Towards a mouse model of depression : a psychoneuroendocrine approach

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Chapter 9

General Discussion

Outline

9.1. Introduction

For decades, treatment of depression has relied on combined behavioral and neuropharmaceutical approaches, which usually take weeks to become effective. These approaches invariably are aimed to reduce the negative symptoms (including anhedonia, a key symptom in depression) patients experience in their daily activities they previously found enjoyable. Still, faster acting and more efficient drugs are needed. The chronic inability to cope with stress is a major risk factor in the precipitation of depression. Hence, there has always been a line of reasoning that intervention in stress system activity *per se* could be a more direct therapeutical approach towards reduction of depressive symptoms. Following, the glucocorticoid antagonist mifepristone (RU38486=MIF) was tested for its efficacy in depressive patients. MIF appeared to be a rapid acting drug primarily abolishing the psychotic symptoms characteristic for a subtype of depression, i.e. psychotic major depression (DeBattista and Belanoff 2006 and the discussion by Carroll and Rubin 2006), while also ameliorating other negative symptoms of depression such as emotional dysregulation, mood disturbance and anhedonia.

The **objective** of the research described in this thesis was to develop a mouse model of depression that would express anhedonia, induced by chronic stress. Anhedonia was assessed by studying the behavioral response to positive stimuli, and reward expectation. We hypothesized that a chronically stressed individual would have a reduced response to such positive stimuli. To test this hypothesis we have exposed mice repeatedly to a psychosocial stressor using our 'rat stress' paradigm. Furthermore, we explored the impact of reward on behavioral performance of the 'depressed' mouse. As a potential therapeutical approach we assessed the outcome of repeated pharmacological blockade of glucocorticoid receptor (GR) function using MIF on stress system activation and behavioral responses.

First we designed a novel method of drug administration to mice, and optimized several behavioral tests. Next, the expression of anhedonia was validated by phenotyping the consequences of chronic stress in our model. Also the outcome of repeated GR blockade with the GR antagonist MIF was examined on neuroendocrine and behavioral response patterns. The last step aimed to compare the impact of chronic stress in healthy human volunteers with its effects in naïve mice on two distinct learning and memory systems.

9.2. Methodology

Drug delivery of glucocorticoid ligands

We deduced the magnitude of the stress response from the enhanced and long-lasting elevations of corticosterone (CORT) secreted by the adrenals. CORT is the endproduct of the HPA axis and feeds back on the brain to modulate the processing of information underlying the neuroendocrine and behavioral response pattern. This action exerted by CORT is mediated by MR (mineralocorticoid-) and GR activation that operate in complementary fashion (Oitzl and de Kloet 1992; De Kloet et al. 1998).

Most often, drug delivery involves hand-restraint administration (e.g., subcutaneous, intraperitoneal or by gavage), which leads to a concomitant, uncontrolled, and unwanted activation of the stress system (Balcombe et al. 2004). This unwanted stress effect would be a serious confounder in our experiments. Therefore, a method of stress-free drug delivery was required. Using oats, we succeeded in designing a novel non-invasive, stress-free method of glucocorticoid administration in mice (**Chapter 3).**

Drug delivery via oats reduced stress system activation as is evident from the following consideration: The method of drug delivery is non-invasive and avoids handling of the mice; they can remain in the home cage (= non-invasive) where the oats are delivered in a separate feeding cup. Although this procedure elicits a short-lasting, slight increase in plasma CORT secretion, its magnitude is not at all comparable to injection-induced effects, neither in quantity nor quality. Removal of physical contact between human and mice also has the advantage that minimal training is required to deliver the drug of choice to the animal; there is no restraint and no injection needed. Hence, the oat procedure minimizes variability of drug effects induced by the experimenter. Animal discomfort is also strongly reduced due to refinement of the method. Moreover, oats containing the glucocorticoid ligand(s) can be offered during both the light and dark period of the 24h circadian cycle. Because oats were given in addition to standard food and water regimes, the mice considered the oats as a treat. This was evident from the observation that all mice readily ate the oats within 10 min after administration. As a result, the need for food and/or water deprivation, which is considered to be stressful to mice, was by-passed (Sommerville et al. 1988; Duclos et al. 2005). We have shown that mice eat the oats with and without glucocorticoid ligands, for at least 7 consecutive days (**Chapter 4**).

The preferred method of administration depends on the experimental design. Drug delivery via oats is not selective as it reaches the entire body. Circulating CORT

has been shown to cross the blood-brain-barrier, although the concentrations in blood plasma vs. brain tissue differ. Penetration of MIF through the blood-brain-barrier is hampered and the drug is rapidly metabolized albeit in active metabolites. Therefore, high concentrations of the antagonist are to be administered (Karssen 2003). Collectively, GR and MR agonists and antagonists delivered via oats likely can activate and block MR and GR functions in brain.

Drug delivery via oats in close-context with behavioral testing is also feasible. However, it might be less suitable to study fast drug effects. The use of oats as a reward in close-context with learning and memory testing might be confounded however, by the postprandial increase in blood glucose, resulting from oats consumption. Glucose is known to modulate cognitive functions (Messier 2004) and can modulate glucocorticoid action (Gagliardino et al. 1984; Peters et al. 2004). However, the observed glucose increase following oat consumption is a mere fraction compared to the glucose concentrations that are required to modulate learning and memory processes in which glucocorticoids are involved (Gold 1986; Messier 2004; Dalm et al. 2009b). Therefore, a role of postprandial glucose is unlikely.

Drug-delivery via oats requires that mice are single housed, at least during the time of drug delivery. In the majority of behavioral tasks the animals are phenotyped one at a time. For experimental designs that require animals to be treated while group-housed, partitioning of the home cage might be a possibility. However, it is likely that interference with the home cage environment will introduce an additional stress factor (Ouagazzal et al. 2003; Chourbaji et al. 2005) to which the animals can be habituated by a handling procedure.

Advantages and disadvantages of drug delivery via oats are summarized in Table 1 below:

Table 1: Advantages vs. disadvantages using drug delivery via oats.

Advantages	Disadvantages
Non-invasive	Time required for the mice to eat the oats is 10 min at a minimum
Minimal corticosterone secretion during delivery, i.e. stress-free	Animals single-housed
Reduces animal's discomfort	
Can be used by any experimenter without extensive training in animal handling	
Administration can be continued for at least 7 days	
Application of drugs in behavioral context	
Application possible throughout the day	
Self-administration resembling human drug delivery	

In conclusion, drug delivery via oats reduces unwanted stress system activation and can be used in close-context with learning and memory tasks. The preferred method of drug delivery will depend on the scientific question addressed and subsequent experimental design.

9.3. Learning and memory reinforcement

Decades ago, Huston and colleagues presented a memory processing theory of reinforcement, and proposed that the reinforcer acts on a memory of either the response or the stimulus-response contiguity (Huston et al. 1974; Huston and Mondadori 1977). It has provided a framework for studies that have demonstrated a close correspondence between memory promoting and reinforcing effects of natural reinforcers like food (Huston and Oitzl 1989). We demonstrated that post-training reward (access to sugar) in close context with learning facilitates spatial memory performance in mice (**Chapter 5**).

Long-term memory was improved by sugar-reward in both spatial tasks, expressed as superior performance in the first trial of the following day. Whereas the memory facilitating effect in the circular hole board was observed already after the first contingency -location of and moving through the exit hole and sugar consumption, it took several days until it was obvious in the Morris water maze. This time-related effect of the reinforcer is most likely due to task-inherent properties (i.e., aversiveness, stress system activity, testing environment). However, common to both tasks is that goal-directed behavior during the training trials and the persistence of the search pattern in the area of the platform and exit hole are strengthened. General activity and velocity as behavioral responses to the task were not reinforced. Thus, the memory trace of how to locate the platform or exit hole is strengthened by the sugar reward. This memory facilitating effect of sugar is most obvious reinforced in the earlier phases of learning.

In line with other viewpoints (Whishaw 1995; Wotjak 2004), we consider the circular hole board test a procedure that is better adapted to the species-specific needs of mice. Moreover, the circular hole board task allows to collect a broader set of variables related to motivation and emotional expression than our water maze paradigm. The limited number of training trials in the circular hole board provides an opportunity to implement pharmacological interventions in close-context with training events (see Table 2 for an overview of circular hole board vs. water maze).

Table 2: Characteristics of the Circular hole board and the Morris water maze

Characteristic	**Circular hole board**	**Morris water maze**
Environment	Dry	Wet
Motivation	Appetive	Aversive
HPA activity	Low CORT	High CORT
Training trials	2 trials/day	4-5 trials/day
Predictive	2nd trial always shorter latency	High Trial-trial variability
Learning and memory	Short- and long term memory	Short- and long term memory
Anxiety measurable	Yes	No
Pharmacological intervention	Can be implemented before and after each trial seperately	Can be implemented following a set of trials

Effects on consolidation processes were achieved by allowing mice free access to sugar in the home cage after the last training trial of the day. As expected, a sugar reward in close-context with training (immediately, but not 4hrs later) facilitated memory in both spatial tasks, albeit within different time domains.

Studies on the effect of sugar reward and other drugs on learning processes include handling, restraining and injecting the animal and thereby, additionally increasing stress-hormone secretion (Meijer et al. 2006). The task-independent activation of the stress system by these manipulations may contribute to the modulation of memory processes. By giving the mice free access to sugar in close context with their performance in the learning task, we have introduced a non-invasive method for sugar reward that is devoid of possible interfering effects of stress hormones on memory processes.

Concluding, in line with the concept by Huston & Oitzl (Huston and Oitzl 1989) post-training sugar in close context with learning facilitates spatial memory in mice, via modulation of consolidation processes. We have utilized this method to study the effect of chronic stress in reward processing in **Chapter 7**.

9.4. Conceptualization of our animal model of depression

We validated our 'rat stress' animal model for depression by three criteria (Willner 1984): (1) *construct validity*: the model mimics the etiology of depression; (2) *face validity:* the model replicates a number of symptoms characteristic for depression; (3) *predictive validity*: treatment of symptoms has identical effects in the mouse model as in humans.

9.4.1. Construct validity

Depression is characterized by disturbances in emotional and cognitive processes, together with a dysregulated circadian and stress-induced secretion of glucocorticoid hormones. The expression of these symptoms may be of genetic- or environmental origin or a combination of both, and can be modulated by cognitive and non-cognitive inputs (Bale et al. 2010; Mann and Currier 2010).

Chronic stress is considered a vulnerability factor for the development of depression (De Kloet et al. 1998; de Kloet et al. 2005; McEwen 2005). Whereas all kinds of stressors induce behavioral alterations and concomitant changes in the regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Endo and Shiraki 2000; Anisman and Matheson 2005), psychological stressors are ethologically relevant and resemble the kind of stress that is related to depression in humans (Calvo-Torrent et al. 1999; Apfelbach et al. 2005; Beekman et al. 2005). Central features of chronic psychological stressors in humans are repeated, unpredictable and uncontrollable exposure to (or imagination of) threatening situations. To mimic such conditions of chronic stress, animal models make use of confrontations with territorial conspecifics and exposure to predators with or without physical confrontation (Apfelbach et al. 2005).

Previously, our group generated a “chronic stress mouse model” by exposing mice repeatedly to the presence of a rat, a procedure referred to as chronic ’rat stress’. Mice and rats could hear, see and smell each other, while preventing physical contact (Grootendorst et al. 2001b). Therefore, we expected that a psychological stressor would target the prefrontal cortex, amygdala and hippocampus. These brain areas show altered synaptic plasticity in rodents exposed to psychological stressors (Diamond and Park 2000; Diamond et al. 2006) and in patients that suffer from depression (Drevets et al. 2008); antidepressants affect this synaptic plasticity (Vouimba et al. 2006). All three brain areas express high levels of GR, indicating a high sensitivity to glucocorticoids secreted during stress (Reul and de Kloet 1985). Although we did not measure synaptic plasticity in mice exposed to our chronic stress paradigm, the observed acute and long term psychoneuroendocrine effects were evident and not detectable in non-stressed mice. These psychoneuroendocrine effects will be discussed in detail in the section related to *face validity*.

We used 3 months old male C57BL/6J mice for all the studies on the animal model described in this thesis for several reasons. First, the C57BL/6J mice is most commonly used in generating transgenic mice, including those with targeted MR and GR expression alterations (Muller et al. 2002; Urani and Gass 2003; Kolber et al. 2008). Hence, this

mouse strain has been extensively phenotyped (i.e., neuroendocrine, emotional and cognitive processes). Secondly, 3 months old mice are regarded as late adolescent or young adults, a period in their lives where brain development is still ongoing. Thirdly, the age of onset of mood/affective disorders has been shown to occur in the median age range of 25-45 years (Kessler et al. 2007). Finally, this age group displays a 24h circadian cycle of CORT secretion which approximates human cortisol secretion during the day (Krieger et al. 1971; Steiger 2003), taking into account the fact that mice are nocturnal animals. The cycle is characterized by peak concentrations of CORT at the onset of darkness, which is the active period for mice. Thereafter, CORT concentrations gradually decrease during the remainder of the dark and into the light period of the circadian cycle (**Chapters 2** and **4**).

Concluding, construct validity of our animal model of depression was achieved by exposing young adult male mice to 'rat stress' which elicits an etiological relevant type of psychological stressor.

9.4.1.1. Stress system activity and multiple memory systems in mice and men

As described in **Chapter 7**, stressed mice reached the same level of performance as non-stressed mice to locate the exit hole in the complex environment of the circular hole board. Interestingly, during the free exploration trial following the training trials, stressed mice preferred using a perservative search strategy, which is less efficient then a serial search strategy (Grootendorst et al. 2001a; Grootendorst et al. 2001b). It was recently discovered that stress may operate as switch between multiple memory systems (White and McDonald 2002; Schwabe et al. 2010). Neurobiological studies demonstrate that memory is organized in multiple brain systems. These memory systems differ with respect to the kind of information they process (Gabrieli 1998; Squire 2004a). Spatial hippocampus-dependent memory supports the acquisition of flexible, consciously accessible knowledge (Scoville and Milner 1957; Eichenbaum 2004). Non-spatial, stimulus-response (S-R) learning processes associations, such as ''stop your car when the traffic lights are red". It is not necessarily accessible to consciousness and relies on the caudate nucleus (Knowlton et al. 1996; Jog et al. 1999).

Whereas the circular hole board is considered a spatial memory task, the setup of the circular hole board experiment described in **Chapter 7**, did not allow to study the use of the two different memory systems that might be used by chronically stressed and non-stressed mice to solve the task. Therefore, we created a learning and memory paradigm for the circular hole board (**Chapter 8**), based on a study performed by Kim et

al (Kim et al. 2001). The same principle was used for the human variant of the learning task. This allowed a translational approach to study the impact of stress on the use of memory systems between mice and human.

Our results show that a period of chronic stress in mice and humans switches the use of either a spatial or S-R learning strategy, in favour of the latter. The observed learning performance refers to a change in the quality of learning, rather than in the quantity of learning (Schwabe et al. 2010).

Acute stress prior to training in a task that could be acquired by a hippocampus-based spatial, and a caudate-based S-R strategy resulted in predominantly caudate-based learning both in rodents and humans (Kim et al. 2001; Packard and Wingard 2004; Schwabe et al. 2007). We show that chronic stress has the same effect. Going back to the results we previously described (Grootendorst et al. 2001a; Grootendorst et al. 2001b, **Chapter 7**: thesis), it is likely that our chronic stress paradigm induces a switch from using S-R over the spatial learning strategy, in both the water maze and circular hole board.

The stress-induced modulation of hippocampus-dependent and caudate-dependent systems is assumed to be influenced by the amygdala (Packard and Wingard 2004). Emotional components like anxiety, punishment, reward, are part of the majority of behavioral tasks for rodents, including the water maze and circular hole board (Dalm et al. 2009b; see **Chapter 5**). The associated stress increases the excitatory amygdala input to the hypothalamus PVN producing enhanced release of CRH and ultimately a larger output of CORT. This additional increase of CORT could affect both the quantity and quality of cognitive performance. Whereas learning is critical for adaptation to the environment, when the adaptation is inappropriate, it can also produce dysfunctional patters of thinking and emotional responding (Schwabe et al. 2010). In fact, heightened activity of the amygdala improves memory consolidation of negative events (Roozendaal et al. 2009).

As a result of stress an organism may switch from perceiving the world from a balanced positive and negative perspective, towards a more negative perception, due to increased use of an S-R learning strategy that maintains focus on threats (negative) rather than reward (positive). Interestingly, a cognitive framework for depression suggests that positive mood promotes associative processes and vice versa (Bar 2009). From an evolutionary standpoint this would be of value as it allows the organism to learn and explore multiple alternatives that a given environment provides regarding coping with rewarding and aversive stimuli. The recent focus on the impact of stress on multiple memory systems actually supports this cognitive framework. Of course, perception

of the environment involves multiple brain regions to work in concert resulting in an adaptive response, either beneficial or maladaptive, but is always aimed to restore homeostasis (McEwen 2000).

Concluding, we demonstrate that chronic stress leads to a shift from elaborate "cognitive" spatial to rather "rigid" S-R/habit learning. Comparable cognitive shifts were observed in patients that suffer from depression (Purcell et al. 1997; Harvey et al. 2004). We suggest that cognitive rigidity, expressed as favoring S-R learning strategy used for problem solving, is an important factor in the etiology of stress-related affective disorders including depression.

9.4.2. Face validity

Affective disorders like psychotic, major and bipolar depression, share several characteristics (de Kloet et al. 2005): emotional changes related to approach or avoidance behavior, loss of interest or pleasure in daily activities, impairment of cognitive functions, reduced motor activity and alterations in the circadian pattern of physiological, neuroendocrine and behavioral responses (Endo and Shiraki 2000; Volkers et al. 2002; Keller et al. 2006). Our chronic stress model was evaluated based on the degree of symptoms expressed as described above; see Table 3 for an overview.

We particularly focused on the expression of anhedonia using different methods aimed at detecting a disturbance in reward processing i.e., reduced responsiveness to rewarding stimuli. Loss of responsiveness to rewarding stimuli indicates a shift in the detection, interpretation and response to negative and positive stimuli that are part of the external world of the organism. Stress has been shown to alter the perception of those stimuli via modulation of stress hormone receptors in the brain, in particular the hippocampus (Oitzl and de Kloet 1992). Hence, we expected that chronically stressed mice would have an altered perception of the environment. To test this, we determined: 1) the behavioral response in a novel environment; 2) modulation of learning and memory processes by reward; 3) sucrose consumption and preference.

9.4.2.1. Anhedonia: behavioral inhibition in a novel environment

For patients that suffer from depression, the negative effects exist in both familiar and novel environments (Volkers et al. 2002; Keller et al. 2006). Chronic 'rat stress' alters the perception of a novel environment as indicated by distinct behavioral response

Table 3: Symptoms associated with depression in humans, and reference to the Chapters in thesis that assessed the expression of the human-like symptoms in our mouse model of depression.

Symptoms of depression *	Assessed in Chapter	Symptoms expressed	Parameter(s) for stressed mice
Anhedonia	6 and 7	Yes	Behavioral inhibition Altered response towards positive stimuli
Appetite/Weight changes	6	Yes	Bodyweight fluctuated
Sleep disturbances/ circadian activity pattern	6 and 7	Yes	Daily organization of behavior in the home cage was different
Psychomotor retardation	6 and 7	Yes	Behavioral inhibition during novelty exposure
Fatigue/loss of energy	not assessed	n.a.	n.a.
Depressed mood	No	n.a.	n.a.
Feelings of worthlessness/guilt	No	n.a.	n.a.
Diminished ability to think/make decisions	6, 7 and 8	Yes	Shift to a more rigid search strategy, likely triggered by an altered perception; sugar reward partially improved memory
Thoughts of death/ suicide	No	n.a.	n.a.

**Source: Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV, American Psychiatric Association 1994.*

patterns, similarly as has been described previously (Grootendorst et al. 2001b). We extended the characterization of behavior to the familiar environment of the home cage of individually stressed, and non-stressed mice. Long-term automatic recordings using the PhenoTyper observation cage (Noldus Information & Technology BV, Wageningen, The Netherlands), revealed a reduction in general activity, and overall, a disturbance in the daily organization of behavior (**Chapter 6**). Most pronounced was the expression of behavioral inhibition measured as a delayed onset to explore the environment.

Animals need to forage for food to meet their energy demands, while at the same time minimizing the risk of being exposed to a life-threatening situation, i.e., a predator or the threat of a possible predator (Lima and Bednekoff 1999). Although mice were exposed to the 'rat stress' outside their home cage, they displayed inhibition of exploration together with focused attention upon return to their home cage. This indicates that the impact of the rat exposure *outside* the home cage was carried over into the situation of the home

cage where the actual exposure to the rat never occurred. The inhibition and the reduced exploratory activity in the home cage has an adaptive value as it temporarily decreases the risk of predation (Nordahl and Korpimaki 1998), even though the actual danger is no longer present. Interestingly, the behavioral inhibition and reduced exploratory activity in the home cage is maintained if the mouse is placed in a novel cage (**Chapters 6, 7** and **8**).

In the novel environment of the circular hole board, stressed mice alternated between serial (sequential hole visits) and perservative (repetitive visits of the same hole) search strategies more often than controls. Exploration of the holes and the rim of the board are important to locate possible routes of escape from the open, unprotected environment. We observed that stressed mice were slower in starting to visit holes and performing rim dips. We also observed changes in head-dipping behavior which have been associated with altered information processing, and therefore may reflect the anxiogenic or anxiolytic state of the mouse (File and Wardill 1975b; File and Wardill 1975a; Takeda et al. 1998). In addition, the anxiolytic state was observed for at least 3 months after cessation of the stressor (Grootendorst et al. 2001b), when mice were tested in the light-dark box. Thus, independent of the environmental context (circular hole board vs. light-dark box), stressed mice displayed behavioral inhibition, indicative for more anxiety-related behavior. Apparently, the behavioral inhibition became part of the daily organization of behavior and lasted weeks after cessation of the stressor (**Chapters 6** and **7**).

Exploration is considered a self-rewarding behavior. In the early 1980's Katz and colleagues studied the effects of stress on open field (novelty exploration) behavior. A history of chronic stress exposure reduced the novelty induced activity, which could be reversed by chronic antidepressant treatment (Katz and Hersh 1981; Katz et al. 1981; Roth and Katz 1981). Whereas the inhibition of exploration is generally related to anxiety, it might also indicate the loss of hedonic responses, as suggested by Bevins and colleagues (Bevins and Besheer 2005). Thus, the exploration patterns in a familiar or novel environment might provide leads to the emotional state of the animal (File 2001; Kalueff et al. 2006).

We showed that chronic 'rat stress' altered the daily organization of behavior such that situations were perceived as threatening and negative, rather than neutral or positive, both in a familiar and novel environment. The chronic stress forced mice into a conflict situation, i.e. approach vs. avoidance, where they have to maintain food and water intake, while lowering the risk of being predated. In 1976, Gray conceptualized two motivation

systems that act in response to environmental stimuli: (i) the Behavioral Activation System (BAS), that controls approach behavior in response to cues of reward via dopaminergic activity in the mesolimbic system; (ii) the Behavioral Inhibition System (BIS), that is sensitive to cues of threat and controls inhibition of behavior via noradrenergic and serotonergic activity in the limbic septohippocampal system and amygdala (Gray 1976; Gray 1987; Gray 1994). Chronically stressed mice in our model displayed increased inhibition of behavior focusing primarily on threats in the environment. Thus, it is likely that the BIS has become more dominant. In fact, this has been shown for patients that suffer from depression (Kash et al. 2002) and schizophrenia (Scholten et al. 2006).

Concluding, chronic stress changes the perception of the environment by focusing on stimuli that might indicate a threat at the expense of possible rewarding stimuli. This in turn shifts the sensitivity between the two proposed motivational systems from the dopaminergic BAS towards a more sensitive limbic BIS. This sensitivity becomes, at least for weeks, embedded into the daily organization of behavior.

9.4.2.2. Anhedonia: modulation of learning and memory processes by reward

The impact of stress on learning and memory processes is described as impairing, improving or ineffective. The key towards disentangling these apparent paradoxical effects exerted by stress is the timing and context of stressor exposure in relation to the learning and memory processes (Joels et al. 2006; de Quervain et al. 2009; Conrad 2010). Glucocorticoids are often examined in this respect as representing 'stress', but obviously these hormones operate in concert with all other stress mediators, and this interaction between the various stress signals adds another level of complexity. Yet, glucocorticoids target the hippocampus, which is considered a key brain structure in the processing of novel information.

The hippocampus functions at the crossroad where novel information is detected, evaluated and appraised. Glucocorticoids operate in the hippocampus through MR and GR, which are implicated during information processing. In fact, these receptors mediate the CORT effects on the appraisal processes of novel information, as well as on memory storage. Additionally, CORT also acts on other circuits impinging on the hippocampal formation, notably the amygdala-entorhinal input through which emotions are regulated. It is well known that the more emotional an experience is, the better it is remembered, and glucocorticoids acting through MR and GR in the hippocampus have a key role in labeling the emotions in space and time (Oitzl and de Kloet 1992; McGaugh and Roozendaal 2002; Brinks et al. 2007c).

Indeed, long term exposure to glucocorticoids or stress has been shown to alter synaptic plasticity in hippocampus in a spatial context, in prefrontal cortex with respect to response selection and in amygdala regarding the emotional value of stimuli, amongst other brain structures, affecting information processing (de Kloet et al. 1999; McEwen 1999b; Mizoguchi et al. 2000; Roozendaal et al. 2009; Conrad 2010). Interestingly, human fMRI studies show that joint activation of hippocampus and brain reward regions, involving dopamine, is crucial for the development of long term memories (Schott et al. 2006b; Wittmann et al. 2007). Changes in the reward processing system belong to the main symptoms of depression (Keedwell et al. 2005; Martin-Soelch 2009). These changes contribute to the expression of anhedonia. We have exploited our method described in **Chapter 5**, to study the impact of chronic stress on the facilitating effect of post-training reward on cognition.

We confirmed and extended our previous results using the chronic 'rat stress' model, and showed that stressed mice displayed a distinct learning curve (Grootendorst et al. 2001b). The stressed mice were slower to locate the exit hole during the first 2 days of acquisition training; thereafter they were as fast as non-stressed mice. Analysis of the free exploration trial after completion of the acquisition learning trials indicated that the stressed mice used a more perservative strategy to locate the exit hole. Controls favored the use of the serial search strategy which became apparent as a pattern resembling a 'see-saw', while the stressed mice showed a smooth though delayed learning curve during training trials. Thus, stress changes the way an animal navigates within a given environment. In fact, we demonstrated in **Chapter 8** that chronic stress produced a shift in the use of search strategies by favoring stimulus response over spatial strategies in mice (Schwabe et al. 2008).

We investigated the impact of stress on reward processing by utilizing our developed method, providing mice post-training free access to 30 mg sugar in close-context with a training trial. As task, we used the circular hole board, which permits multiple readout parameters on emotional and cognitive responses (see **Chapter 5**).

In stressed mice post-training sugar partially restored performance to the level of control mice that did not receive sugar post-training. The graphical representation of the latency to exit hole displayed a similar 'see-saw' pattern (i.e., latency during 1st trail of the day higher than during the second trial). However, controls without-sugar gradually improved performance (latency in trial 1 approximating latency in trial 2), whereas stressed mice that received sugar remained slower during all 1st acquisition trials of the day. This could be due to the overall behavior expressed in stressed mice, namely the initial inhibition which was present during circular hole board training and

up to 1 month later in the light-dark box novelty paradigm. The question then becomes which processes are affected by stress, and which of those are partially restored to function, under these conditions of post-training sugar.

An organism will need to learn to predict which environmental stimuli are biological meaningful, rewarding or aversive. In complex natural environments, like the circular hole board, this requires the integration of different sensory modalities into coherent memories and the coordination of various motor systems. In particular, the brain must learn and store representations of the biological value of appetitive or aversive stimuli, and recall these representations to control adaptive experience-dependent behavior (for example, when to approach or to retreat; Hammer et al. 1997).

The process of integration during learning and memory processes involves 3 stages: acquisition, consolidation and retrieval. During acquisition, both the task inherent proximal and distal environmental cues are perceived by the organism. In the circular hole board spatial memory task, the goal is to locate the accessible exit hole that is connected via a tunnel to the home cage. Because of the complexity of this task, it will take several training trials before the organism understands the 'rule'. It needs to create a map of the environment, and recognize its own position in relation to the exit hole (note: the mouse is placed in a non-transparent cylinder before it is free to explore the circular hole board environment). In addition, it will start to value the predictable access to sugar reward post-training. Analysis of the training trials revealed that path length, walking velocity and time to leave the start center were not affected by post-training sugar during the course of the learning paradigm. The performance however, did improve from trial to trial and from day to day in sugar-rewarded mice. It is likely that this is due to modulation of short- and long-term memory following re-exposure to the circular hole board environment.

Immediately after learning the memory is still in a labile form prior to being fixed or consolidated in a more permanent form (McGaugh and Herz 1972). This implies that during the post-trial, post-training period memory remains susceptible to disruptive or facilitating treatments. Thus, environmental manipulation and changes in circulating CORT concentration or a reward can act during this labile period and affect spatial learning in rats (Sandi et al. 1997; De Kloet et al. 1998; Akirav et al. 2001; Joels et al. 2006), and mice (**Chapter 5**; (Dalm et al. 2009b). Because the sugar-reward was provided post-training and, importantly, in close-context with the learning task, memory consolidation was affected in both stressed and control mice. Although to a different degree, both groups displayed improved performance due to the reward. This raises the question which process(es) are involved in the observed cognitive enhancement.

Several studies have shown that the intake of palatable food (e.g., calorically dense food containing high amounts of carbohydrates or fats) is increased during periods of stress. In fact, the increased intake results in an improved emotional state in humans (Dube et al. 2005) and lowers the cortisol response to a stressful event (Pecoraro et al. 2004; Ulrich-Lai et al. 2007). In our own studies we observed that exposure to the circular hole board increased corticosterone concentrations in mice (see **Chapter 5** discussion). It is speculated that the free access i.e. self administration, to 30 mg sugar corns of the stressed and non-stressed mice during the (pre-)training phase of the circular hole board learning and memory paradigm might have dampened HPA axis activity, resulting in improved spatial performance and emotional state.

As previously mentioned, the impact of CORT on learning and memory is described as impairing, improving or ineffective, and becomes manifest depending on the timing and context of stressor exposure in relation to the learning processes, described as a U-shaped curve (Joels et al. 2006; de Quervain et al. 2009; Conrad 2010). Previously we showed that chronic 'rat stress' increased basal CORT concentration 7 days after the last rat exposure (Grootendorst et al. 2001b). We did not measure the CORT response induced by circular hole board exposure in stressed and non- stressed mice that had received 30 mg sugar prior. However, as other studies do suggest, daily access to palatable food attenuates the stress response (Ulrich-Lai et al. 2007). Moreover, the intake of palatable drink as stress relief also affects reward pathways and metabolic circuitry in the brain (Ulrich-Lai et al. 2010; Ulrich-Lai et al. 2011).

In conclusion, as observed in human studies the improved performance in stressed mice by post-training sugar might result from the attenuation of HPA axis activity following this reward, which shifts the CORT value towards the optimal range required for modulation of learning and memory processes.

9.4.2.3. Anhedonia: sucrose consumption and preference

Measurement of sucrose consumption or preference is in widespread use in preclinical psychopharmacology to show an alteration in reward responsiveness induced by stress by a change in hedonic responses. Authors describing animal models of chronic stress generally report a decrease in sucrose consumption as a measure for anhedonia (Katz 1982; Pothion et al. 2004; Strekalova et al. 2004; Anisman and Matheson 2005; Willner 2005). We used a 5% sucrose solution (i.e. dissolved table sugar in water), based on a study by Pothion and colleagues (Pothion et al. 2004). They showed that a sucrose

solution between 4-8% resulted into the highest intake (± 11-16 ml) and preference (± 97-98%) in naïve C57BL/6J male mice.

Prior to the start of the 'rat stress' paradigm, mice showed an impressive consumption of (12 ml) and preference for (85%) the 5% sucrose solution (**Chapters 6** and **7**). Control mice further increased their consumption and preference over the course of the experiment. There was a clear time-dependent pattern in the development of increasing consumption of and preference for sucrose in the control mice which was absent in stressed mice. In contrast, stressed mice consumed less sucrose during the initial phase of the 'rat stress' paradigm; since baseline levels were reached only one day after the cessation of rat exposures. During the stress paradigm the stressed mice spent less time near the bottles than controls, which most likely reduced their fluid intake. However, since the preference of the stressed mice for sucrose did not change during the course of the experiment, and water intake was comparable to controls, we feel confident that 'rat stress' affected the hedonic properties of sucrose. Stressed mice did not increase their preference and consumption alike the controls. Based on the findings described in **Chapter 6,** we may conclude that chronic stress induces anhedonia.

However, the outcome of the sucrose preference task does not necessarily indicate expression or absence of anhedonia, as indicated by reduced sucrose consumption and preference. The results of the sucrose testing in **Chapter 7** showed that mice exposed to the same chronic stress paradigm, consumed more volume of both sucrose and water, 35 days after cessation of the stressor. In fact, we found a stress-induced increase of caloric intake. It is known that glucocorticoids stimulate behaviors that are mediated by the dopaminergic mesolimbic "reward" pathways, and also increase the intake of food with high carbohydrate and fat (see review Dallman et al. 2007), so-called "comfort" food. Consequently, we propose that the rat stress procedure affected the reward system, in a manner that also counteracted to some extent the addictive properties of sucrose (Avena et al. 2008).

The volume overload of 300% due to sucrose drinking most likely affected the body's fluid and energy balance. Sucrose by itself is rich in energy, which is utilized directly, stored in adipose tissue or secreted from the body (Peters et al. 2004). Drinking sucrose might have lowered stress-induced CORT secretion as shown by Bell and colleagues (Bell et al. 2002). Indeed, absolute CORT values in response to rat exposure were lower than measured in previous studies (Grootendorst et al. 2001a; Grootendorst et al. 2001b). Our additional experiment revealed **(Chapter 6**), that the 'rat stress' control procedure (placement in another cage) reduced the nocturnal activity for at least two days, while sucrose overload affected the activity pattern only on the day of consumption. Therefore,

the reduced consumption and lesser preference for sucrose is a distinct feature of the rat stressed mice. Consequently, we conclude that the 'rat stress' procedure affected the reward system.

As described in **Chapter 7,** the initial 24h sucrose testing was followed by measuring the preference for the bottle that previously contained the 5% sucrose solution. Despite the fact that the content of the sucrose bottle was changed to water, stressed mice preferred to drink from that water bottle. This can be seen as a sign of habitual learning. The rigidity in behavior might also suggest reduced extinction learning as a consequence of stress (Brinks et al. 2009; Schwabe and Wolf 2009; Schwabe et al. 2011). Exposure to a psychosocial stressor before training in an instrumental task rendered the participants' behavior insensitive to the change in the value of the food outcomes: i.e., stress led to habit performance at the expense of goal-directed performance in humans (Schwabe and Wolf 2009). This proves that recognizing a change in rewarding values is differentially perceived under stress. Also, chronic social stress enhances habit-based learning in mice (Ferragud et al. 2010). We performed the sucrose testing > 1 month after cessation of the stressor and still, stressed mice displayed the apparent habit-like behavior. This suggests that, next to behavioral inhibition (see section 9.4.2.1.) the use of habit-like responses over goal-directed flexible response prevails in chronically stressed mice. The shift in memory systems and response selection has been discussed in more detail in section 9.4.1.1.

What could have triggered the shift in response strategy in stressed mice? Did they perceive the taste of sugar as highly rewarding, strengthening the memory for this location? It would be of great interest to study the time-dependent effects of chronic stress with respect to stress-induced metabolic changes and food intake.

Overall, we conclude that 'rat stress' model induces the expression of anhedonia in mice, and more generally, that chronic stress alters reward processing, leading to shifts in response strategies. As we focused on responsiveness towards positive stimuli, the shift in response strategy is likely due to an altered perception of the environment. Ultimately, rigidity might occur, making the organism less flexible when faced with new challenges.

9.4.2.4. Dysregulation of HPA axis activity

The circadian cortisol secretion pattern is disturbed in patients that suffer from a mood disorder like depression (Belanoff et al. 2001b; Flores et al. 2006). Although numerous mouse models for a wide range of human stress related mood disorders like depression

have been developed, the circadian secretion pattern of mice has received relative little attention. We showed that naïve male C57BL/6J mice display a circadian rhythm that is affected by age (**Chapter 2**). The secretion of CORT was highest for the 9 months old mice and lower in the 3 months and the 16 months old mice, during the light / inactive period, with no differences in total CORT secretion over the dark / active period. In **Chapter 7** the 24h circadian pattern of glucocorticoid secretion is affected by chronic stress in 3 months old mice. One day after termination of the chronic stress procedure the CORT concentration was not increased. However, 6 days after cessation of the stressor, the CORT concentration during the light inactive period of the day was nearly 1.5 times higher.

Concluding, although this finding is suggestive, clearly more data need to be collected on the HPA reactivity regarding face validity of the neuroendocrine system in our 'rat stress' model for depression.

9.4.3. Predictive validity

In our experiments we have used naïve mice, stressed mice and mice that received the GR antagonist MIF. We have not tested whether the short-lasting treatment did ameliorate stress induced alterations in mice exposed to our 'rat stress' paradigm. We did show that post-training sugar reward partially restored learning and memory performance in stressed mice to the level of non-stressed mice (see section 9.4.2.2. for discussion).

The efficacy of GR antagonism in clinical studies could be due to the following factors: (1) The detrimental effects of high CORT levels via GR activation are prevented by the GR antagonism. On the other hand, (2) as a result of GR blockade and subsequent rise in CORT levels, only the MR becomes strongly activated. The recent discovery of MR being located in the membrane and sensitive to high amounts of CORT, exerting non-genomic actions (see Karst et al. 2005; Joels et al. 2008; Groeneweg et al. 2011; Groeneweg et al. 2012) in addition to the well-known genomic action mediated by MR, opens new avenues to discover MR functions. (3) As the pharmacological action of the GR antagonist wanes, GR becomes activated by the high circulating levels of CORT and via negative feedback, shuts off the CORT secretion. By means of recurrent blockade of GR we might force a pronounced circadian pattern of enhanced CORT secretion followed by its suppression. This effect might be of importance for the development of a new balance and threshold for HPA axis activation. Acting in such a way, drugs that antagonize GR or boost MR are likely candidates for novel antidepressants.

9.4.3.1. Effect of daily mifepristone administration on circadian and stress-induced HPA axis activity.

The secretion of CORT (cortisol and corticosterone in humans and corticosterone in rodents) exhibits a circadian pattern (Windle et al. 1998a; Buckley and Schatzberg 2005; Dalm et al. 2005). In humans dramatic changes in circadian patterns of corticosteroid hormones have been described in aging and also in psychiatric and neurological diseases like depression and Alzheimer's disease (Hatfield et al. 2004; Peeters et al. 2004). These changes in cortisol are linked to resistance in GR-mediated negative feedback (Ribeiro et al. 1993; Heuser et al. 1996; Pariante and Miller 2001).

In response to blockade of GR the circadian CORT secretion was altered (**Chapter 4**). Two hours after a single dose of MIF (200mg/kg RU38486) was administered to naïve mice, the CORT concentration was significantly higher and remained elevated for 14h due to interference with negative feedback at the level of GR. Interestingly, during the 7th day of GR antagonist administration no apparent alteration in circadian CORT secretion was observed. The repeated GR antagonism resulted overall in lower CORT secretion during the light period of the day, compared to controls and acute GR antagonism.

How does this paradoxical effect of GR blockade come about? We reason that by blocking GR mediated negative feedback with the antagonist a long-lasting elevation of CORT occurs which persists beyond the actual blockade of the receptor, since MIF is very rapidly degraded and eliminated (see Karssen 2003). Next, the elevated CORT levels will exert a negative feedback action which persists for many hours suppressing CORT even below baseline at 32h post injection. However, CORT secretion remains highly responsive to stressors at that time, which is also evident from the increased adrenal weight after GR antagonist treatment. Hence, we propose that a renewed GR antagonist administration will each day show a diminished effect on the CORT secretion until the HPA axis does not respond at all anymore at the 7th day of administration; this is what was observed.

Repeated MIF administration to rats for 5 days in a 10 fold lower dose than we did, also caused a reduction in ACTH and CORT release. Moreover, c-fos expression in the prefrontal cortex and amygdala, and decreased expression in hippocampal region (Wulsin et al. 2010) was observed. These data suggest that MIF is enhancing inhibitory and suppressing excitatory inputs to the PVN that collectively may account for downregulation of HPA axis activity as well. This downregulation of HPA axis activity is also further enhanced by CORT action via hippocampal MR suggesting a coordinate control by both MR and GR at longer time intervals after antagonist treatment.

In a study by Bachmann and colleagues (Bachmann et al. 2003) MIF and other more specific GR antagonist, administered for 3 weeks in a dose range comparable with that of Wulsin studie, did not increase hippocampal and pituitary GR mRNA expression. However, prefrontal cortex GR mRNA expression was increased after 3 weeks of MIF treatment. We observed that after the first MIF administration MR expression was downregulated in the hippocamus, but upregulated after the 7th administration, specifically in the hippocampal CA2 cell field. The observation that MR function becomes more prominent after GR antagonism is also evident from the shift in hyperactivity displayed on the circular hole board to a more serial search pattern (Oitzl et al. 1994; Oitzl et al. 1997b).

Also the mode of MIF administration appears important. Episodic administration of very high concentrations of MIF down regulates the HPA axis over a few days. This effect can be prevented by increasing the frequency of MIF administration to twice a day. Such a condition of continuous blockade of central GR's enhances the amplitude in circadian and stress-induced activations probably because of a changed set point of the HPA axis. This increase in feedback resistance caused by the chronic infusion of the GR antagonist follows the theoretical prediction by Walker & Lightman (Walker et al. 2010): any system that has a delay between activation and inhibition has to oscillate. However, in all cases, irrespective of the route and dose of administration, the size and weight of the adrenal cortex is increased suggesting hyperfunction of the adrenals during GR blockade.

Concluding: the above finding in our chronic 'rat stress' model may explain how 'normalization' of aberrant cortisol secretion may occur in patients suffering from psychotic major depression that are treated daily with very high doses of MIF (Belanoff et al. 2001a; Thomson and Craighead 2008). It appears that the dose and mode of administration of the GR antagonist is essential for this downregulation of HPA axis activity. The paradoxical strengthening of negative feedback inhibition of CORT secretion by recurrent administration of MIF is most likely achieved by the integration of MR and GR-mediated effects.

9.5. Concluding remarks

In this thesis, the studies were described with mice that had a history of repeated and unpredictable exposure to rats. Our 'rat stress' paradigm is known to elicit an etiological relevant type of psychological stressor, which we found causing neuroendocrine and behavioral changes in the mouse that show features of human depression. The CORT secretion was altered, while an impaired cognitive performance and a reduced preference for positive rewarding stimuli developed. Accordingly, these behavioral changes induced by the 'rat stress' paradigm display features fulfilling *construct* and *face* validity of a mouse model of depression.

In the cognitive domain, chronic 'rat stress' led to a shift from a spatial learning strategy to a rather rigid stimulus-response strategy. This shift towards so called habit learning reflects a state of cognitive rigidity in problem solving behavior. Moreover, exposure to novelty revealed behavioral inhibition in our chronic stress model. Collectively, the behavioral inhibition, the rigidity and reduced flexibility to novelty, as well as the preference for habit learning present a phenotype that provides leads for translational studies. Central to these translational leads is the behavioral phenotype of our mouse model that signals vulnerability for pathogenesis of human stress-related affective disorders including depression. In **Chapter 8** we report our discovery that this shift from spatial to habit learning and rigidity occurred in both mice and humans, after a history of chronic stress exposure.

Our mouse model of depression demonstrated features predicted by the BIS/BAS theory of the late Jeffrey A. Gray (Gray 1982). This theory points to a reciprocal relationship between the limbic noradrenergic / serotonergic behavioral inhibition system (BIS) and the mesolimbic dopaminergic behavioral approach or activation system (BAS) that corresponds to the conflict between two major personality traits i.e., the conflict between the motivation to avoid fear vs. the desire to approach an award or to anticipate joy and happiness. In particular because chronic stress seemed capable to drive the perception of the mice towards focus on potential threatening rather than rewarding stimuli, or in other words by enhancing BIS over BAS activity. This change in activity between the two postulated motivational brain circuits appeared to become part of the daily behavioral organization in our mouse model.

Anhedonia is a prominent behavioral feature of our 'rat stress' model, that is manifested as a consequence of the presumed reduced activity of the mesolimbic dopaminergic circuit. In order to test for anhedonia our studies were performed along the conceptual framework developed by Huston and Oitzl (Huston and Oitzl 1989) aimed to integrate naturally occurring reinforcers as memory promoting rewards. Here we report that access to sugar as post-training reward in close context with learning indeed partially restored the spatial memory impairment of the chronically stressed mice. This discovery is a further demonstration of the *predictive* validity of our chronic stress model.

Our findings raise the following questions for further study:

(i) ***Translational perspective***. Since the negative inhibitory bias of stressed mice was partially counterbalanced by exposure to a sugar reward, it is tempting to translate this finding to a therapeutical perspective in the human. Environmental enrichment of the home situation has been shown to increase the well-being of both humans and mice by lowering anxiety levels with a positive influence on learning and memory performance (Walker and Mason 2011). Alternatively, the combination of extinction training in a fear conditioning paradigm with anti-depressant treatment was recently shown to remodel the memory circuit through local neurotrophic activity (Karpova et al. 2011).

These two examples demonstrate that external cues can help (at least partially) to overcome the effects induced by previous stressor for better or worse. It would be of interest to study whether exposure to positive stimuli can also be used as 'animal therapy', translating human findings back to the design of the experimental procedures and measurements. This implies that positive stimuli during behavioral testing may increase the well-being of the animals. Alternatively, in translational perspective, the combination of this type of psychotherapy based on reward may help the efficacy of anti-depressants in remodeling neural circuitry underlying the BIS/BAS reciprocity. In further experiments the actual measurement of BIS/BAS activity, obviously, is required to substantiate this notion.

(ii) ***Dopamine and opioid signaling in the reward circuitry.*** A diminished functioning of the reward mechanism is evident in patients suffering from depression, especially in the mesolimbic dopamine system (Nestler and Carlezon 2006). In our chronic stress model, post-training reward modulated memory performance, and sucrose preference eventually led to an increase in sucrose consumption over time. To account for this phenomenon Treadway and Zald (Treadway and Zald 2011) differentiated between

consumatory (hedonic – 'liking') and motivational ('wanting') aspects of reward. Whereas the mesolimbic system is involved in motivational processes, the opioid system underlies the sensation of the pleasure obtained from drinking a sweet solution or disgust when something is bitter. To better understand this dissociation we would need to examine the impact of chronic stress in our animal model of depression on dopamine and opioid signaling.

(iii) ***HPA reactivity.*** More data needs to be collected on the HPA reactivity to support the *face* validity of the neuroendocrine system in our 'rat stress' model of depression. This refers to the neuroendocrine characterization of the 'rat stress' model. Also, the improved performance in stressed mice by post-training sugar might result from the attenuation of HPA axis activity following this reward. It would be of interest to examine if CORT dynamics acquires the optimal range required for promotion of learning and memory processes.

(iv) ***The "antistress" drug mifepristone.*** The daily recurrent blockade of the GR with the very high doses of mifepristone triggered in the mouse a rebound surge of endogenous CORT that subsequently mediated a negative feedback action and progressively downregulated HPA axis activity. This paradoxical effect of mifepristone in the mouse occured in the face of a persistent limbic MR activation, which incidentally also explained the concomitant change in explorative behavior towards a serial search strategy. The finding points to the mechanism underlying the beneficial effect of the "anti-stress'' drug mifepristone for stress-related mood disorders like depression.

The above mentioned conclusion is based on findings in the naïve unstressed mouse and needs to be extrapolated to the chronic stress model. If this extrapolation holds, it provides a criterium for *predictive* validity that explains how 'normalization' of aberrant circadian cortisol secretion may occur in patients suffering from psychotic major depression treated daily with very high doses of mifepristone (Belanoff et al. 2001a; Thomson and Craighead 2008). However, the kinetics of mifepristone is very different in mouse and men, because in human the antiglucocorticoid is protected against rapid metabolism and clearance by binding to a circulating α_1-glycoprotein lacking in the mouse. It seems that the dose and mode of administration of the GR antagonist is essential for downregulation of HPA axis activity and therapeutic efficacy, but this needs to be further examined.

(v) ***MR:GR balance.*** The paradoxical strengthening of negative feedback inhibition of CORT secretion by recurrent administration of mifepristone is most likely achieved by integration of MR- and GR-mediated effects. Hence, besides anti glucocorticoid modulation of the MR:GR balance also enhancing the MR- mediated actions in the brain may provide an interesting alternative lead towards a novel class of antidepressants and/or antipsychotics. That one particular gene variant (haplotype) of the MR is associated with optimism therefore provides a highly exciting novel lead (Klok et al. 2011).