuroplasticity is indeed of causal importance for reduced depression-like
behaviors after antidepressant treatment. Additionally, considering the undisputed relation
between neuroplasticity and learning and memory, it is tempting to speculate that cognitive
impairments seen in depressed patients are associated to these neuroplastic changes.
Future studies using animal models of depression should provide evidence for a causal
relationship between depression-related cognitive impairments and reduced neuroplasticity,
and in particular, what molecular changes might account for these impairments.
### Box 2. Animal models used in depression research

<table>
<thead>
<tr>
<th>Model</th>
<th>Main features of symptoms and antidepressant effects</th>
</tr>
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<tbody>
<tr>
<td>Forced swim test</td>
<td>Lack of struggling when placed in a chamber of water, thought to represent a state of despair. Antidepressants acutely increase the time an animal struggles.</td>
</tr>
<tr>
<td>Tail suspension test</td>
<td>Lack of struggling when suspended by its tail, thought to represent a state of despair. Antidepressants acutely increase the time an animal struggles.</td>
</tr>
<tr>
<td>Learned helplessness</td>
<td>Animals exposed to inescapable foot shock take a longer time to escape, or fail to escape entirely, when subsequently exposed to escapable foot shock. Antidepressants acutely decrease escape latency and failures.</td>
</tr>
<tr>
<td>Chronic mild stress</td>
<td>Animals exposed repeatedly to several unpredictable stresses (cold, disruption of light-dark cycle, foot shock, restraint, etc.) show reduced sucrose preference and sexual behavior. Chronic antidepressant treatment reverses these symptoms.</td>
</tr>
<tr>
<td>Social stress</td>
<td>Animals exposed to various types of social stress (proximity to dominant males, odors of natural predators, defeat by a dominant conspecific) show behavioral abnormalities. Chronic antidepressant treatment reverses these symptoms.</td>
</tr>
<tr>
<td>Early life stress</td>
<td>Animals separated from their mothers at a young age show some persisting behavioral and HPA axis abnormalities as adults, some of which can be reversed by antidepressant treatments.</td>
</tr>
<tr>
<td>Olfacotry bulbectomy</td>
<td>Chemical or surgical lesions of the olfactory bulb cause behavioral abnormalities, some of which can be reversed by antidepressant treatments.</td>
</tr>
<tr>
<td>Anxiety-based tests</td>
<td>The degree to which animals explore a particular environment (open space, brightly lit area, elevated area) is increased by anxiolytic drugs (e.g., benzodiazepines).</td>
</tr>
<tr>
<td>Reward-based tests</td>
<td>Animals show highly reproducible responses to drugs of abuse (or to natural rewards such as food or sex) in classical conditioning and operant conditioning assays.</td>
</tr>
<tr>
<td>Cognition-based tests</td>
<td>The ability of animals to attend, learn, and recall is measured in a variety of circumstances. This possibly models cognitive impairments.</td>
</tr>
</tbody>
</table>

### 3. Rodent models of depression

Animal models of depression are evaluated for their validity based on four criteria: (1) similarity in the symptom profile presented, such as decreased interest in pleasure (face validity), (2) amelioration or attenuation by treatments effective in treating the human condition i.e. antidepressants and behavioral therapy (predictive validity), (3) provocation by events thought to be important in eliciting the human disorder, such as stressful life events (etiological validity), and (4) involvement of similar neurochemical processes, like decrease hippocampal BDNF expression (construct validity). This is a challenge. Many of the core symptoms of depression (e.g., depressed mood, feelings of worthlessness and suicide) cannot be easily measured in laboratory animals. As a result, most available animal models of depression rely on one of two principles: 1) effects of known antidepressants and 2) responses to stress, thereby modeling depressive symptoms (Box 2).
3.1 The forced swim test
Some of these tests, in particular the forced swim test, have been very effective at predicting the antidepressant efficacy of pharmacons (predictive validity)\textsuperscript{80}. In this test, rodents immersed in a vessel of water develop an immobile posture after initial struggling. This immobility is considered as behavioral despair. Most antidepressants, administered acutely before the test, reverse immobility and promote struggling\textsuperscript{80}. Obviously, models that use an acute stressor (for example, forced swimming) are better thought of as read-out of coping style, and cannot recapitulate a long-lived multidimensional syndrome such as depression. Therefore, this test should be considered as a fast and cost-efficient screening paradigm for potential antidepressants, rather than an animal model reflecting multiple aspects of the disorder. In this thesis this test was used to screen for the possible long-lasting antidepressant effect of the novel antidepressant ketamine.

3.2 The social defeat paradigm
Animal models that try to incorporate the multidimensional aspects of depression in order to study its neurobiological underpinnings are limited to stress models such as social defeat or chronic mild stress. These are more technically challenging paradigms, however, they show unique sensitivity to chronic and not acute antidepressant administration, which is comparable to the therapeutic delay of 3–6 weeks that is required for all available antidepressant drugs to adequately treat depression in humans\textsuperscript{26}. In the social defeat model the experimental male rodent is placed in the territory of a large dominant male, after which a fight takes place and the intruder gets (socially) defeated. Apart from having etiological validity, because of the social stressor used as trigger, this model also has face validity, in which certain behavioral changes brought about by stress superficially resemble depressive symptoms. For instance, the defeated animal shows decreased sucrose intake or a reduced anticipation towards sucrose after chronic stress, which is thought to model anhedonia\textsuperscript{33,81}. However, most studies of these models mimic the effects of acute stress, i.e. effects are studied acutely after the last stress-encounter, whereas we know from human studies that depressive symptoms are still apparent long after cessation of the stressor\textsuperscript{82}. Moreover, these paradigms do not model the effects of more passive forms of stress, such as social isolation\textsuperscript{83}. This is unfortunate since the lack of social support is known to be important especially for maintenance of depressive symptoms\textsuperscript{33}. Therefore, to be able to study the long-term consequences of social defeat stress on depressive-like behavior, I adopted a social defeat paradigm\textsuperscript{33} in which experimental rats get socially defeated after which they are kept in social isolation for an additional 10-12 weeks. This paradigm might match human
depression in which active stress is often involved in the onset of depression, while passive stress, e.g., in the form of impaired social interaction, has strong precipitating effects on the development of the disease.\textsuperscript{84}

4. Aim and outline of the thesis

MDD is a devastating brain disease that negatively impacts on the lives of many people worldwide. The aim of this thesis is to identify novel molecular targets that can be used to address the cognitive dysfunction that comes with MDD. For this I made use of two animal models, 1) temporal effects of ketamine in the mouse forced swim test, 2) social defeat (SD) stress in rats, which has best validity in terms of modeling aspects of MDD. In particular, I investigated the molecular and cellular neurogenic and plasticity changes that might affect hippocampal function, short and long-term after SD stress. I investigated whether typical antidepressants have a positive effect on the changes that are brought about by SD stress. Also I investigated the effect of behavioral therapy in this model. In this thesis there are several specific questions concerning stress-induced hippocampal plasticity that are addressed in the four experimental chapters.

First of all the antidepressant action of ketamine was investigated. This NMDA receptor antagonist produces rapid (1 hour), robust and sustained (up to two weeks) antidepressant actions in treatment-resistant MDD patients\textsuperscript{31} and in preclinical models of depression following a single systemic infusion\textsuperscript{32}. The rapid antidepressant effect of ketamine is of great interest since all other available antidepressants take weeks to exert their antidepressant effects. However, the mechanism through which ketamine exerts its effects is largely unknown. In chapter 2 I investigated the synaptic mechanism through which ketamine produces its antidepressant effect.

Then I went on to get better insight into the neurobiology of MDD by using an animal model with profound validity for the human disorder. First, I investigated cognitive functioning and molecular changes at the level of the synapse that might occur directly after severe SD stress exposure when compared to the long-term effect of SD. These molecular alterations are involved at the onset of neuroplastic changes and the installment of a depressive-like phenotype, and are addressed in chapter 3 of this thesis.

A second issue concerns the longitudinal effects of stress on neuroplasticity. As we know from human studies (see\textsuperscript{82} for review), depressive symptoms are often still apparent long after the end of a stressful life event and MDD is considered a chronic disease.
Therefore, it is of interest to determine whether the long-term effects of SD stress on depressive-like (cognitive) symptoms are related to altered hippocampal plasticity. In chapter 4 I questioned whether the depressive symptoms that are seen long after the cessation of social defeat stress exposure and are maintained by social isolation, are associated with reduced hippocampal memory performance. Ongoing, I investigated which type of cellular and molecular plasticity occurred at hippocampal synapses that could be involved in hippocampus-dependent memory impairment long after SD stress, and whether a form of behavioral therapy or chronic antidepressant treatment could restore these changes. In addition, I investigated whether long-term effects of SD stress were also associated with changes in the neurogenic process in the hippocampal dentate gyrus, which is discussed in chapter 5. Here, I also investigated the antidepressant action on SD-affected neurogenesis.

Finally, chapter 6 of this thesis brings together my data, and here the most important conclusions are discussed in the context of MDD.
Chapter 2

Hippocampal AMPARs mediate the enduring antidepressant effects of a single treatment with ketamine

*Biological psychiatry, under review*

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