Chapter 7: General Discussion

4. "πιας τ’ αυγό και κούρευτο" — Greek proverb
Social Defeat-Induced Persistent Stress as a preclinical model for depression

Characteristics of the depressive state
Major depression is a chronic mental disorder that affects approximately 1 out of 7 individuals during their lifetime4, independently of their cultural, societal, occupational or financial status. Depression is accompanied by severe health burden and increases the risk of mortality4-31. Depression onset is traditionally linked to genetic predisposition (inherited vulnerability)4-32, exposure to adverse conditions (extreme, prolonged stress)4-33 or interaction of the two4-34, such as the adoption of maladaptive stress-coping styles4-35. The depressive state is characterized by anhedonia3, i.e., reduced ability to experience pleasure from otherwise rewarding activities, for example, social interaction. Anhedonia encompasses a variety of motivational deficits, including difficulties in reward anticipation, cost-to-benefit evaluation and goal directed decision-making21. Furthermore, depression is accompanied by mild cognitive symptoms, such as reduced concentration and impaired memory3, which persist even after mood recovery. This phenotype manifests in combination with cognitive biases31, i.e., attentional allocation towards negative experiences and difficulty in processing of emotionally charged information4-35. Together, depression includes various endophenotypes with probable multifactorial causes. Due to this, and despite coordinated efforts from researchers and clinicians alike, the neurobiology of depression has remained poorly understood. Consequently, therapeutic options against this disorder are limited13, with ample side effects and only a third of patients responding to available treatments4-36.

SDPS employs a combination of social stressors
The heterogeneity in origins and manifestations of depression has been a matter of intensive research and discussion for decades. At the preclinical level, this resulted in questioning the suitability of behavioral readouts assumed to model depressive states4-37. As extensively discussed in the general introduction of my thesis, in recent years several animal models of depression have been developed1-38. Each of these displays advantages and disadvantages in terms of reliably mimicking depressive symptoms1-38,4-39, and

5. "The best material model for a cat is another, or preferably the same cat" — N. Wiener & A. Rosenblueth
most cover only one or a few of the endophenotypes of the depressive state. Models employing social stress are gradually becoming popular, since they are considered as ethologically most relevant. This is based on the notion that adverse events of social nature are implicated in onset, variability and persistence of depressive symptoms. In the studies described in this thesis, the Social Defeat-induced Persistent Stress (SDPS) paradigm was employed. SDPS offers a naturalistic exposure to social stress, by combining an acute phase of severe social hierarchy-based stress (social defeat) with a prolonged phase of sub-threshold social isolation stress (single housing). In an anthropomorphic view, this aims to emulate the adverse socio-cultural settings that are shown to be conducive to depressive pathologies in humans, including exposure to aggression and violence, deprived environments, feelings of loneliness and unmet social support.

Social isolation aggravates the depressive state
Perceived solitude is predictive of the depressed state in aging adults and has been associated with suicidal ideation during adolescence. Vice versa, the establishment of the depressive state is accompanied by social withdrawal and degrading interest towards social activities. The vicious cycle of loneliness and social avoidance facilitates the progression of depressive mood. In the SDPS paradigm, after social defeat, animals are single-housed for a prolonged period of time, mirroring unnatural social settings for rats, that, similar to humans, are social creatures that function optimally within groups. This protracted social deprivation is assumed to facilitate incubation of the depressive-like state in the longterm. Recently, a neuronal substrate for the subjective experience of social isolation was described in mice, implicating the dorsal raphe nucleus (DRN), the brain’s primary source of serotonin. Together with the well-documented role of DRN serotonergic transmission in depression and antidepressant response, these data further support the notion that prolonged social isolation, as in the case of SDPS, acts to maintain chronic depression. Supporting this notion, SDPS effects last for up to 6 months, representing ¼ of the expected lifespan of the rat.

Importantly, SDPS allows for investigation of the molecular, cellular and behavioral underpinnings of depression long after the stressful incidence and independently of the initial stress response. This has large impact on the clinical implications of the study: SDPS does not examine acute stress reactivity, rather, similar to human depression, it employs a combination...
of stressors that progressively lead to a maintained depressive-like state (see chapter 4). The gradual exposure to adversity and the consequent development of depressive symptoms thereby provides a solid foundation for investigation of the neurobiological mechanisms that mediate sustained depression, a valuable asset in the quest for novel treatment approaches.

**SDPS and the diagnostic criteria for depression**

A thorough discussion about the SDPS paradigm with respect to the four validity criteria for preclinical models, i.e., i) face; ii) ethological; iii) construct; and iv) predictive validity, is presented in the general introduction. Here, I aim to further this debate, highlighting the parallels between essential diagnostic criteria for major depression and the equivalent behavioral expressions as observed in the rat (Table 1), namely, its face validity. In addition, I drew similar analogies between the effects of SDPS and neurobiological manifestations of depression in humans (construct validity), and in response to treatment (predictive validity) (Table 1). In the following sections I will focus on two core features of the depressive-like state induced by SDPS, as its behavioral aspects can be classified into two major categories, i.e., perturbations of the affective (including reward-related) and of the cognitive domain. The two processes are interdependent and develop in conjunction. As such, no individual brain region or molecular process is known to be uniquely implicated in any of the two. Rather, it seems likely that interplay between dysregulated brain networks and their unbalanced output accounts for the observed behavioral impairments. Nevertheless, in the next sections, I attempted to summarize the behavioral data, and where possible the neuronal underpinnings, of the effects of SDPS based on these two phenotypic pillars of the depressive state.

<table>
<thead>
<tr>
<th>Major Depressive Disorder</th>
<th>Social Defeat-induced Persistent Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>Face validity (symptoms</em>)</em>*</td>
<td></td>
</tr>
<tr>
<td>Depressed mood (sadness)</td>
<td>Not examined</td>
</tr>
<tr>
<td>Diminished interest or pleasure (anhedonia)</td>
<td>↓ Social Motivation (AA) ↓ Social Recognition (SR) ↑ Anhedonia (Sucrose)</td>
</tr>
<tr>
<td>Weight loss / gain</td>
<td>↓ Acute / - Long-term</td>
</tr>
<tr>
<td>Food consumption</td>
<td>↓ Acute / - Long-term</td>
</tr>
<tr>
<td>Insomnia / hypersomnia</td>
<td>Not examined</td>
</tr>
<tr>
<td>Psychomotor agitation / retardation</td>
<td>↓ Open field</td>
</tr>
<tr>
<td>Fatigue or loss of energy</td>
<td>Not examined</td>
</tr>
</tbody>
</table>
Table 1. Comparison of the diagnostic features of major depressive disorder with the corresponding behavioral manifestations of the depressive-like state following SDPS. Included are SDPS-triggered phenomena that correspond to the four validity criteria (face-, etiological-, construct-, and predictive-validity) for animal models of neuropsychiatric diseases.

<table>
<thead>
<tr>
<th>Symptoms/Features</th>
<th>N/A</th>
<th>Reduction in working &amp; recollection memory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worthlessness &amp; guilt</td>
<td>N/A</td>
<td>Spatial ecollection (OPR)</td>
</tr>
<tr>
<td>Loss of concentration &amp; indecisiveness</td>
<td>N/A</td>
<td>Social Recognition (SR)</td>
</tr>
<tr>
<td>Reduction in working &amp; recollection memory</td>
<td>N/A</td>
<td>Discriminative ability (NOR)</td>
</tr>
<tr>
<td>Suicidal ideation</td>
<td>N/A</td>
<td>Attentional Biases (AA)</td>
</tr>
<tr>
<td>*Comorbidity (alcohol)</td>
<td></td>
<td>Context-dependent extinction (alcohol)</td>
</tr>
</tbody>
</table>

**Ethological validity**

Biological predispositions (genetic, hormonal, neuronal)
Not examined

Severe life-events, stress
Social Defeat & Social Isolation

Genes x Environment interaction
SDPS-prone individuals

**Construct validity (underlying pathology)**

Hippocampus (structure, function)
- Neurogenesis
- Long-term LTP
- Inhibitory tone
- CSPGs expression

HPA axis
- Acute / Long-term

**Predictive validity**

Pharmacotherapy
- Imipramine
- Chondroitinase AB

Cognitive therapy
- Environmental enrichment

*According to DSM (5th edition); symptoms should a) persist over time (at least for a period of 2 weeks) b) cause significant loss of functionality c) not be a result of medical condition nor be substance-induced. (↓) reduced; (↑) increased; or (-) unaffected by SDPS; N/A: not applicable at the preclinical level; #Not a symptom per-se; $Not examined in human patients as yet; (+) Responsive to antidepressant treatment and other interventions.
Affective component

Depressive disorders are often referred to as affective disorders, given that disturbances in mood and emotion are at the core of the observed pathology. It is of no surprise that the two prerequisite diagnostic criteria for major depressive episodes concern severe and persistent changes of the affective state: i) negative affect, such as sadness and guilt, and ii) anhedonia, such as diminished capacity for pleasure. Although depressed mood is the salient feature of MDD, patients might reach this state via different routes. In particular, pervasive negative thoughts and emotions, which are elicited by and exaggerated upon stress exposure, might act as the basis for maintained depression. Likewise, a sustained lack of positive reinforcement, which diminishes intrinsic motivational processes, might exacerbate the manifestation of the disorder. Both these processes are addressed by SDPS, in the form of social defeat (introducing acute, severe stress) and social isolation (prolonged environmental impoverishment, lack of positive stimuli).

SDPS, social motivation and avoidance behavior

The most evident example of disturbed affective function following SDPS is the decreased interaction with a social target, namely, a presumptive opponent of the Long-Evans strain (and chapters 4-6). In SDPS, avoidance behavior persists up to 6 months and is a prominent characteristic of animals susceptible to its effects (chapter 4). In animals living in communities, social approach is considered rewarding from an evolutionary perspective and experimental evidence implements the mesolimbic system, the brain’s reward center, in the expression of social behaviors. Following defeat exposure, deficits in social approach have been directly correlated with molecular and neurophysiological disturbances of the mesolimbic dopaminergic network. Likewise, inter-individual differences in social approach and antidepressant response are both mediated by neuroadaptations seen in the mesolimbic pathway, including alterations in the firing patterns of dopaminergic neurons.

In depressed patients disturbed approach-avoidance performance is associated with diminished behavioral reinforcement derived from presentation of positive stimuli. This indicates dysfunctional processing of reward-associated information in combination with difficulties in processing of affective material (see below – cognitive component). In a similar manner, social avoidance after SDPS might reflect motivational...
deficits that are mediated by aberrant relay of reward-related information. In this respect, presentation of an unfamiliar social target, although rewarding for control rats, might elicit abnormal affective reappraisal in the SDPS group, leading to maladaptive approach behavior. It is of note that SDPS-induced alterations in reward circuitry occur in parallel with abnormalities in stress and emotional centers (e.g., the amygdala), and in areas dictating higher-level decision-making (e.g., PFC). Given the extensive connectivity between these areas, their influence on the expression of avoidance behavior is highly plausible.

SDPS and incentive motivation
Another example of SDPS-triggered disturbances in the affective domain is the profound dysregulation in motivation to seek and acquire natural and drug-related rewards, reflecting aspects of anhedonia. SDPS resulted in excessive motivation to seek alcohol and sucrose, whereas SDPS-susceptibility was correlated with exaggerated motivational deficits (chapter 5). According to literature and in agreement with our data, the anhedonic state manifests itself in various ways including impairments in cost-to-benefit evaluation and subsequent reward-based decision-making and action planning. The term "decisional anhedonia" has been used to describe a state during which over-evaluation of the incentive salience of rewards and reward-associated cues leads to inappropriate behaviors. Our data support this notion, as SDPS-triggered maladaptive reward-seeking was observed during effort-for-profit computations in progressively demanding reinforcement schedules. Notably, in healthy individuals, motivation to approach and/or acquire a given reward is proportional to the degree of satisfaction the reward serves (anticipatory hedonic valence). Depressed patients on the other side do not adjust their behavior based on reward satisfaction. Rather for them, liking and motivation (as in effort required to obtain the reward) are dissociated. In SDPS this is exemplified by the unaffected consumption of rewards when these are available abundantly (e.g., home-cage consumption).

Hypersensitive stress response or dysfunction of the reward system?
Together with a dysfunctional reward system in SDPS, the involvement of an over-responsive stress circuit is hypothesized, which supersedes mechanisms that are activated in the presence of rewards. According to this hypothesis unbalance among the pathways governing positive
reinforcement vs. anxiety and fear leads to maladjusted behavioral manifestations, as observed in depression. In favor of this notion, amygdala activation, e.g., after presentation of negative emotional faces, is exaggerated and long-lasting in depressive disorders\textsuperscript{462} and predicts depression severity\textsuperscript{463} and individual response to antidepressant treatment\textsuperscript{464}. In a similar manner, it is possible that increased reactivity of the stress pathways overshadows reward-driven behavior in drug dependence. Indeed, stress-induced negative reinforcement is crucial in transitioning to addiction\textsuperscript{465} and might contribute to the often seen coexistence between depression-like pathologies and extreme preoccupation with drugs of abuse\textsuperscript{41,74}.

The two mechanisms described above are not mutually exclusive. In our understanding, excessive or continuous stimulation of stress pathways and concomitant dysfunctional reward-related information processing might contribute to phenotypic manifestations observed in depressed patients and in addicts alike. These behaviors include anhedonia or reward sensitization and social avoidance or reduced motivation for non-drug rewards, respectively. In addition, these parallel processes could occur together with a reduction or loss of cognitive control, possibly leading to maladaptive decision-making as observed in both afflictions. When referring to the comorbid, depressive-addictive phenotype, our own observations on the effects of SDPS in alcohol-seeking behaviors further support a simultaneous dysregulation of the two systems\textsuperscript{535}. Indeed, we showed that the severity of avoidance behavior, reflecting the depressive state, is predictive of subsequent vulnerability to dependence-like behaviors, such as increased motivation to acquire alcohol\textsuperscript{535} and impulsive alcohol seeking (chapter 5). Together, these data argue in favor of concurrent and interdependent adaptations of the reward- and stress-systems.
Cognitive component

Albeit less acknowledged, cognitive dysfunction is consistently met in major depressive disorder, with up to 30% of patients displaying clinically significant cognitive impairments. In MDD, aberrant cognition manifests itself mainly in deficits in working memory, concentration and attention and executive function. Cognitive difficulties are associated with the degree of depression-induced disability in every-day life and functional recovery. It has been proposed that impairments in cognitive function can be categorized in two major groups, namely cognitive biases, which include maintained attachment to stimuli of negative valence, and cognitive deficits, which include disturbances in short-term memory, planning and problem-solving.

SDPS and cognitive bias

Disturbed emotional processing in depressed individuals is thought to promote aberrant attentive, perceiving and motor reactivity to stimuli of emotional valence, depending on the charge of the given stimulus, namely, cognitive bias. Commonly, depressed patients persistently focus on stimuli or memories of adverse nature, and they show hypersensitivity to perceived punishment and negative feedback. From clinical studies, there is a consensus that excessive bottom-up influence (e.g. hyperactive amygdala) and reduced top-down regulative role (e.g. dysfunctional PFC) diminish cognitive control over the emotional response, thereby mediating this bias in depression. Indeed, in depressed patients, increased amygdala reactivity during processing of emotional information contributes to attentional allocation towards negative cues and increased memory of negative items.

An example of hypersensitivity to perceived punishment can be observed in SDPS rats, as they exhibit avoidance of a potentially threatening social target (LE rats). Although approach behavior was under all circumstances safe, defeated animals showed persistent over-generalized avoidance behavior, reflecting reduced control over their emotional response to the presentation of the social target. Furthermore, in patients, invasive memories of a traumatic life event, such as the loss of a loved-one, contribute to an unrealistic representation of the possible outcome when placed in similar environments and/or confronted with reminding conditions. It is possible that after exposure to social defeat, SDPS rats exhibit avoidance towards the resident LE rats as they experience a
“perceived” re-occurrence of the traumatic incident. Together, SDPS reliably mimics depression-induced difficulties in cognitive control over negatively charged affective information. Notably, the reduction in interaction with an unfamiliar social target was limited to the resident opponents (LE rats), as SDPS-exposed animals did not show deficits in exploration of a positive target, i.e., a Wistar juvenile rat (own unpublished observations). This implies that defeat-induced social avoidance is specific to social stimuli of negative salience, further arguing in favor of the establishment of an affective bias after SDPS.

Moreover, SDPS elicited deficits in cognitive control over non-social rewarding stimuli, such as the alcohol- and sucrose-associated cues within the self-administration apparatus (context). As discussed above, a tendency towards anhedonia is prominent following SDPS, and can manifest itself as increased reinstatement of alcohol-seeking behavior, driven by the presentation of stimuli previously paired with alcohol delivery. These results parallel findings in humans that link the magnitude of alcohol craving with over-evaluation of and attentional biases towards alcohol-associated cues. Furthermore, during extinction of the reward-associated context, SDPS induced an extinction-resistant phenotype that was correlated with the severity of the depressive-like state (chapter 5), possibly involving dysregulation of higher cortical areas that mediate extinction learning. Likewise, SDPS-prone animals exhibited impulsive-like responding independently of reward delivery per se (chapter 5, cf. time-out responding), a behavioral manifestation traditionally associated with poor function of the cortico-striatal circuitry, known to regulate impulse control. It is noteworthy that SDPS-vulnerable rats resemble the behavioral profile of sign-trackers, animals that are prone to assign incentive salience to reward-associated cues rather than the reward itself. Sign-trackers show aspects of addiction-vulnerability such as increased impulsive-like action, delayed extinction, and facilitated reinstatement. These phenotypes are displayed by the SDPS-prone subpopulation, further supporting the notion of poor cognitive control and a subsequent inability to disengage attention from reinforcing cues or contexts that is exaggerated in depression-susceptible individuals.

SDPS and cognitive deficits

*Cognitive deficits,* for example failure in short-term memory, are thought to originate from disturbances in attention and concentration and thus are considered secondary symptoms in depressive pathology. Depressed patients display mnemonic impairments in a wide range of experimental
tasks, such as when testing verbal memory, in which the degree of the deficit correlates with the duration and persistence of the depressive state. Additionally, spatial memory performance is disturbed in depression and this effect is associated with hippocampal dysfunction. The most prominent example of cognitive deficits in SDPS was the inability of animals to retain place-related information following a short training phase at the object place recognition task. SDPS elicited long-lasting impairments in short-term spatial memory, which weighed on the severity of the depressive-like state (chapter 4). Similar to the human disease, these memory deficits were associated with functional deterioration of the hippocampus (chapter 6), a brain area that is thought to coordinate initial memory formation. Of note, our data argue that these memory impairments develop and stabilize subsequent to impairments in the affective domain (chapter 4), providing a unique temporal profiling of depressive symptoms at the preclinical level. This resembles the clinical progression of depression in which primary mood-related symptoms precede attentional deficits/biases, which in turn are considered a triggering factor for memory impairment.

Difficulties in managing affective information are thought to contribute to cognitive impairment in depression, particularly under conditions viewed as cognitive-demanding. For example, depressed patients show reduced cognitive capacity when presented with effortful tasks or when the task success is based on ignoring or circumventing stimuli of affective salience. Accordingly, SDPS induced a persistent decline in the social recognition memory task, a challenging behavioral readout, which to a large degree calls for emotional processing. Long-term social recognition requires intact episodic memory, i.e., the ability to form and recall memories of events in temporal and spatial detail. Episodic memory is thought to necessitate emotional arousal, and experiences of emotional valence are better consolidated and retrieved than non-emotional ones. SDPS animals showed reduced memory retention of the emotionally relevant target, possibly pointing at i) attentional deficits during pre-task (consolidation phase), and/or ii) a poor retrieval of affective information during the task (retrieval phase). Noteworthy, as mentioned above, there is a third factor that might influence social recognition performance, namely the motivational potential, i.e., the intrinsic urge of a rat to engage into social activities or the lack thereof.
Hippocampus and the SDPS-induced disruption of cognitive function

It is worth mentioning that the hippocampus (HPC) might be the locus of convergence for both cognitive biases and cognitive deficits that develop after SDPS. Several lines of evidence argue in favor of hippocampal dysfunction being central to the cognitive aspects of the depressive state, as well as the associated SDPS-driven alcohol-related pathology. Particular examples and the parallels drawn by our own studies are listed below:

i) The crucial role of the HPC in spatial memory\textsuperscript{227,311} and context-associated social recognition\textsuperscript{486} is well described. Both these types of memory are severely disrupted after SDPS\textsuperscript{152,153,155} and are exaggerated in animals susceptible to its effects (chapter 4 and own unpublished observations);

ii) SDPS induces imipramine-reversible changes in hippocampal neurogenesis\textsuperscript{145}, which is required for its antidepressant behavioral effects\textsuperscript{65};

iii) SDPS induces imipramine-reversible reduction in hippocampal plasticity\textsuperscript{166}, which underlie deficits in short-term spatial memory performance. Remodeling of hippocampal extracellular matrix and subsequent normalization of hippocampal function restores these cognitive deficits (chapter 6);

iv) Both clinical\textsuperscript{487} and preclinical\textsuperscript{488} studies showcase the importance of intact hippocampal function in context-dependent extinction learning, as it is required for determination of relevance and adaptive memory update\textsuperscript{30}. SDPS delayed extinction learning\textsuperscript{152}, an effect that was further aggravated in the vulnerable population (chapter 4);

v) The hippocampus is required for cognitive control, as hippocampal lesions induce impulsivity\textsuperscript{489} and disrupt cost-to-benefit decision-making\textsuperscript{490}. Likewise, SDPS-prone animals exhibited increased impulsive-like behavior (chapter 5);

vi) Context-associated reinstatement of drug-seeking depends on the hippocampus and its communication with cortical and subcortical areas\textsuperscript{66,491}. SDPS affected context-mediated relapse of natural and drug-related rewards\textsuperscript{152,155} and this phenotype was exacerbated by SDPS-proneness.

Together, extensive literature and our data support the idea of a dysfunctional HPC that is associated with reduction in context-dependent memory, dysregulation of context-regulated emotional response and the effects of antidepressants.
The communication of the HPC with other key brain structures (PFC, amygdala, NAc) support a role for the HPC as a hub mediating these aspects of the depressive pathology. Thus, selective manipulation of hippocampal plasticity should be at the forefront of future depression research and should be extensively probed for novel therapeutic entries.

Molecular dissection of cognitive deficits after SDPS

As mentioned before, the hippocampus is heavily implicated in depression-induced cognitive impairment, such as poor memory and reduced cognitive flexibility. Amongst the most consistent findings in depression research are volume loss, decreased neurogenesis and functional decay of the HPC, observed both in clinical and preclinical settings. Hippocampal atrophy is associated with both greater memory impairments and poorer clinical outcomes in depressed individuals. Moreover, extensive literature supports detrimental effects of chronic stress in context-associated HPC-dependent spatial learning and memory. Based on these observations, stress-based animal models of depression have focused on recapitulating hippocampal dysfunction, assessing reduction in hippocampal neurogenesis and plasticity, as well as deficits in HPC-dependent memory. Recent studies have illustrated the complex anatomical and functional underpinnings of cognitive deficits in depression, unmasking glutamatergic, GABAergic and monoaminergic contributions. Our work identified a novel molecular mechanism for hippocampal dysfunction in depression and highlighted the extracellular matrix (ECM) as a novel substrate for the antidepressant response (chapter 6).

Non-neuronal contributions in SDPS-induced dysregulation of the hippocampus

The identification of ECM as an active mediator of cognitive deficits in depression poses several novel questions. Whereas ECM molecules are synthesized and released by neurons and astrocytes alike, chondroitin-sulphate proteoglycans (CSPGs), such as Brevican, Neurocan and Phosphacan, are all primarily expressed by astrocytes. In SDPS, aberrant expression of CSPGs correlates with the magnitude of depression in the SDPS-prone subpopulation (own unpublished data). Although not addressed

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6. "If you can't explain it simply, you don't understand it well enough." — Albert Einstein
in our studies, it is possible that astrocyte-derived ECM contributes to the SDPS-induced deficits in hippocampal physiology and plasticity. Indeed, recent experimental evidence showcased the functional role of glia-derived ECM molecules on synaptic function and plasticity⁵７. Astrocytes shape synaptic connectivity and functional dynamics locally at the tripartite synapse⁴⁹⁶, while coordinated synaptic activity from neighboring astrocytes regulates excitation/inhibition balance in large networks, e.g., via gliotransmission⁴⁹⁷. Taken together, astrocytes are perfectly located and well equipped to alter hippocampal physiology and to modulate ECM assembly. At present, a large body of evidence supports the involvement of astrocytes in depression⁴⁹⁸ and antidepressant response⁴⁹⁹, although the molecular substrate of this is yet to be identified. Alterations in the molecular composition of astrocytes, including expression of glutamate transporters, is commonly seen in post-mortem material of depressed patients⁵⁰⁰,⁵⁰¹, particularly at the hippocampal CA1⁵⁰². Importantly, dysfunction in glia-mediated glutamate uptake, which is Tenascin-R-dependent⁵⁰³, is implicated in cognitive symptoms in depression⁵⁰⁴, indicating a significant role for astrocyte-facilitated glutamate transmission in depressive pathology. Future research should focus on deciphering the role of astrocytes and astrocyte-derived ECM in the developing cognitive symptoms in SDPS, and in particular, on addressing whether astrocyte dysfunction precedes -and even triggers- ECM changes or whether SDPS-induced alterations in ECM assembly lead up to astrocyte (mal)adaptations.

**Extracellular matrix remodeling: from stress to depression**

A question of importance concerns the temporal profile of ECM dysregulation in SDPS. Only recently the effects of environmental adversity on ECM started to be explored. For example, pericellular ECM, in the form of perineuronal nets (PNNs) is necessary for the maintenance of a fear memory following exposure to acute stress (foot-shock)⁴¹⁶. Furthermore, chondroitinase ABC (ChABC)-triggered disruption of PNNs renders the fear memory susceptible to extinction-assisted erasure. This mechanism has been described in the amygdala, hippocampus and the medial prefrontal cortex, depending on the nature of the fear memory, i.e., cued-, context- or remote-memory, respectively⁴⁰⁰,⁴¹⁶. In addition, there is evidence that this mechanism underlies erasure of fear memories following antidepressant administration when combined with extinction training⁴¹⁵. These seminal studies indicate an active role of the ECM in adaptations following exposure to acute stress and pinpoint to a more general mechanism that is not restricted to a particular brain region, nor is limited by the intrinsic characteristics of the (type of) memory.
It is tempting to hypothesize that, in a similar fashion, ECM participates in the dysregulation of the hippocampus acutely following social defeat stress, with implications for the development of the depressive state in the long term. In our model, ECM changes were causally related to cognitive deficits (short-term place recognition), as observed following 2–3 months from the last defeat episode. As we showed in chapter 4, this cognition-related impairment is temporally delayed, i.e., it emerges after affective disturbances and it is established at ≥8 weeks following stress exposure. Assuming that the pericellular and perineuronal increase in ECM levels are causal to the observed cognitive deficit, it is plausible that gradual alterations in ECM composition and PNNs organization take place during the isolation period of the SDPS paradigm and become maladaptive as the depressive-like state develops.

**Extracellular matrix remodeling: adaptations at the molecular, cellular and circuit level**

Furthermore, in chapter 6 we explored the effects of ChABC-facilitated ECM reorganization on long-lasting depressive-like symptoms. Several studies that employ ChABC report positive or negative effects of treatment on cognition depending on the model and the type of cognitive assessment used. For example, acute ChABC has been shown to improve hippocampus-mediated contextual memory in a preclinical model of Alzheimer’s disease\(^{505}\). Effects of ChABC include improved object recognition up to 1 week in transgenic\(^{506}\) and up to 3 weeks in wild-type\(^{381}\) mice following intraperirhinal cortex administration. Maintenance of drug-related memories is disrupted up to 9 days after ChABC administration in the prelimbic PFC\(^{507}\) and, when combined with extinction training, intra-amygdalar ChABC application prevents reinstatement of drug-related memories at 3 weeks following conditioning\(^{508}\). In SDPS, cognitive improvement was seen at 10–12 days following ChABC application, a time-point that coincides with partial CSPG and PNN recovery. In addition to its behavioral effects, ChABC reversed SDPS-induced deficits in hippocampal plasticity (long-term potentiation), as observed up to 3 weeks after administration. Taken together, existing literature and our own observations imply that both acute and delayed downstream effects of ECM remodeling account for severe alterations in cognitive function.

In the rat, acute ChABC application induces an almost complete ECM removal (chapter 6). It is possible that structural plasticity occurring acutely following ChABC accounts for enhanced synaptic communication, for example, by facilitation of AMPA lateral mobility\(^{392}\) and NMDA clustering\(^{509}\). 

General Discussion
Likewise, enhanced signaling might occur following the release of ECM-bound molecules, such as growth factors that, among other effects, are known to initiate structural changes\textsuperscript{510}. Of note, the contribution of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) proteins in SDPS-induced ECM reorganization and in ChABC effects is assumed. MMPs and ADAMTS activity is crucial for endogenous ECM composition, as they regulate its enzymatic proteolysis, making them a tangible alternative target to ChABC for counteracting SDPS-induced cognitive effects. Although not examined in our studies, it is possible that MMP inhibitors, such as tissue inhibitor of metalloproteinases (TIMPs), take over during early time-points following ChABC administration, to ensure balanced and timely recovery of the ECM.

At later stages (2-4 weeks), post-ChABC ECM recovery can affect the synaptic network within the hippocampus, e.g., via its effects on parvalbumin (PV)-expressing interneurons. In chapter 6, we proposed that reduced excitatory input onto PNN‘/PV‘ neurons, a neuronal population that is increased in SDPS rats, can alter their inhibitory output, thus creating a low-plasticity network configuration in the hippocampus with the observed cognitive effects. This is substantiated by data supporting activity-mediated changes in excitatory/inhibitory input onto PV‘ neurons that eventually lead to adaptive learning and memory processes\textsuperscript{384}. Furthermore, we showed that a single ChABC application reverses the changes in inhibitory transmission and hippocampal plasticity. This is in accordance with the effects of long-term antidepressant treatment on PNN‘/PV‘ neurons that is shown to reactivate the period of enhanced plasticity, promoting memory formation\textsuperscript{416}.

Finally, at a later stage following ChABC treatment (≥4 weeks), which is denoted by the conformation of a new ECM network (chapter 6), recovery of neuronal communication at the circuit level is hypothesized. This is supported by the fact that, besides its putative cognitive-boosting effects, ChABC partially restored SDPS-induced deficits in social recognition (long-term memory) and approach-avoidance behavior. These complex behaviors are beyond hippocampal control\textsuperscript{146,511} and are subjected to robust neuromodulation\textsuperscript{512}. Thus, their partial normalization by ChABC could indicate restoration of the brain inter-regional connectivity. It would be of great interest to experimentally test this hypothesis, for example by probing the effects of inactivation of DRN-to-HPC projections in social avoidance\textsuperscript{513} in ChABC-treated rats. Likewise, it would be essential to functionally assess communication between HPC and the PFC\textsuperscript{514,515} in SDPS,
for example by analysis of the physiological properties of anterograde traced (HCP-to-PFC) neurons.

Future Directions

Novel behavioral approaches in SDPS

It is tempting to consider the SDPS paradigm a flawless preclinical model of depression, however, there are still many unanswered questions over its utility. To start with, although we examined various behavioral expressions of SDPS in detail, several landmarks of the depressive pathology that can be experimentally assessed in rodents, such as behavioral despair, attentional allocation and sexual communication were not addressed in my work (Table 1). Thus, it would be of interest that future research would probe these manifestations in order to explore the disorder in its entirety and to identify endophenotypes\(^\text{17}\) and their correspondent molecular substrates.

Furthermore, the SDPS paradigm would greatly benefit from assessing the effects of defeat exposure at different time points within the life course of an animal. Adolescence, for example, is a crucial developmental period during which environmental stressors can trigger depressive pathologies both in humans\(^\text{516}\) and in rodents\(^\text{517}\). Notably, adolescent defeat exposure elicits differential reactivity towards drugs of abuse later in adulthood, with severe implications for the development of addictive behaviors\(^\text{518}\). Similarly, it renders animals prone to late-life hippocampus-dependent cognitive decline\(^\text{519}\). It would thus be of great interest to examine whether adolescent SDPS exposure is characterized by the same neuroadaptations that mediate cognitive deficits seen in adulthood (e.g. alterations in hippocampal PNNs and inhibitory neurotransmission).

As mentioned earlier, genetic predisposition likely confers vulnerability to disease onset and facilitates the development and maintenance of depressive symptoms. Specifically, individual reactivity to environmental conditions, such as adverse life events that elicit subsequent adaptive homeostatic processes, “personalizes” the manifestation of the depressive state. This interaction between genetic predisposition and environment adds an extra level of complexity in the search for the neural basis and ontology of depressive disorders\(^\text{8}\). For example, a family history of depression increases the risk of multiple depressive episodes\(^\text{520}\), and the occurrence of each episode exacerbates the probability of a subsequent one\(^\text{521}\). In addition, it is though that the threshold of episode-triggering stress decreases following initial occurrence, so as sub-threshold stress...
exposure can kindle the continuation of the disease\textsuperscript{522}. We just tapped into these matters when examining the effects of SDPS in the depression-prone subpopulation (chapters 4, 5). Selective breeding of SDPS-prone and -resilient individuals would further facilitate the elucidation of mechanisms promoting depression susceptibility and those underlying resilience, potentially leading to clinical breakthroughs.

Most important, the SDPS model is applied in male rodents as it is partly based on dominance-submission interaction. It is challenging to apply such a model in female rodents, since they do not show hierarchical dynamics similar to males. Yet, depression prevalence is doubled in women\textsuperscript{5}, who experience more presumed vulnerability to external triggers, such as stressful environmental challenges, and more frequent disease onset compared with men\textsuperscript{522}. Likewise, women diagnosed with major depression are in sevenfold risk of developing alcoholism when compared with men, and vice versa female alcoholics are in ~30\% higher risk of comorbid depression versus their male counterparts\textsuperscript{508,524,525}.

Together, these facts demonstrate the imperative need to examine the effects of SDPS in a female population. Although it is more technically demanding, this might be feasible by using lactating females as aggressors\textsuperscript{526,527} during the resident-intruder phase of the SDPS paradigm. Similar to our model, social defeat in females precipitates social avoidance\textsuperscript{528}, anhedonia and altered response to drug of abuse\textsuperscript{529,530}. In addition, using this paradigm, epigenetic effects of stress have been identified, such as dysregulation of the HPA axis and corticosteroid signaling\textsuperscript{531}, which could confer risk for the development of depression.

**Mechanisms underlying SDPS-induced hippocampal dysfunction**

Despite our efforts, numerous questions concerning the effects of SDPS at the cellular level and in particular on cell-to-cell communication at the hippocampal CA1 remained unanswered. First, although we discussed the effect of SDPS on perisomatic excitation (\textit{cf.} decreased input onto PNN\textsuperscript{-}/PV\textsuperscript{+} cells, chapter 6), the contribution of dendritic excitatory input onto PNN\textsuperscript{-}/PV\textsuperscript{+} was not addressed. In the hippocampus CA1, PV\textsuperscript{+} interneurons receive the majority of excitatory input onto their dendrites\textsuperscript{532}. As SDPS altered the expression of perisynaptic ECM (\textit{cf.}, upregulation of synaptic CSPGs) it is possible that these observed plasticity effects were extended to input onto PV\textsuperscript{+} dendrites. If true, this would further contribute to reduced PV\textsuperscript{+} inhibitory output. Another essential element that is missing from our analyses is the direct assessment of the excitatory input onto PNN\textsuperscript{-}/PV\textsuperscript{+} and PNN\textsuperscript{-}/PV\textsuperscript{+} interneurons in SDPS, e.g., by examining evoked...
EPSCs in PV+ cells, as well as their accumulated inhibitory output, e.g., by measuring presynaptic GABAergic release. Although conceptually straightforward, these experiments are technically very challenging in the rat model, and need to be addressed using transgenic (PV-Cre) animals and/or viral delivery of constructs that allow for visualization of PV+ interneurons in vivo.

Using the same tools, the contribution of PNN-free PV+ interneurons to inhibitory transmission and hippocampal plasticity in SDPS might be further elucidated. PNN/PV+ cells constitute a large population (~40–50%) of interneurons at the CA1 subfield. These cells show increased excitatory perisomatic input vs. their PNN-coated counterparts. Notably, this is in parallel with an SDPS-induced reduction in the density of the inhibitory perisomatic puncta (presynaptic terminals) these cells receive (own unpublished data). Together, these data point towards possible over-excitation of PNN/PV+ cells in SDPS, which might lead to increased inhibitory output of that particular interneuron population. The most likely explanation for such an event would be an attempt to compensate for the reduced inhibitory output of PNN/PV+ interneurons, an adaptive change aiming to maintain the excitation/inhibition balance in the depressed hippocampus. Alternatively, PV+ cells are shown to contact other GABAergic cells in closed loops at the CA1 stratum pyramidale for example, cholecystokinin (CCK)-expressing basket cells. Provided that PNN/PV+ interneurons would preferentially target GABAergic cells it is possible that their over-excitation contributes to the observed decreased inhibitory frequency onto pyramidal cells. Finally, the contribution of other types of interneurons of the CA1, such as somatostatin-expressing and CCK+ cells, in the effects of SDPS needs further attention. Of particular interest are CCK+ cells, which project both back to PV+ interneurons and principal cells, with the latter effect being targeted at the stratum pyramidale cell bodies and at dendrites that receive excitatory input from the CA3. Together, CCK-expressing interneurons are well located within the hippocampal circuitry and their modulation might be sufficient for SDPS-induced disruption of this functional network.
Conclusion

Despite large efforts in recent years, research on affective disorders still suffers in two major ways. First and formost, as they present great phenotypic complexity (e.g., melancholic, atypical endophenotypes of MDD; bipolar; and comorbid anxiety disorders), one of the biggest challenges is to model these afflictions, with all their variants, in a preclinical setting. Indeed, the inability to mimic key depressive features such as feelings of worthlessness, guilt and suicidal ideation questions the face validity of the existing animal models. Adopting questionable behavioral readouts that reflect an over-simplified interpretation of the human condition might actually hinder progress in the field. Second, as the quest for better animal models carries on, the complexity of the neuronal mechanisms involved in this heterogeneous disorders only now started to appear, rendering targeted therapeutic manipulations hard to identify and achieve.

In the preceding sections I attempted to assess the validity of the SDPS paradigm as a preclinical model of depression. SDPS, is by default insufficient to examine the whole spectrum of behavioral constructs that develop during human depression, such as ruminations over one’s own death. While acknowledging these limitations, it can be used to faithfully model several core phenotypic manifestations of the depressive state. As briefly mentioned in the general introduction, we approached depression in a practical manner, attempting to model independent dimensions of the disorder, for example, the anhedonic state or putative cognitive deficiencies (chapter 6), emerging long after stress exposure. In the process of doing so, and sometimes to our own surprise, we achieved modeling far more complex constructs, including depression comorbidity and susceptibility (and chapters 4, 5). The net result was to illustrate that by employing the SDPS paradigm, we can both parse depression in its individual components, e.g., the affective and cognitive symptoms, and assess these as interdependent variables that act jointly during depression.

Preclinical models emulating depressive-like phenotypes are central in furthering our understanding on how external triggers (e.g., persistent adverse environmental factors) as well as endogenous contributions (e.g., genetic predisposition) interact to induce this complex disorder. The SDPS model holds promise towards the elucidation of depression endophenotypes and their unique neuronal substrates. Collectively, my work highlights the usefulness of the SDPS paradigm and lays the foundation for future research that focuses on the identification of novel molecular and cellular targets for the treatment of depression.