Chapter 1:
General Introduction
Major Depressive Disorder

Mood disorders are amongst the most prevalent psychiatric disorders worldwide, affecting 1 out of 8 Europeans, with higher incidence in women. Major Depressive Disorder (MDD) is a lifelong, recurrent affliction, which involves significant societal, medical and financial burden. MDD is considered a leading cause of disability in the Western World.

According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), MDD is characterized by persistent (>2 weeks) negative mood and/or reduced interest for daily activities, with both symptoms critically influencing an individual's occupational, educational or social functioning. Additional symptoms include neurovegetative (sleep, appetite and weight regulation), psychomotor (overall activity and fatigue) and cognitive (concentration, guilt and suicidal ideation) aspects.

Adding an extra level of complexity, MDD is amongst the most comorbid mental disorders, with more than 70% of patients being co-diagnosed with at least one other psychiatric disorder, most commonly anxiety (60%), substance abuse (24%) and/or impulse control disorders (30%).

Symptoms of MDD are heterogeneous, varying in onset, duration and frequency. Readily available therapeutic approaches against MDD are mostly limited to pharmaceutical agents that were developed more than 60 years ago under the assumption that they counteract a neurochemical monoaminergic unbalance that underlies the disease (see monoamine depletion hypothesis). As it is now understood, complex actions of antidepressants, such as neurotrophic effects that mediate structural plasticity, account for the delayed-onset in beneficial effects against MDD. Pharmacotherapy is oftentimes complemented with other type of treatments such as psychotherapy (e.g., cognitive behavioral therapy). In treatment-resistant patients more invasive techniques, such as electroconvulsive therapy and deep-brain stimulation, have been employed.

Despite advances in understanding the neurobiological substrate of depression and the actions of antidepressants, current (combinations of) treatments are suboptimal, with more than 65% of treatment-receiving patients still presenting symptoms and as much as 20% of patients remaining treatment-unresponsive. Notably, no more than 37% of treatment-receiving patients display at least 50% reduction in symptoms following first-level pharmacotherapy with citalopram, which is one of the most widely prescribed antidepressant agents. In addition, the cumulative remission effect of combined pharmacotherapy and
psychotherapy does not exceed 67%, even following consequent treatment steps12.

Depressive disorders are hard to combat due to complex associated triggers and the resulting diversity in pathological manifestations. While genetic factors might render an individual prone to the development of MDD, the relatively low levels of heritability (40–50%), as compared with other neuropsychological diseases13, have hampered the identification of MDD-unique genetic substrates. Likewise, environmental factors, such as adverse life-events and exposure to severe unescapable stress, result in a full-scale depressive episode only in a small fraction of the population14, further illustrating the complexity of MDD. This individual variability in the underlying causes of MDD has been a major obstacle in developing adequate treatments, and data suggests that novel therapeutic solutions can be best derived from personalized approaches15,16. Together, it has been proposed that the depressive state of an individual can be decomposed into endophenotypes, considerably facilitating the identification of common genetic loci and leading to higher treatment efficiency17,18.

There is pressing need for preclinical research to model this repertoire of endophenotypes, addressing disease manifestation at the level of the individual19. In my thesis, I adopted a similar approach, i.e., I addressed specific endophenotypes of the depressive state in order to identify their unique-or shared- molecular substrates. In the following paragraphs, I will first briefly describe the two main endophenotypes of the depressive state that my dissertation deals with, namely anhedonia and disturbances in cognitive function. Subsequently, I will introduce the common type of comorbidity between MDD and alcohol abuse disorders, for which I developed a preclinical model.

Anhedonia

According to the DSM-5 criteria, one of the core characteristics of the depressive state is a persistent inability to experience pleasure when confronted with otherwise rewarding activities and stimuli. This failure in behavioral reinforcement has been termed “anhedonia” and it primarily reflects a general flattening of the emotional response. Historically, the anhedonic phenotype has been linked to deficits of the limbic system a network of brain areas mediating the experience of reward in the presence of evolutionary meaningful stimuli (e.g., sex, food). In a more recent concept, anhedonia encompasses a broad spectrum of consummatory and motivational deficits, including reward-related decision-making and goal-
directed behavior. Thus, anhedonia, as seen in depression, might convey failure in i) anticipation or prediction of expected rewards; ii) association of relative values and costs with rewards; iii) determination of the effort required to obtain rewards; iv) integration of this information to decide whether it is worthwhile to obtain rewards; and finally v) motivation to perform the necessary actions to obtain rewards. In support of this notion, recent studies have described maladaptive integration of learned and retrieved reward-coding information and, consequently, misguided behavioral adaptations in response to reward-related stimuli in depressed patients. Importantly, the variety of anhedonic symptoms indicates the implication of distinct neuronal circuitries, originally serving diverse functions, such as the prefrontal cortex (PFC) in decision making, the amygdala in response to affective stimuli and emotional regulation, and the basal ganglia (e.g., nucleus accumbens (NAc)) for motor coordination and motor impulsivity. In the experimental chapters that follow, I evaluated which of the above-mentioned anhedonia components can be modeled in the rat, and which brain areas and circuitries could be involved in their manifestation.

Cognitive Dysfunction
A less prominent, but equally debilitating, symptom of depressive disorders is cognitive dysfunction, which can persist beyond remission of the mood disorder. Deficits of the cognitive domain are thought to facilitate the onset, as well as the development of MDD. These include deficits in verbal, working and long-term memory, which reflect well documented impairments of brain areas primarily mediating learning and memory, such as the hippocampus (HPC). In agreement, reversal of depression-induced neurogenesis deficits at the HPC predicts behavioral recovery from memory-related symptoms and the efficacy of antidepressant treatment. Furthermore, mild cognitive decline in depression includes loss of concentration and hesitation in decision-making. These clinical symptoms are attributed to dysfunction in brain systems mediating executive control, such as the prefrontal cortex (PFC). Under a perpetuating depressive state, poor executive control in combination with over-reactivity of areas regulating the emotional response to stimuli (e.g., amygdala) might lead to the development of strong attentional biases and aberrant affective processing. In agreement, depressed individuals show distinct negative bias, i.e., persistence of stimuli or memories of adverse nature, as well as hypersensitivity to perceived punishment and negative feedback. In the experimental chapters that follow, I aimed to model cognitive dysfunction in presence of an induced depressive state, with
respect to both memory failure and attentional biases. Furthermore, I addressed the neuronal substrates underlying these pathological manifestations.

Comorbidity

One of the challenges taken upon in the studies described in my thesis was to ascertain a causal relationship between depressive and addictive disorders, bridging the gap between clinical and preclinical research. Drug dependence is a chronic relapsing disorder, characterized by excessive preoccupation with, craving of and, upon abstinence, withdrawal from the substance of abuse, with MDD being one of the most prevalent psychiatric disorders that co-exists with alcohol dependence. It is estimated that more than 30% of depressed individuals are in fact diagnosed with comorbid alcohol use disorder (AUD). Conversely, depressive symptoms are present in about 80% of the treatment-seeking alcoholics.

Coexistence of the two pathologies is detrimental for the patients’ disease prognosis. Comorbidity predicts increased severity of the symptoms of both diseases, e.g., frequent episodes of aggression and higher risk of suicide. Comorbid patients exhibit earlier onset and prolonged duration of the symptoms, with alcohol dependence persisting after the amelioration of the mood disturbances, and vice versa, depressive symptoms lingering (or even worsening) following discontinuation and protracted abstinence from alcohol use. The latter contributes to the high rates of relapse amongst comorbid patients. In these individuals, occurrence of a depressive episode predicts relapse to alcohol use. Conversely, alcohol abuse can trigger (during remission) or worsen depressive mood. Lastly, comorbidity affects the therapeutic outcome in treatment-seeking individuals. In fact, prolonged treatment periods are needed and the final outcome of different approaches varies, with only modest to small effects reported. This might be related to the fact that none of the treatment strategies and available medication specifically targets the comorbid phenotype.

There are several hypotheses on the origins of comorbidity between depression and addictive disorders. Due to their reciprocal relationship, it is thought that the primary disorder is a risk factor for the secondary, thus conferring increased vulnerability. Shared risk factors, such as genetic predispositions or stress triggering onset or relapse, are amongst the plausible scenarios as well. Finally, addictive disorders
might manifest in an attempt to self-medicate from mood disturbances. In the following sections I will discuss the contribution of brain regions implicated in the different aspects of MDD as presented above, highlighting the commonalities in circuitries involved in depressive and addictive disorders. In view of the aims and scope of my dissertation, this will be limited to the most relevant brain systems, i.e. cortical and hippocampal contributions.

**Brain Circuitries**

As emphasized above, depression is a complex psychiatric disorder manifested in varying pathological endophenotypes, including the comorbid endophenotype. Although I will exclusively discuss the frontal cortex and hippocampus, it is likely to assume that aberrant function in more than one brain area is implicated in MDD. Indeed, as the network hypothesis proposed, depression emerges as dysfunctional communication between several brain systems. For example, depressed mood reflects emotional dysregulation, and it is widely accepted that dysfunctional communication between amygdala and the PFC might promote sadness. Similarly, the mesolimbic system and the PFC are both responsible for reward deficits observed in depression, with maladaptive reward evaluation and reward-related decision-making contributing to disturbed behavioral reinforcement.

**Frontal-cortical contributions**

The frontal cortex, including the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC), is considered the main mediator of complex cognitive processes, such as executive functioning, planning and decision making and inhibitory control. There is a plethora of studies implicating frontal cortical areas in both the expression of depression and substance abuse, with evidence originating from clinical and preclinical research. It is hypothesized that under normal conditions, frontal subregions serve behavioral flexibility, regulating a variety of inputs of emotional and hedonic salience. As such, when the flow of information within the circuitry, as well as towards sub-cortical regions is disrupted, pathological manifestations, like the ones seen in depression or addiction, could emerge. In clinical settings, both increases and decreases in frontal activation have been recorded, depending on the subregion under examination, the task at hand and the diagnosis of the
participants. Hyperactivation towards drugs and drug-related paraphernalia in ACC and both the dorsal and ventral areas of the PFC are seen in addicts, coupled to hypoactivation of the same areas in response to neutral stimuli\textsuperscript{48}. These functional changes are linked to attentional biases in favor of drug-associated environments and stimuli, promoting craving. Furthermore, during abstinence from the substance of abuse, these patients display hypoactivation in ACC and mPFC\textsuperscript{48}, which is thought to contribute to reduced inhibitory control, promoting pathological drug-wanting\textsuperscript{36}. Together, both in presence and in absence of the drug, PFC dysfunction promotes maladaptive decision-making, culminating in continuation of drug use despite adverse consequences, and in relapse. Likewise, neuroimaging studies illustrated the role of frontal-cortical areas in depression, with morphological changes, such as reductions in cortical volume of the ACC and OFC, consistently reported\textsuperscript{50-52}. These alterations are thought to account for the observed functional abnormalities, which, in turn, are hypothesized to contribute to reduced top-down control (e.g., towards the NAc and amygdala), facilitating dysfunctional reward- and emotional processing\textsuperscript{29,34}.

In terms of anhedonia and dysfunctional reward processing, the PFC and in particular the ACC are thought to play pivotal roles. Under normal conditions, the ACC mediates reward anticipation, i.e., future representation of a reward (or the avoidance of a punishment) that contributes to behavioral reinforcement\textsuperscript{44}. Furthermore, the ACC is implicated in the integration of cost-to-benefit signals, and by extension, in the control of reward-related decision making\textsuperscript{21,53}. Thus, the volume loss and aberrant function of the ACC of MDD patients could contribute to the expression of the depression-induced anhedonic phenotype\textsuperscript{54-56}.

Notably, after correcting for the volume differences between depressed individuals and healthy controls, the majority of neuroimaging studies have linked acute or recurrent depressive episodes to increased frontal activity, as seen mainly at the OFC, mPFC and ACC\textsuperscript{51}. This excessive activation is generally viewed as a need for a greater degree of engagement in order to achieve control-level performance in emotionally relevant tasks\textsuperscript{29}. For example, while processing positive stimuli, depressed patients exhibit increased ACC activation, indicative of additional effort made when elaborating on optimistic outcomes\textsuperscript{21}. On the other hand, hypoactivation in MDD is particularly observed in the dorsolateral PFC (dIPFC). The dIPFC promotes sustained attention under cognitive challenging conditions and exerts inhibitory control over the amygdala, regulating emotional responses to stimuli. In depressed patients, dIPFC hypofunction is thus linked to
attentional biases and working memory deficits. Importantly, reduced dlPFC activation, and the accompanied diminished ability for disengagement from negative stimuli, is considered as a major vulnerability factor in MDD. 

Hippocampus
The hippocampus (HPC) is traditionally linked to learning and memory processes, as it mediates the transition of information from short to long-term storage states. Long-term potentiation and depression occurring at the HPC are considered to be the cellular basis of learning. As such, there are many pathological states, varying from trauma to neurodegenerative disorders that are attributed to reduced hippocampal function. In many cases, memory-related deficits, as observed in aging-related dementia or Alzheimer's disease, originate from the HPC. Importantly, amongst the most well validated symptoms of depression is reduction in hippocampal grey matter, which, in turn, is linked to diminished cognitive capacity. 

As discussed below, the HPC is profoundly affected by protracted stress exposure, the consequent activation of the hypothalamic-pituitary-adrenal (HPA) axis and finally, the release of glucocorticoids (GCs), such as cortisol. Prolonged GCs signaling severely reduces dendritic arborization and negatively impacts on hippocampal cell survival, leading to hippocampal atrophy, one of the neurobiological hallmarks of the depressed state. Importantly, intact function of the HPC is necessary for termination of the stress-related HPA response, in a negative feedback loop configuration. In depression, subsequent stress exposure can promote an even greater increase in GCs, leading to a variety of pathological manifestations.

Based on these observations, the neurogenesis hypothesis of depression emerged, which postulates that following severe stress, reduced hippocampal neurogenesis (and inability to control HPA reactivity) contributes to the development of depressive symptoms, such as memory impairments and maladaptive responsiveness to subsequent stress. This notion is supported by the fact that prolonged administration of most antidepressant agents reverses depression-induced HPC-related neurogenic defects. Similarly, antidepressant-triggered behavioral relief from depression depends on hippocampal neurogenesis.

Importantly, on account of its connectivity with the mesolimbic reward circuitry (e.g., NAc), the HPC is implicated in memory encoding of
emotionally salient events, such as reward-stimulus associations\textsuperscript{46}. In depression, aberrant hippocampal function can lead to encoding errors\textsuperscript{34}, possibly bypassing retrieved details on the rewarding properties of a given stimulus and eventually contributing to anhedonia. In this respect, in addiction, encoding errors might lead to over-representation and temporal persistence of reward memories, thereby promoting addictive behaviors\textsuperscript{37}. In favor of this notion, and similar to stress, addictive substances can reduce hippocampal neurogenesis\textsuperscript{68}, contributing to HPA disinhibition. Reduced neurogenesis is in turn implicated in increased sensitivity to the effects of drugs, such as the development of extinction-resistant drug memories. This perpetual loop confers vulnerability and precipitates maladaptive learning focused on drug-driven reinforcement, thus promoting dependence\textsuperscript{37}. 

In a riveting essay, Davidson and colleagues (2002) proposed that depressive disorders can be viewed as disorders relating to inappropriate context-regulation of affect\textsuperscript{53}. According to this view, patients exhibit aberrant responses to contexts after failing to regulate affective reactivity to stimuli that should no longer be perceived as negative, e.g., a persistent fear response in absence of an actual threat. The hippocampus is considered essential to this process, being the hub for context encoding and given its direct connections with higher cortical areas (e.g., PFC) and the amygdala\textsuperscript{69}. The vast literature supporting structural and functional deficits of the hippocampus in depressed patients and reversal by antidepressants favors this idea\textsuperscript{70}.

In the preceding sections, I attempted to summarize the main characteristics of depressive pathologies and their comorbidities, as well as major circuitries mediating these deficits from a clinical perspective. An illustration of the effects of uncontrollable, prolonged stress, a major trigger of MDD and drug-related disorders, follows as a prelude to my own work, which focuses on modeling depression and comorbid AUD at the preclinical level.

**Stress**

Stress exposure initiates a cascade of physiological and psychological events that interrupt the homeostatic state of an organism\textsuperscript{71}. These are predominantly manifested in a “fight or flight” response that has countless evolutionary advantages. Some individuals display excessive stress reactivity, seen at the hormonal, neurochemical and neurocircuitry levels, in erroneous settings, e.g., in absence of a stressful stimulus. In
addition, stress reactivity in these individuals persists well-beyond the appropriate autonomic response, hindering the restoration of homeostatic balance. Finally, exposure to persistent or uncontrollable stress can result in the adoption of maladaptive coping mechanisms altogether, which in turn can evoke a broad spectrum of depressive symptoms. In a similar fashion, stress exposure triggers the development and perpetuation of substance abuse disorders, especially in the vulnerable individual. In conditions of severe stress, neuroadaptations take place that promote the continuation of addictive behaviors, by impeding abstinence or by triggering relapse. Among these processes, disturbances in emotion regulation, overwhelming drug craving and drug withdrawal-precipitated negative reinforcement prime the individual to drug-related cues and contexts, increasing the frequency of intake and the possibility of relapse.

The main biological processes that govern the adoption of inappropriate strategies in response to stress are discussed below for both depressive and addictive disorders. The focus is on deficits that concern the affective domain, such as disturbances in motivation, and those affecting cognition, such as attentional biases.

In healthy conditions, acute stress activates the HPA axis, which in turn promotes glucocorticoid (GC) release and prepares the organism for fast and fitting behavioral responses. In these settings, activation of the amygdala connects environmental stimuli and experiences to emotional valence. In turn, the hippocampus processes contextual information that regulates the affective response. Putative reward-processing systems, such as the mesolimbic network, drive the hedonic tone that results in behavioral reinforcement. Finally, higher cortical areas, such as the PFC, coordinate upcoming actions, exerting top-down control and promoting adaptive decision-making.

In contrast, following exposure to chronic stress, or moderate stress in vulnerable individuals, excessive reactivity of the HPA axis contributes to unfit physiological responses in subsequent adverse, or perceived as adverse, conditions. For example, as discussed before, hyperactivation of the HPA axis results in prolonged release of GCs, which has been repeatedly shown to contribute to cellular death, with major implications for normal brain function. In particular, in the hippocampus, GC-mediated neuronal degeneration supports a role of exaggerated stress responses in cognitive impairment, seen both in memory-related deficits and in dysfunctional context-reflect regulation. Furthermore,
as GC signaling directly influences dopaminergic (DA) neurotransmission, prolonged stress-induced neuroadaptations at the mesolimbic circuitry can trigger aberrant reward processing. This in turn can lead to anhedonic or hyper-sensitized reinforcement states, seen in depression or in addiction, respectively. Finally, at the PFC, GC signaling-mediated neuronal atrophy has devastating effects on executive and cognitive control, especially under challenging cognitive conditions, e.g., when exerting inhibitory control. Taken together, it is possible that, in prone individuals, stress leads to dysfunctional motivational and cognitive processes. A behavioral state emerges that is characterized by aberrant emotional processing and attentional biases. Communication between various brain structures, involved in decision-making, response inhibition, learning and memory, reward and motivation, decays. Finally, failure of adaptive coping to subsequent stress or subsequent drug exposure promotes the continuation of the depressive or the addictive state, respectively. In agreement with this, a blunted response to positive stimuli, characteristic in depression, is partly mediated by hypoactivation of the brain reward centers, and is partly sustained by overcompensation from stress systems. Along this line, reward over-evaluation and exacerbated incentive states, as seen in addiction, stem from hyper-reactivity of the mesolimbic system and from stress-induced neuroadaptations that disrupt appropriate processing of stimuli of rewarding valence.

In the present section, I illustrated the inductive role of stress in depression and in perpetuation of drug abuse. Based on this, it comes as no surprise that often enough preclinical paradigms aiming to model these afflictions employ exogenous stressors as triggers of pathology. As these models have been instrumental in defining a mechanistic basis for depression, a brief description of the most commonly used follows below.

**Animal models**

“Essentially, all models are wrong, but some are useful”

- George E.P. Box, 1987

For more than four decades, translational approaches modeling the development of psychiatric disorders in animals have been promoted and extensively used. Distinct approaches have been adopted leading to behavioral assays that employ non-social (e.g., chronic mild stress, learned
helplessness) or social (e.g., maternal deprivation, early-life isolation) stressors for the induction of the depressive state. In addition, behavioral readouts that assess different aspects of the depressive state, such as behavioral despair (e.g., forced swim test), anhedonia (e.g., sucrose preference) and deficits in social behavior (e.g., social interaction, social memory) have been developed. It is widely accepted that such animal models must retain specific properties in order to faithfully, and if possible multi-dimensionally, represent the disease, the so-called validity criteria. For an animal model to be valid, depressive-like symptoms must be triggered by etiologically relevant factors, such as exposure to invasive stress (etiological validity). Similarly, the model needs to induce a pathological state parallel to the one observed in depressed patients (face validity), e.g., sustained negative affect, cognitive decline. Construct validity refers to the ability of the animal model to emulate pathophysiological hallmarks of the human disorder (e.g., decreased hippocampus volume in depression), which either contribute to the onset of, or affix proneness to the disease. Finally, additional efforts have been made in order to establish depression models with predictive validity, which entails responding to current treatment approaches in humans (e.g., pharmacotherapy). This allows screening for novel antidepressant agents in order to extrapolate treatment outcomes of the human disease.

In light of the above mentioned validity criteria, I will discuss here the most relevant animal models for depression used by others, namely, the chronic mild stress model, learned helplessness, deprivation of maternal care and early-life isolation stress (Figure 1, Table 1). This serves as introduction for the more extensive description of the preclinical model of choice for our work, i.e., the social defeat-induced persistent stress (SDPS) paradigm.

**Chronic mild stress**

*The chronic mild stress model* (CMS) consists of a range of mild stressors given at an unpredictable rate for a prolonged period of time. Animals are subjected to different stressful stimuli or environmental conditions in randomized order and varying intervals. These include reversal of light/dark cycle, use of stroboscopic and other intense light sources, presentation of sudden, loud noises, home-cage tilting, housing in overcrowded conditions, dampening the home-cage bedding material, food and/or water deprivation and others. CMS exploits the uncontrollable nature of novel and chronic stress exposure, as a major trigger in the development of depression in humans. However, it is of note that its etiological validity is
weak, given the fact that the stressors employed are of little resemblance with adverse events marking the development of the human disease. In addition, such manipulations might promote anxious (sub)phenotypes, with symptoms corresponding better to comorbid anxiety and mood disorders. Nevertheless, using CMS, central symptoms of the depressive state can be modeled, with a focus on anhedonia and behavioral despair. Furthermore the CMS model has been repeatedly shown to mirror neurobiological substrates (e.g., hippocampal atrophy) and neurochemical disturbances (e.g., alterations in serotoninergic transmission) as observed in depressed patients, justifying its wide use in translational approaches aiming to model depression. Finally, CMS holds good predictive validity, as administration of a broad spectrum of antidepressant agents ameliorates the deficits seen in CMS-exposed animals. It should be noted though that in the vast majority of CMS studies, antidepressant agents are applied during the last weeks of stress exposure. Thus, antidepressant relief in the presence of acute stress should be interpreted with caution when extrapolating to the human situation.

**Learned helplessness**

Learned helplessness (LH) consists of repeated exposure to unavoidable stress aiming to evoke a state of despair. Animals are subjected to multiple inescapable electrical shocks in a given environment and subsequent escape behavior is assessed. Behavioral despair is expressed as disrupted ability to acquire an escape response when the stressor can be avoided. The LH paradigm aims to mimic conditions of prolonged unpredictable stress that lead to a depressive state. However, similarly to the CMS model, LH provides poor etiological validity due to its non-naturalistic approach of severe stress. Another limitation of the LH model is its technical replicability, which relies heavily on methodological details, such as the available apparatus and shock application. Nonetheless, LH confers large face validity, as it triggers aspects of the anhedonic phenotype (e.g., reduction in sucrose preference) and cognitive disturbances (e.g., deficits in spatial memory), which are at the core of depressive pathologies. Notably, hyperlocomotion and increased sensitivity to novelty is also seen after LH, supporting an anxiogenic effect. Animals exposed to LH display many neurobiological correlates to the human disease, such alterations in hippocampal spine morphology and changes in serotoninergic and noradrenergic transmission. Additionally, LH induces congenital disturbances in reward sensitivity and fear extinction, arguing for strong genetic contribution to depression susceptibility.
Finally, a stronghold of LH as a valid model of depression is its predictive validity, as the paradigm is highly responsive to a variety of therapeutic interventions, including prolonged antidepressant administration\textsuperscript{108,109}, mimicking the typical dynamics of delayed treatment response in humans.

**Figure 1.** Most frequently used preclinical models of depression. **a)** Chronic mild stress, in which animals are exposed to variable mild stressors, such as intense illumination of the home-cage, given at an unpredictable, chronic rate. **b)** Learned helplessness, in which animals are exposed to repeated unavoidable stress, such as electrical foot-shocks, that elicit disrupted escape response. **c)** Maternal deprivation, in which animals are deprived of maternal care in early postnatal days. **d)** Early-life isolation, in which animals are isolated from conspecifics during critical developmental periods, such as adolescence. **e)** Social defeat, in which animals are exposed to and physically defeated by a dominant male conspecific, resulting in subordination.
Deprivation from maternal care

*Maternal deprivation* (MD), e.g., following separation at early postnatal days, is one of the paradigms employed to model adverse environmental factors that lead to depressive states. In humans, early-life adverse events, such as emotional or physical neglect and abuse, can render an individual vulnerable to subsequent stressors, thus increasing the probability to develop a pathological state such as depression. Exploiting extensive etiological validity, MD utilizes early-life social stress as a trigger of depressive-like symptoms. Although an anhedonic state is hard to validate following deprivation of maternal support, behavioral despair and cognitive deficits are consistently seen after MD and are related to the physiological effects of stress in the hippocampus. Repeated maternal separation leads to a marked stress response and is shown to induce aberrant function of the HPA axis that persists during adulthood, mimicking the neurochemical status of depressed patients. MD stress effects are reversed following late antidepressant treatment, arguing in favor of its predictive validity. Notably, the effects of MD follow gender dichotomy, with only female subjects exhibiting phenomena that partially reflect a depressive state, such as cognitive disturbances or reduction in hippocampal neurogenesis. These results indicate sex-specific vulnerabilities following MD stress and, despite the fact that depression is more prevalent in women than men, they highlight the inefficiency of MD to exhaustively model the depressive state.

Early-life isolation

*Early-life isolation* stress (EI), in the form of social isolation during a critical period for social development, has been used to model the effects of early-life adverse events in depression. Deprivation of contact with conspecifics during the pre- and peri-adolescence period induces a variety of depressive-like symptoms later in adulthood, such as reduced social motivation, anhedonia, behavioral despair and hyper-responsivity to subsequent stressors. The neurochemical profile of animals exposed to EI stress resembles that of depressed patients, with alterations in monoamine, neurotrophin and hormonal levels. Similarly, EI leads to depression-mimicking alterations in hippocampal morphology and function. Interestingly, environmental enrichment, an equivalent of cognitive psychotherapy, can reverse behavioral despair, memory deficits and aberrant hippocampal physiology seen in adults following EI, adding predictive value to this preclinical model of depression. It is of note that
Table 1. Most frequently used stress-based preclinical models of depression. For each model, and based on existing literature, depressive-like manifestations that correspond to the four validity criteria (face-, etiological-, construct-, and predictive-validity) for animal models of neuropsychiatric diseases are summarized. Included are limitations that each paradigm presents.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Face Validity</th>
<th>Etiological Validity</th>
<th>Construct Validity</th>
<th>Predictive Validity</th>
<th>Limitations</th>
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<tr>
<td>Chronic Mild Stress</td>
<td>Anhedonia&lt;sup&gt;17,24&lt;/sup&gt;</td>
<td>Poor (non-naturalistic)</td>
<td>Hippocampus&lt;sup&gt;17,24&lt;/sup&gt;</td>
<td>Antidepressant response</td>
<td>Antidepressant administration during / acutely after stress</td>
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<td></td>
<td>Behavioral despair&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Cognitive decline&lt;sup&gt;17,24&lt;/sup&gt;</td>
<td>HPA axis&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
<td>Anxiety&lt;sup&gt;24&lt;/sup&gt;</td>
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<tr>
<td>Learned Helplessness</td>
<td>Anhedonia&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Poor (non-naturalistic)</td>
<td>Hippocampus&lt;sup&gt;17,24&lt;/sup&gt;</td>
<td>Antidepressant response</td>
<td>Technical replicability</td>
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<td></td>
<td>Cognitive decline&lt;sup&gt;17&lt;/sup&gt;/</td>
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<td>Genetic component&lt;sup&gt;1&lt;/sup&gt;</td>
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<td></td>
<td>Anxious&lt;sup&gt;17&lt;/sup&gt;</td>
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<tr>
<td>Maternal Deprivation</td>
<td>Behavioral Defeat&lt;sup&gt;12,19,11&lt;/sup&gt;</td>
<td>Good</td>
<td>Hippocampus&lt;sup&gt;18,198&lt;/sup&gt;</td>
<td>Antidepressant response</td>
<td>Vulnerability to develop depression rather than depressive state</td>
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<tr>
<td></td>
<td>Cognitive decline&lt;sup&gt;12,19,11&lt;/sup&gt;</td>
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<td>HPA axis&lt;sup&gt;1,12&lt;/sup&gt;</td>
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<td>Gender Dichotomy&lt;sup&gt;12,19,11&lt;/sup&gt;</td>
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<tr>
<td>Early-life Isolation</td>
<td>Anhedonia&lt;sup&gt;17&lt;/sup&gt;</td>
<td>Good</td>
<td>Hippocampus&lt;sup&gt;17,24&lt;/sup&gt;</td>
<td>Environmental enrichment</td>
<td>Vulnerability to develop depression rather than depressive state</td>
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<tr>
<td></td>
<td>Cognitive Decline&lt;sup&gt;17&lt;/sup&gt;</td>
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<td>Monamines&lt;sup&gt;24&lt;/sup&gt;</td>
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Similar to maternal separation, EI confers vulnerability to the development of depression following later-life environmental challenges rather than inducing a depressive-like state per se<sup>120</sup>.

**Social Defeat-induced Persistent Stress**

The main goal of my thesis is to examine the diversity in endophenotypes of depressive-like disorders in rodents, i.e., to assess their behavioral manifestations and to elucidate their neuronal substrates. As mentioned above, there are many different ways to approach modeling depression at the preclinical level, each highlighting different facets, endophenotypes, of the depressive state. In this thesis I employed the social defeat-induced persistent stress (SDPS) paradigm to reach my aims. The SDPS paradigm is an adapted version of the social defeat model widely used in rats and mice to induce depressive-like pathology<sup>142,142</sup>. In SDPS, the standard resident-intruder protocol that includes physical and perceived exposure to a larger territorial male and subsequent defeat, is combined with a prolonged period

1. “Never confuse a single defeat with a final defeat” — F. Scott Fitzgerald
of social isolation (single housing). The basic principle in SDPS, similarly to other animal models employing social stress, is that repeated exposure to social stressors, such as inescapable aggression, physical defeat (injury and pain infliction), threat of defeat, and hierarchy-based subordination can and will induce a depressive-like state. This state is maintained by constant exposure to subthreshold stressors, such as social exclusion that in itself will not trigger long-lasting depressive symptoms.

It has become clear that social defeat-based paradigms hold good etiological validity, since they exploit enduring psychosocial stressors that parallel the social nature of adverse life events triggering depression in humans. In terms of face validity, rodents exposed to defeat stress show decreases in food and water intake, reduced interest for reproductive activities, low general activity and exploration (reflecting fatigue) and heightened anxiety, similar to depressed humans. Importantly, social defeat has been repeatedly shown to emulate anhedonic (sub-)phenotypes, including aberrant responses to natural rewards (motivational deficits) and loss of interest in engaging into social activities (social withdrawal). In addition, a large body of literature validated the multifaceted effects of defeat stress on the cognitive domain, including deficits in spatial and emotional memory. Notably, social defeat stress has been proven extremely useful in characterizing depression-prone and -resilient subpopulations, mirroring the diverse effects of stress in humans. By extension, social defeat facilitated the investigation of the neurobiological substrates that govern vulnerability and resilience to stress, delineating protective factors and possible biomarkers that might be translated for use in the clinic.

Acute or repeated defeat stress triggers a neuroendocrine state characterized by sympathetic activation (HPA axis, corticotropin-releasing factor -CRF- and corticosterone -CORT- release). Similarly, increased responsivity (cellular activity, expression of immediate early genes) of stress-related brain structures occurs, in parallel to activation of areas that contribute to the emotional and cognitive dysfunction seen in depressive-like states, such as the PFC and amygdala. A well-replicable effect of social defeat stress exposure is structural and functional deterioration of the hippocampus, strongly associated with reduced cognitive flexibility and memory deficits seen in depressed patients. Indeed, several studies have shown that following defeat, changes in HPC volume and neurogenesis occur, paralleling loss of hippocampal volume.
seen in humans. In addition, reduction in hippocampal long-term potentiation and depression (LTP, LTD) has been recorded, implying altered information processing that could contribute to the observed cognitive decline. Taken together, compelling evidence indicate that the social defeat model retains strong construct validity, triggering neurobiological endophenotypes characteristic of the human affliction. Of note, reversal of the observed deficits by antidepressant therapy restores the behavioral phenotypes, supporting the notion that these neuroadaptations are causal to the development of the depressive state. In agreement, several depressive-like manifestations induced by defeat stress have been shown to remit following chronic -but not acute- administration of antidepressant agents, similarly to human observations. Indeed, following social defeat, chronic administration of antidepressants has been shown to reverse social withdrawal, anhedonia and helplessness, all core manifestations of the depressive state in humans. Importantly, forms of cognitive or behavioral therapy, such as environmental enrichment and re-exposure to social conditions (paired housing), are sufficient to reverse several of the behavioral and neurobiological effects of defeat, further supporting its predictive validity.

In conclusion, SDPS is one of the most comprehensive preclinical models of depression, meeting all validity criteria for a useful animal model. As such, SDPS can induce a well-established, protracted depressive-like state that reflects the multifaceted character of depression, including negative affect, deficient motivation and cognitive dysfunction. This in turn gives a solid base to further investigate the neurobiological substrates and mechanisms underlying the delirious effects of depressive disorders and allows for identification of novel treatment targets.

Aims and scope
In the preceding sections, key behavioral and neurobiological characteristics of depression were presented, with focus on dysfunction of the affective and cognitive domains. In the following chapters, experimental evidence for the use of SDPS in modeling the depressive-like state will be presented. Central point of my work was to highlight a SDPS-induced behavioral state that reliably mimics depressive symptoms, thus providing the basis for molecular probing and testing of pharmacological interventions against depression.
Starting with chapter 2, I described the reciprocity between the sustained depressive state and alcohol abuse. Despite the frequent clinical comorbidity and the detrimental consequences of comorbidity in disease prognosis, to date, no preclinical model has been employed to specifically study their co-occurrence. Thus, in an attempt to develop a first animal paradigm that models concurrent primary depression and secondary alcohol abuse disorder, I employed SDPS in combination with alcohol self-administration procedures. With this approach, the unique influence of the depressive state on alcohol-seeking and -taking behaviors was identified.

In chapter 3, I examined the effects of SDPS in sucrose self-administration, aiming to further explore the influence of the depressive-like state on the evaluation of a natural reward. Historically, depression-induced anhedonia has been limited to behavioral readouts examining acute phases of sucrose consumption during or immediately after stress exposure. Instead, I aimed to broaden our understanding of anhedonia, by inquiring the effects of SDPS in instrumental responding for sucrose long after the application of stress. This approach allowed dissecting different aspects of the anhedonic phenotype, such as motivation and reward evaluation, in a preclinical setting.

In chapter 4, I examined individual variability to the effects of stress in subsequent development of the depressive pathology. By subjecting an outbred population of rats to the SDPS paradigm, I reliably identified depression-prone vs. depression-resilient rats, long after stress exposure. This led to a detailed profiling of depressive symptoms in the affective (social behavior) and the cognitive (spatial memory) domain, as they develop over time. Furthermore, this approach laid foundations for studying resilience in this preclinical model of depression.

In chapter 5, I questioned the impact of depression susceptibility in subsequent vulnerability to alcohol abuse. Based on the diagnostic criteria of addictive pathologies in humans, aspects of addiction-vulnerability were examined in depression-prone vs. depression-resilient rats. My work unmasked dependence-like phenotypes that were specifically associated with depression susceptibility. Likewise, I demonstrated that depression resilience limits the development of these behavioral manifestations.
In chapter 6, I aimed to underpin the molecular substrates of the depression-induced reduction in cognitive function. It is becoming largely understood that cognitive deficits not only accompany mood perturbations in depression, but are crucial mediators of the maintenance of the depressive state. To better understand the molecular mechanisms underlying SDPS-triggered cognitive deficits, I examined alterations of the hippocampal synaptic proteome and hippocampal physiology and intervened at the cellular level aiming to reverse cognitive dysfunction.

In the last part of my thesis, chapter 7, I brought together all experimental data on SDPS. In light of our results, the validity of the model is discussed, followed by an evaluation of the ways our work furthered our understanding of depression. In addition, I discuss the limitations we faced and the questions that remained unanswered. The chapter ends with future research perspectives for the study of SDPS-triggered depressive-like states.