Chapter 1

General Introduction

1. Introduction

Mood disorders such as major depressive- and bipolar disorder, share several characteristics (de Kloet et al. 2005): emotional changes related to approach/avoidance behavior, loss of interest or pleasure in daily activities (i.e. anhedonia), 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). Chronic stress, specifically a dysregulation of the glucocorticoid system, is thought to be a precipitating factor in the etiology of depression.

The **main objective** of this thesis is to develop a mouse model that expresses signs and symptoms as seen with patients that suffer from depression, with special focus on the expression of anhedonia as the common denominator. Cognitive and emotional consequences of chronic psychosocial stress are studied with emphasis on changes in the responsiveness to positive stimuli. In addition, circadian patterns of neuroendocrine and behavioral activity are monitored in response to novelty, and within the familiar environment of the mouse's home cage.

The experiments are divided into three categories addressing:

1. Methodological optimization: since timing, context and duration of a stressor determine the experimental results, interference by unintentional stressors resulting from the experimental procedures has to be controlled and minimized. For example, separation of the stress effects induced by an injection from the action of the drug, and reduction in the adversity of the learning environment. We designed and optimized drug administration methods, and behavioral tests that minimized unwanted stress system activation (**Chapters 3, 4** and **5**).

2. Longitudinal studies in the home cages of mice, and in novel environments: these were conducted to measure circadian neuroendocrine activity, behavioral patterns, learning and memory, and emotional processes. Combined, the measurements will indicate whether anhedonia is expressed in our chronic stress model (**Chapters 2, 5, 6** and **7**).

3. Translational approach: chronically stressed mice, and chronically stressed healthy humans are subjected to comparable experimental designs that allow to test the use of distinct memory systems (**Chapter 8**).

Glucocorticoid functionality in mice was manipulated using two approaches:

1. Environmental challenges mimicking chronic psychological and psychosocial stress conditions in humans, namely: repeated, unpredictable and uncontrollable exposure of mice to rats (chronic 'rat stress').

2. Pharmacological intervention that compromises the functionality of the glucocorticoid receptor via repeated administration of the glucocorticoid receptor antagonist RU38486, also known as mifepristone (MIF).

The **goal** of the research described in this thesis is to characterize behavioral and neuroendocrine features in mice during, and in response to our chronic psychosocial stressor ('rat stress'), and during and after pharmacologically-induced dysfunction of the glucocorticoid receptor. We expect that the results will contribute to the understanding of the etiology of depression. Especially on the processes possibly underlying the expression of anhedonia, and may provide leads for alternative therapeutic approaches in humans.

2. Stress: Activity of the Hypothalamic-Pituitary-Adrenal axis

The original term *stress* was first used by Hans Selye for the biological phenomenon of a disrupted homeostasis (Selye 1937; Selye 1950). Since the 1950s, the definition of the term stress has evolved. **Box 1** describes the definition of stress against which the experimental designs in this thesis were created.

Box 1: Concept of stress

For operational use of the stress concept we favour the view of one of the pioneers in stress research, the late Seymour (Gig) Levine who defined 'stress' as a composite, multidimensional construct, in which three components interact: (i) *input*, when the stressor is perceived and appraised, (ii) *processing* of stressful information and (iii) *output* or stress response. The three components interact via complex self-regulating feedback loops with the goal to restore homeostasis through behavioral and physiological adaptations. These adaptations need to be coordinated in brain and body. The major communication systems, the autonomic nervous system and the HPA axis, are extremely important in this respect (Levine 2005).

Stressors that are of psychological nature occur due to uncertainty, lack of information and lack of control, and elicit the most profound neuroendocrine and behavioral responses. The ability to cope with such a psychological stressor is dependent on experience- and gene-related factors, and is affected by cognitive, non-cognitive and environmental inputs. Moreover, coping resources rely on the *context* in which the stressor is experienced. Powerful determinants of context are psychosocial factors such as social position, social support or attachment to a care giver. If any of these factors is disrupted - e.g., loss of control in a social environment, expulsion from social support, homelessness or deprivation of (maternal) care – an acute stressor may exceed the coping resources and produce strong emotional reactions, which ultimately may lead to a condition of chronic stress, exhaustion or burnout and enhanced vulnerability to mental diseases such as depression or anxiety disorders.

These modulations of the stress response have been defined by McEwen and Wingfield (McEwen and Wingfield 2003; McEwen and Wingfield 2010) as variations in an *allostatic state* that cumulatively strive towards homeostasis. *Allostasis* is defined as the process of achieving stability, or homeostasis, through physiological or behavioral change. In principle these changing allostatic states are adaptive, self-preservative and short-lasting. In terms of communication, successful *allostasis* (in establishing homeostasis) would mean that e.g., the HPA axis hormones involved are turned on rapidly when needed, and turned off efficiently when homeostasis has been achieved. The hormonal responses however may be inadequate, or excessive and prolonged and the cost to maintain homeostasis may become high. This leads to wear and tear, or *allostatic load*, ultimately enhancing the vulnerability to disease. Depression may be interpreted as a consequence of sustained hyperactivity of HPA axis activity resulting in excess circulating glucocorticoids.

The stress hormones cortisol and corticosterone (from here on abbreviated as 'CORT' respectively) follow a rhythmic secretion pattern. They are secreted in hourly pulses, exhibit a circadian pattern and can be induced by stressors superimposed on the rhythmic secretion (De Kloet et al. 1998; Windle et al. 1998b). These modes of CORT secretion are regulated by inputs from the suprachiasmatic nucleus, paraventricular nucleus of the hypothalamus, prefrontal cortex, amygdala, and hippocampus, among others. Both stress-induced changes in CORT levels and circadian patterns of CORT will be addressed in this thesis. Following, the concentration of CORT will be our marker indicating stress system activation as a result of our experimental procedures, which is controlled by the Hypothalamic-Pituitary-Adrenal (HPA) axis (see Figure 1 in section 2.1).

2.1. Stress system activation

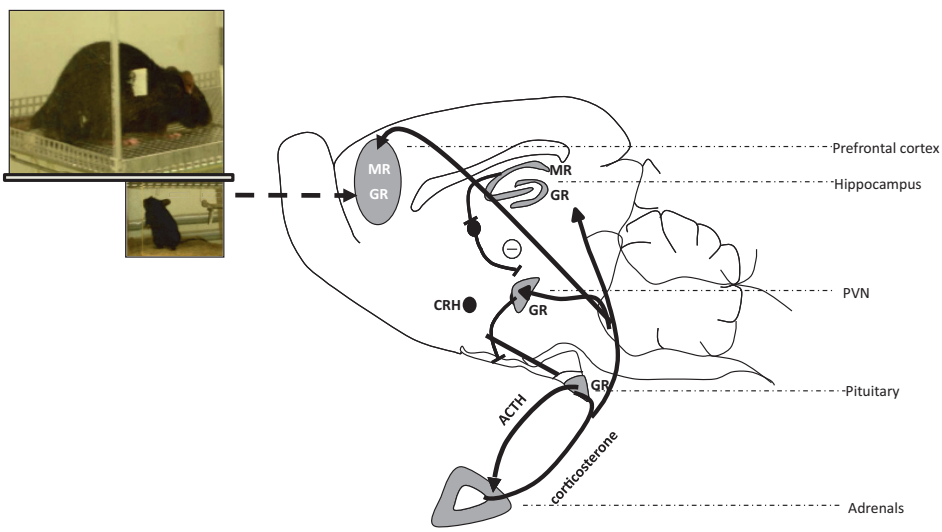
To efficiently cope with threatening situations, the organism requires a set of emotional, behavioral and neuroendocrine responses, summarized as the *stress response*. The stress response is an essential component of the natural defense/response mechanism, providing energy resources in order to react in the most efficient way for the organism. Ultimately, the stress response allows the organism to integrate new with previously learned response strategies, often leading to a new set point of psychological and biological reactivity. However, stress, especially chronic stress, is predominantly

associated with a negative emotional state. As will be described in the following sections, a period of stress may become deleterious when it remains uncontrollable.

The origin of stressors can be *systemic,* directly disturbing physiological integrity (e.g., infections, temperature or blood volume changes) and *psychological* or *psychosocial* (e.g., social conflict, traumatic life event); both able to disturb mental integrity. Exposure to a demanding, threatening event either real or imagined will result in a freeze, fight or flight stress response. This response is governed by two main systems that process the perceived information into a reaction. First, the rapid activation of the sympathetic nervous system increases the release of catecholamines: adrenaline and noradrenaline. These catecholamines stimulate the peripheral organs and increase the blood flow to the central nervous system and muscles within seconds. This allows the organism to promptly respond to the stressor with heightened arousal and attention. The second, slower regulatory response is activation of the HPA axis, characterized by secretion of the glucocorticoid hormones (mainly cortisol in humans, corticosterone in rats and mice (De Kloet et al. 1998; de Kloet et al. 2005).

Activation of the HPA axis by a stressor (see Figure 1) rapidly induces the parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN), to secrete corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) in the portal vessel system; the portal system being the vascular link between the

Figure 1 **Hypothalamic-Pituitary-Adrenal axis**



hypothalamus and the anterior pituitary. Within the anterior pituitary, CRH stimulates cells to synthesize adrenocorticotrophin hormone (ACTH) from its precursor pro-opiomelanocortin (POMC). AVP potentiates the effect of CRH, leading to more release of ACTH. Subsequent increases in circulating ACTH then drive synthesis in and secretion of CORT from the adrenal cortex into the blood.

CORT serves a wide variety of functions in the body. They enhance catabolism, mobilizing lipid and glucose reserves, suppress the immune system and increase the cardiovascular tone (Munck and Naray-Fejes-Toth 1994; De Kloet et al. 1998). In addition, CORT regulates their own secretion by facilitating recovery and inhibiting HPA axis activity. This negative feedback is exerted at several levels of the HPA axis that are activated by the given stressor, thereby normalizing the activity of the stress system and preventing it from overshooting. HPA axis activation enables the organism to respond with the required energy resources to meet the demands of the event.

Prominent in the brain's stress circuitry are the amygdala nuclei for regulation of emotional responses (McGaugh 2004; Phelps and LeDoux 2005), the hippocampus (which defines context in terms of time and place) for learning and memory processes (Sanders et al. 2003) and prefrontal cortex regions for planning and control of actions. Depending on the magnitude of CORT signaling i.e., non-stressed, acute- or chronic stress, the functionality of these brain systems will be affected and thereby alter neuroendocrine-, as well as emotional and cognitive processes (Quirk and Beer 2006; Oitzl et al. 2010), while also changing the circadian pattern of HPA axis activity. The latter will be addressed in the next section.

2.2. Circadian pattern of HPA axis activity

The daily pulses in glucocorticoid concentration follow a circadian rhythm in blood plasma. This rhythm is characterized by peak concentrations of CORT and ACTH at the start of the active period, which is early in the morning for diurnal animals like humans (Krieger et al. 1971; Steiger 2003), and at the onset of darkness for nocturnal animals like rats and mice (Windle et al. 1998a; Windle et al. 1998b; Barriga et al. 2001); lower concentrations occur during the course of the day/night.

Depending on when a stressor is applied during the phase of the rhythm, the amplitude and duration of the stress response differs (Young et al. 2004). Underlying the circadian rhythm, CORT secretion exhibits an ultradian rhythm which is characterized by approximately hourly bursts (de Kloet and Sarabdjitsingh 2008; de Kloet 2009). These circadian and ultradian rhythms of CORT are also expressed in the brain (Droste et al. 2009), and aimed to prepare the organism for environmental changes ahead, i.e., light-

dark cycle and foraging activity. Daily variations in CORT secretion are thought to be fundamental for the maintenance of physiology and well being. Disturbances in the normal secretion pattern, for instance due to chronic stress, are considered to enhance vulnerability to stress-related disorders (Young et al. 2004; de Kloet et al. 2005).

Dramatic changes in circadian patterns of glucocorticoids hormones have been described in aging and psychiatric disorders like depression and Alzheimer's disease (Hatfield et al. 2004; Peeters et al. 2004). The excessive activity of the HPA axis is generally associated with impaired mental and physical health (Sapolsky 1999; Lupien and Wan 2004) and characterized by increased basal and/or stress-induced levels of glucocorticoids and ACTH (Van Eekelen et al. 1995; Herman et al. 2001).

Although mouse models for a wide range of human stress-related disorders have been developed, surprisingly little is known about the impact of age, chronic stress, and repeated blockade of glucocorticoid receptors on basal regulation subserving circadian activity of the HPA axis in mice. All this factors are of importance to further understand how chronic stress can precipitate the development of stress-related disorders, like depression. In this thesis, we will focus on the circadian patterns of neuroendocrine and behavioral activity during and after chronic stress, in mice. In the following sections the role of the glucocorticoid receptors in stress system regulation is introduced.

2.3. Mineralo- and Glucocorticoid receptors

The actions exerted by CORT depend on the functionality of two brain nuclear receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). The pharmacological properties, distribution pattern, and function are distinct for MR and GR (De Kloet et al. 1991; Veldhuis et al. 1992; McEwen 1996; De Kloet et al. 1998; Oitzl et al. 2010).

MR has a 10-fold higher affinity for the naturally occurring CORT than GR (Kd = 0.5 and 5.0 nM, respectively (De Kloet and Reul 1987). Consequently, MR is almost fully saturated at low circulating levels of CORT, whereas GR becomes occupied at increasing levels of CORT as seen during stress and the circadian peak. MR expression in the brain is more restricted to certain areas, with the highest density in hippocampus, and to a lesser extent in the amygdala, septum, PVN and brain stem. GR is expressed throughout the brain (De Kloet et al. 1998), with high expression in the hippocampus, septum and parvocellular part of the PVN of the hypothalamus, brain stem; moderate levels are reported in the central amygdala.

Upon binding of CORT to MR and GR a complex is formed. The corticosteroid-receptor-complex dissociates from a large protein-complex and translocates from

the cytosol to the nucleus as homodimers (MR/MR) or heterodimers (MR/GR). In the nucleus, the dimers bind to glucocorticoid response elements (GREs) in the promotor areas of genes, where they recruit components of transcriptional machinery and activate transcription (McEwen et al. 1986; De Kloet et al. 1991; Morsink et al. 2007). MR and GR can enhance (transactivation) or repress (transrepression) gene expression (Truss and Beato 1993; Beato et al. 1996), and thus influence target genes that are involved in the emotional, behavioral and neuroendocrine response.

MR and GR mediate different aspects of CORT signaling. Studies have emphasized the critical functionality of MR in the sensitivity and feedback of neuroendocrine responses at all stages: (i) primarily the binding of CORT to MR controls the release of ACTH during both the circadian trough and peak (Dallman et al. 1989; Ratka et al. 1989; Bradbury et al. 1994); (ii) blockade of MR by a specific antagonist increases the level of circulating CORT under basal, resting conditions and in response to novelty stress (Ratka et al. 1989). It was concluded that one of the MR-mediated effects of CORT is the initial constraint of HPA axis activity (Oitzl et al. 1995). After acute stress, MR mRNA is quickly upregulated via CRH which is associated with increased inhibition of HPA axis activity, leading to normalization of the disturbance (Hugin-Flores et al. 2003). In hippocampus, MR activation maintains excitability, while GR occupancy suppresses excitability, which is transiently raised by excitatory stimuli. MR and GR distinctively mediate the actions of CORT secretion and its effects throughout the day. Thus, MR activation by CORT maintains basal activity of the HPA axis and controls the sensitivity or threshold of the system's stress response, known as the "proactive" mode. MR promotes coordination of circadian events (e.g., sleep/wake cycle, food intake) and is involved in processes underlying selective attention, integration of sensory information and response selection (Oitzl and de Kloet 1992; Oitzl et al. 1995).

In the second "reactive" mode, when CORT concentrations increase as a result of circadian rhythm and stress, GR becomes activated. GR activation will terminate HPA axis activation via negative feedback leading to reduction in CORT concentration. GR feedback takes place in different brain sites including the pituitary and PVN (Dallman et al. 1987; Levin et al. 1988). GR activation enables an organism to incorporate the neuroendocrine and behavioral responses deployed by facilitating learning and memory processes (De Kloet et al. 1998).

As described, MR and GR are expressed in brain regions involved in emotional, cognitive and neuroendocrine regulation. The receptors mediate rapid mono-genomic CORT actions within seconds to minutes, until the slow and long lasting genomic actions start after an hour lasting hours to days. In this thesis, we set out to alter HPA axis activity

by activation or blockade of the corticosteroid receptors, using either an environmental stressor and/or pharmacological manipulation with a GR blocker. Subsequently, we expect to find alterations in emotional, cognitive and neuroendocrine regulation as indicators of symptoms as seen with depression. Next, the role of MR and GR in the domain of emotional and cognitive processes is discussed.

2.4. Hypotheses of glucocorticoid action and cognition

Aberrant CORT concentrations as seen during periods of chronic stress are causally related with hippocampal, prefrontal cortex and amygdala dysfunction. However, the underlying mechanism is still unclear. Next, we will describe two hypotheses that provide clues to the underlying mechanisms.

The glucocorticoid cascade hypothesis (Sapolsky 1992; Sapolsky 1999): The elevated CORT is believed to arise from a GR dysfunction. When GR function is normal, the rise in CORT concentrations is terminated following GR activation. However, in patients that suffer from a mood disorder like psychotic major depression where CORT levels remain elevated, reduced GR expression in brain (Webster et al. 2002) and in peripheral tissue (Gormley et al. 1985; Pariante 2006) is found. The GR reduction weakens the negative feedback action and induces CORT resistance (De Kloet et al. 1997; Pariante et al. 2004; Ridder et al. 2005). As a result of decreased GR expression or function, circulating levels of CORT are elevated as a compensatory reaction to overcome the CORT resistance at the GR (Sapolsky et al. 1986; Pariante 2003). Prolonged hypersecretion of CORT damages brain structures essential for HPA axis functioning e.g., hippocampus, prefrontal cortex and amygdala. Following, the reduced functioning of brain structures leads to a feed-forward circuit in which ongoing stressors drive overproduction of CORT.

An important role for GR in control of aberrant CORT concentrations is apparent. However, next to GR the existence of another corticosteroid receptor was proven: the MR (Reul and de Kloet 1985).

The MR-GR balance hypothesis is based on (dys)functioning of either one or both receptors, creating an imbalance in MR-GR activation in context with the event. Whereas MR operates in pro-active mode to prevent homeostatic disturbance, additional GR activation promotes the reactive recovery after stress and following circadian peaks (Oitzl and de Kloet 1992; de Kloet et al. 1993a). MR and GR activation in the context of an event facilitate learning and memory, whilst MR and GR activation out of context impair memory (Joels et al. 2006). Studies with transgenic MR and GR mouse models show that

overexpression or inactivation of either two receptors seriously impair physiological and psychological responses to stress (Gass et al. 2001).

MR and GR are co-expressed in most cells of the hippocampus (van Steensel et al. 1996). The hippocampus is a key structure for learning and memory processes, and stress response regulation in general. Processing of spatial information can be specifically modified depending on activation of MR and GR. GR facilitates consolidation of the employed behavioral response. It is evident that dysfunction of MR and GR signaling may have profound effects on emotional, behavioral and neuroendocrine responses.

Blockade of MR activation with antagonist or genetic deletion of forebrain MR, interfered with memory formation (Zhou et al. 2010), and also chronic MR activation impairs spatial memory (Douma et al. 1998; Yau et al. 1999). Furthermore, MR affects emotional behavior. Predominant MR activation alters the behavioral response in novel situations and subsequent explorative search patterns, influencing what is learned and memorized (Oitzl and de Kloet 1992; Zhou et al. 2010). Blockade of MR results in an increased exploration on the elevated plus-maze (time spent in open arms indicates that animal is less anxious), which can be interpreted as an anxiolytic effect (Korte et al. 1996; Smythe et al. 1997; Bitran et al. 1998).

GR promotes memory processes and facilitates consolidation of a learned behavioral response. Mice with alterations in GR functionality, either by a mutation of the GR (e.g., GR-knockout, GR $^{dim/dim}$ mice) or by pharmacological intervention (treated with a GR antagonist intracerobroventricularly), showed impaired spatial memory. In addition, GR activation affects anxiety related behavior, with reduced anxiety in conditions of decreased GR functionality (Tronche et al. 1999; Jakovcevski et al. 2008). Since GR blockade interferes with anxiety motivated behavior, this can be considered an anxiolytic effect, as demonstrated by Korte and colleagues (Korte et al. 1996).

An imbalance in MR or GR activation, due to genetic, environmental, and/or pharmacological intervention is thus suggested to underlie the emotional, behavioral and neuroendocrine disturbances that make the organism more vulnerable for stress related mood disorders like depression (De Kloet et al. 1998; Brinks et al. 2007c; Oitzl et al. 2010).

The experiments described in this thesis aimed to modulate the activity of the glucocorticoid stress system, thereby changing the pattern of MR and GR activation. The psychoneuroendocrine effects were assessed before, during and after onset of chronic stress, and following GR antagonist administration.

2.5. Stress, learning and memory

Memory formation is modulated by task-inherent appetitive and aversive characteristics. Other stimuli occurring in close context with the task can impair or enhance memory (Dawson and McGaugh 1971; McGaugh et al. 1972). These stimuli can be either negative stressors or positively rewarding. The learning and memory process can be described as follows. When a situation is encountered, gain of information (acquisition) about the event takes place. During and directly after the event (Joels et al. 2006), a memory trace of the gathered information and response selection is created and stabilized (consolidation). Upon return to a similar situation the previously acquired response selection is retrieved and either used to deal with the situation at hand, or the response is modified as a result of environmental and cognitive stimuli (retrieval).

The impact of stress on learning and memory processes is described as being impairing, improving or even apparently ineffective (see for extensive review: (Joels et al. 2006; de Quervain et al. 2009; Conrad 2010). Several parameters are important to notice: (i) context – close association between the stress and the learning task facilitates performance. Extremely low or high CORT concentrations in close-context impair performance, demonstrating the inverted U-shaped dose-effect curve of CORT; (ii) convergence in time - stress hormones present around the time of learning and retrieval, i.e., during the actual performance of the behavioral task, can facilitate learning. However, high concentrations of stress hormones before or after learning impair performance; (iii) stressor specificity – different stressors activate different and overlapping brain regions. Whereas physical stressors activate lower brain regions and ascending pathways into the forebrain (e.g., regions involved in pain responses), psychological stressors activate the higher brain regions (hippocampus, prefrontal cortex, amygdala); (iv) frequency of stressor occurrence – single or repeated exposure to a stressor. Characteristics of the stressor, context, timing, memory phases (acquisition, consolidation, retention) during which stress is experienced are important variables contributing to the effect of stress on cognition. In addition, age and gender effects are known. Moreover, there is considerable individual variation in the effects of stress due to genetic background and life history.

Stress can shape the memory trace and subsequent response during future encounters by modifying learning and memory processes that occur before, during and after an initial event. These effects exerted by stress operate in brain circuits that primarily were pronounced by genetic and experience-related factors in preparation of upcoming events.

In order to study the full range of stress effects on learning and memory processes, we designed and optimized learning and memory tasks (**Chapter 5**). In

addition, we were able to perform a unique translational study from mouse to human (**Chapter 8**), where we tested the impact of chronic stress on the use of different memory systems. The following section will give a short impression on different memory systems.

2.5.1. Memory systems

Memory systems differ regarding the kind of information they process, the performed operations and the underlying neural structures (Gabrieli 1998; Squire 2004b). Researchers predominantly focused on the stressor and its impact on memory, but have rather neglected that memory consists of multiple systems processing information in parallel (Squire 2004a; Squire et al. 2004b). For example, changing catecholaminergic activity in the amygdala, a brain structure involved in emotional memory, (Cahill et al. 1995) can modify hippocampal spatial memory (de Quervain et al. 2009; Roozendaal et al. 2009).

Interactions between memory systems are most evident in situations in which multiple memory systems can support behavioral performance. The well-known water maze (Morris 1984) task where rodents navigate to find a platform provides an example. When the platform is visible and in a fixed location, the performance can rely on both hippocampus-dependent spatial (''cognitive'') and neostriatum-dependent stimulus-response (stimulus-response: S-R; ''habit'') memory. In an elegant study, Kim et al (Kim et al. 2001) demonstrated in rats that stress prior to training facilitated the use of an S-R strategy and reduced the use of a spatial strategy to find the platform. Spatial memory, which is considered to rely on more complex processes than S-R memory, supports the acquisition of flexible, consciously accessible knowledge (such as the event of your birthday) that is particularly ascribed to the hippocampus (Scoville and Milner 1957; Eichenbaum 2004). Non-spatial S-R learning process associations, such as ''stop your car when the traffic lights are red", are not necessarily accessible to consciousness and relies on the caudate nucleus (Knowlton et al. 1996; Jog et al. 1999). The two memory systems can work in parallel and process information simultaneously. Cognitive tasks can be designed that allow a differential use of spatial and non-spatial memory systems.

Acute stress prior to training in a task that could be acquired by a hippocampus-based spatial, and a caudate-based non-spatial 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). This stress-induced modulation of hippocampus-based and caudate-based learning and memory systems is assumed to be influenced by the amygdala as well (Packard and Wingard 2004). Emotional components (including anxiety, punishment, reward) are included in the majority of behavioral tasks for rodents.

Stress before the learning task affects the performance, which might be due to the differential use of memory systems. Psychosocial stress before training in an instrumental task rendered the participants' behavior insensitive to the change in the value of a reward: i.e., stress led to habit non-spatial performance at the expense of goal-directed spatial performance in humans (Schwabe and Wolf 2009). This study proves that the recognition of change in the rewarding values of stimuli is differentially perceived under stress.

The modulation of non-spatial habit, and spatial cognitive memory systems by stress has attracted a lot of scientific attention the past decade (Kim et al. 2001; Packard and Wingard 2004; Schwabe et al. 2007; Dias-Ferreira et al. 2009). However, the effects of prolonged or repeated periods of stress on the modulation of these two memory systems have not been described. In this thesis we determined the impact of chronic stress in both mice and humans. The results could indicate the underlying processes that drive behavioral alterations as seen in patients that suffer from depression.

2.6. Depression: emotional and cognitive disturbances

Depression is characterized by several symptoms (see **Box 2**).

Box 2: Symptoms of depression

The Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV-TR), describes that at least five of the following signs and symptoms must be present for at least 2 weeks as to be characterized as a depressive disorder: (1) anhedonia: loss of interest for or inability to experience pleasurable emotions from normally pleasurable life events, (2) appetite/weight disturbances, (3) sleep disturbance / circadian activity pattern, (4) psychomotor retardation, (5) loss of energy, (6) feelings of depressed mood, (7) worthlessness/guilt, (8) concentration difficulties/ indecisiveness and (9) thoughts of death/suicide. It is recognized that the latter five symptoms are typical human characteristics and cannot be modeled in mice. Our experimental setup described in this thesis aimed to induce symptoms (1), (2), (3), (4) and (8) using our 'rat stress' paradigm in mice.

The core symptom of depression is anhedonia, which is defined as the inability to experience pleasure (Ribot 1897). Anhedonia is indicative for alterations in the perception of emotional and other environmental stimuli, which in turn affects cognitive processing, and vice versa. In psychopathology disturbances in the detection, response to, and interpretation of emotions are common, and can produce altered emotional responses.

Patients suffering from psychotic major depression show reduced emotional reactivity. More specifically, a deficit in processing of positive stimuli is evident, while a bias towards the perception of negative stimuli exists. This imbalance in emotional processing results in depressed mood and anhedonia (Phillips et al. 2003; Leppanen 2006; Bermpohl et al. 2009). However, the neural substrates for mood disorders are poorly understood. Next to disturbances in limbic and prefrontal brain regions, alterations in the brain reward mechanism (the mesolimbic dopamine system) are likely. Neuroimaging studies show reduction in hippocampal volume, and alterations in prefrontal cortex, amygdala and brain regions associated with the mesolimbic dopamine system (i.e., nucleus accumbens and the ventral tegmental area; (Nestler and Carlezon 2006; Martin-Soelch 2009). Studies in depressed patients revealed a decrease in reward sensitivity toward positive stimuli (Shankman et al. 2007) and altered reward-related decision making (Forbes et al. 2007) functions are restored following classic antidepressant treatment in a subset of patients (Drevets 2000). Disturbances in emotional processing affect cognitive processing, like memory formation (Stiedl et al. 2000) which in turn affects the emotional response to stimuli (Blair et al. 2007). Depending on the CORT concentration, and subsequent binding to MR and GR, emotional and cognitive processes can be modulated (Brinks et al. 2007b).

Patients suffering from depression exhibit hyperactivity of the HPA axis even before the onset of clinical symptoms. The CORT concentrations are elevated during the circadian cycle (Keck and Holsboer 2001). Remarkably, patients with a severe form of depression (psychotic major depression) appear to be relieved of symptoms following treatment with the GR antagonist MIF (Belanoff et al. 2001a; DeBattista and Belanoff 2006). Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV-TR), describes that at least five of the signs and symptoms referred to in **Box 2** must be present for at least 2 weeks as to be characterized as a depressive disorder. It is recognized that symptoms (5), (6), (7) and (9) are typical human characteristics and cannot be modeled in mice. Our experimental setup described in this thesis aimed to induce signs and symptoms (1), (2), (3), (4) and (8), using our 'rat stress' paradigm in mice.

2.6.1. Treatment of depression

The classic antidepressants (e.g., tricyclic antidepressants; Serotonin and Noradrenaline Reuptake Inhibitors: SSRI, SNRI) have shown therapeutic effect. However, the therapeutic effect may take weeks before being expressed, with increased risk for the patient to experience unwanted side effects, and the increased likelihood that the risk on suicide remains high during the first weeks of treatment (Schatzberg 2002). Hence, new antidepressants are warranted. We know that the classic antidepressants affect MR and GR expression in brain, and normalize CORT secretion patterns (Reul et al. 1993). The normalization was thought to be partly due to restoring the negative feedback mechanism at the level of GR (Ribeiro et al. 1993; Heuser et al. 1996; Pariante and Miller 2001). Thus, targeting the receptors that mediate CORT secretion, MR and GR, might open up potential new drug treatment for patients that suffer from depression.

Indeed, clinical trials revealed that high doses (600 - 1200mg/day) of the GR antagonist mifepristone (i.e., RU38486) show therapeutic efficacy for the most severe form of depression, psychotic major depression (Murphy et al. 1993; Belanoff et al. 2001a; DeBattista et al. 2006). Treatment for several days only, already improved emotional and cognitive processes, together with restoration of aberrant levels of CORT. The 'antidepressant' effect is thought to arise via the following pathway: GR antagonism leads to increased amplitude in pulsatile and circadian CORT levels, which induce a resetting of the HPA axis activity, with a subsequent change in GR sensitivity, and a distinct action via MR by CORT (Sartor and Cutler 1996; De Kloet et al. 1998). The rhythmicity of the circadian activity is enhanced (van Haarst et al. 1996). In addition, GR resistance could be compensated via increased MR expression (Wodarz et al. 1992; Calfa et al. 2003).

Additional evidence for GR dysfunction comes from depressed patients that received the GR agonist dexamethasone. These patients showed non-suppression of ACTH and CORT (Nemeroff 1996; DeBattista et al. 2006), suggestive for an impaired negative feedback at the level of GR. It has become clear that GR dysfunction is associated with stress-related psychiatric disorders. This shift in the balance of MR and GR activation renders the organism more vulnerable to diseases (Holsboer 2000; de Kloet et al. 2005).

2.6.2. Effects of GR antagonism

The role of GR has been studied specifically by pharmacological modulation, using the GR antagonist RU38486 (Roussel-Uclaf 38486; first synthesized in 1981) also known as mifepristone or in short RU486. It has both antiglucocorticoid and antiprogesterone

activity. The latter is utilized in early termination of pregnancy. RU38486 is readily absorbed via the oral route in humans and rodents. The α1-acid glycoprotein binds RU38486 in humans, increasing its bioavailability (Agarwal 1996). However, the bioavailability of RU38486 in rodent is 40% partly because the rodent's α1-acid glycoprotein does not bind RU38486 explaining the low levels in plasma and fast plasma clearance (Philibert and Teutsch 1990). RU38486 is distributed to all tissues, thereby exerting a generalized antiglucocorticoid activity (Heikinheimo and Kekkonen 1993). Intracerebroventricular (ICV) administration of RU38486 was performed. GR antagonism does not interfere with basal resting activity of the HPA axis at the trough of circadian activity. However, RU38486 increases the circadian peak secretion of CORT and prolongs stress induced activity (Gaillard et al. 1984; Ratka et al. 1989; van Haarst et al. 1996).

GR antagonism has been shown to protect mice and rats against the negative impact of high CORT and chronic stress on hippocampal functioning. Mice with streptozotocin-induced type I diabetes and high CORT for eleven days showed hippocampal alterations; treatment with mifepristone for 4 days during the early phase of diabetes attenuated the morphological signs and protected the mice from cognitive deficits (Revsin et al. 2009). Neurogenesis was normalized in rats that underwent a chronic stress paradigm for 21 days, and were treated with mifepristone during the last 4 days (Mayer et al. 2006; Oomen et al. 2007). The protecting, and therapeutic efficacy of GR antagonism is most pronounced in conditions of high CORT levels. Although CORT concentration increases due to GR antagonism, there is no receptor to act on. It thus appears that the resulting shift in MR-GR activation is responsible for the positive effects of GR antagonism.

Taken together, the GR antagonist mifepristone (MIF) increases HPA axis responsiveness and resilience in humans (Lamberts et al. 1991). Similar effects are found in rats (van Haarst et al. 1996). Whereas much is already known on GR functioning, the mechanism underlying the apparent therapeutic efficacy of GR antagonism is unclear. Before we would study the impact of GR antagonism in our chronic stress model we will determine the effects of GR antagonism in naive mice. We will collect data related to circadian HPA axis activity, emotional, behavioral and neuroendocrine responses (**Chapter 4**). This data will provide parameters that can show whether in stressed mice similar processes are affected and the possible protecting/normalizing effects of MIF on those processes.

3. Rodent models of depression

Animal models of depression can be of genetic origin, induced by (social-) environmental challenges (usually exposure of rodents to various types of stressors) or via pharmacological modulation. The resulting neuroendocrine and behavioral changes are indicative for certain signs and symptoms (Willner 1990; Willner et al. 1992; Willner 1995). Chronic stress is believed to render the organism more vulnerable to the development of stress related psychiatric disorders. Therefore, most animal models use long-term manipulations of the stress system to model the predisposition to depression (Willner and Mitchell 2002). Although the subtypes of depression are typical human disorders, a subset of human characteristics can be assessed in animal models; see Table 1.

Table 1: Symptoms associated with depression in humans and reference to Chapters in the thesis that determined the expression of the human-like symptoms in our mouse model for depression.

Symptoms of depression*	Measurable in animal models?	Determined in Chapter
Anhedonia	Yes	6 and 7
Weight changes	Yes	6
Sleep disturbances/circadian activity pattern	Yes	6 and 7
Psychomotor retardation	Yes	6 and 7
Fatigue/loss of energy	Yes	not determined
Depressed mood	No	n.a.
Feelings of worthlessness/guilt	No	n.a.
Diminished ability to think/make decisions	Yes	6, 7 and 8
Thoughts of death/suicide	No	n.a.

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

Table 1 indicates which human characteristics were determined in our chronic stress model. A valid model of depression would ask for multiple symptoms to be induced and measured (see also **Box 3**). Preferably, multiple behavioral tests need to be performed to approximate the characteristic mood symptoms (Anisman and Matheson 2005).

First, a brief introduction to rodent models for human stress, primarily targeting GR and MR is provided, followed by a description of our chronic 'rat stress' paradigm in more detail.

The pivotal role of GR for survival has been shown in mice with a total deletion of GR ($GR^{null/null}$). Ninety-five percent of these mice died shortly after birth because of impaired lung development (Cole et al. 1995; Reichardt and Schutz 1996). The remaining 5% survived because of an incomplete knockout of the GR. However, partial inactivation of the GR produced depression-like changes in behavior and a mild HPA axis dysregulation. Anxiety-associated locomotor activity was increased and adrenal responsiveness was augmented. This occurred in GR heterozygous mice (GR+/- with a 50% reduction of GR; (Ridder et al. 2005; Chourbaji and Gass 2008) and in mice with postnatally induced deficiency of GR in the forebrain (Boyle et al. 2005; Boyle et al. 2006). A reference to the variety of genetically modified GR mouse models can be found in (Muller et al. 2002; Urani and Gass 2003; Kolber et al. 2008).

Box 3: A model is defined as any experimental preparation serving the purpose of studying a condition in the same or different species. In developing and assessing an animal model, it is critical to consider the explicit purpose intended for the model, to determine the criteria required to establish its validity. Validation of models for psychiatric disorders include consideration of the following: *construct validity* (theoretical rationale for designing the model based on clinical expression of the disorder); *face validity* (phenomological similarity between the model and the disorder); *predictive validity* (the correspondence between drug actions in the model and the clinical setting; (Willner 1997; Bloom and Kupfer 2001). It depends on the scientific question addressed which animal model is to be used (e.g., social stress paradigms with or without physical contact).

Numerous animal models of 'depression' are available that predominantly focus on the expression of negative emotions. We set out to develop a chronic stress paradigm that would allow investigation of immediate and long-term consequences for emotional and cognitive responses, in relation to positive rewarding stimuli and stress system activity patterns. In addition, behavioral measurements were designed to cover a wide range of effects.

We aimed to disturb the MR-GR balance in two ways, using (1) an environmental challenge –chronic 'rat stress' - to modulate the activity of the stress system over a longer

period, and (2) pharmacological modulation of GR activity using the GR antagonist mifepristone. Neuroendocrine, emotional, cognitive and behavioral patterns were assessed. The next section provides more detail on the features of our chronic stress model.

3.1. Environmental stress paradigms

One of the precipitating factors in the development of depression is a disturbed reactivity to novel situations. This reactivity is a combination between genetic predisposition and past learning experiences. In humans, chronic psychological stress during adulthood can precipitate psychiatric disorders (Corcoran et al. 2003). Central features of chronic psychological and psychosocial stressors in humans are repeated, unpredictable and uncontrollable exposure to (or imagination of) threatening situations. To mimic these central features, animal models are based on social confrontations with or without physical contact (Apfelbach et al. 2005). To clarify the difference between a stressor with- and without the ability of physical contact, an example for each is described below.

3.1.1. Social stress paradigm with physical contact

Chronic stress in mice can be induced by social defeat. A mouse is rendered subordinate by repeated exposures and defeat by a dominant mouse during several weeks. Consequences are: decreased locomotor and exploratory activity, increased anxiety. HPA axis activity is affected as indicated by low body weight, elevated CORT and ACTH concentrations, and low hippocampal MR mRNA expression (Koolhaas et al. 1997) (Koolhaas et al. 1997; Veenema et al. 2003). Schmidt et al., developed a chronic social stress paradigm where mice are exposed to different cage members every 3 - 4 days, which creates an unstable social hierarchy; an unavoidable stressor. The consequences are expressed by increased adrenal and reduced thymus weight, flattened circulating circadian CORT concentrations patterns, reduced mRNA expression of hippocampal MR and GR, increased expression of AVP in the PVN, increased anxiety and lower responsivity to a sucrose solution (Schmidt et al. 2007; Schmidt et al. 2008; Sterlemann et al. 2008). However, these stress paradigms are a mix of physical and psychological stressors. Whereas physical stressors affect predominantly lower brain areas (e.g., brain stem) that subsequently affect the forebrain, psychological stressors are processed in higher brain areas (e.g., prefrontal cortex, amygdala, and hippocampus).

Psychological and psychosocial stressors are ethologically more relevant compared to physical stressors, and resemble the kind of stress that is related to

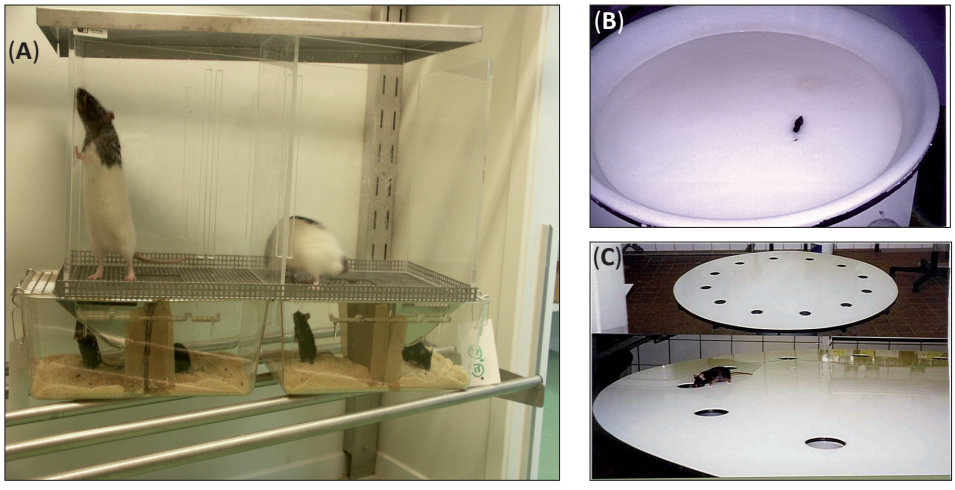
depression in humans (Calvo-Torrent et al. 1999; Apfelbach et al. 2005; Beekman et al. 2005). In rodents, the behavioral effect of predator exposure becomes manifested in the defeated subject as increased anxiety-like behavior, risk-assessment in novel environments and learning and memory impairments (Calvo-Torrent et al. 1999; Grootendorst et al. 2001a; Grootendorst et al. 2001b; Adamec et al. 2004; Diamond et al. 2006). For our stress model we induce chronic psychosocial stress by means of exposing mice to the presence of a rat, without physical contact.

3.1.2. Social stress paradigm without physical contact

Already sensory stimuli (visual, auditory and olfactory) are sufficient to activate the stress system associated with the release of CORT (Blanchard et al. 1998; Diamond et al. 1999; Linthorst et al. 2000; Beekman et al. 2005). In nature, mice and rats avoid each other and it was shown that exposure of mice to rats in a laboratory setting increased CORT concentrations in blood plasma and in the extracellular fluid of the mouse brain, as measured using microdialysis (Linthorst et al. 2000). Previously, our group created a chronic stress model for mice by exposing mice repeatedly to the presence of a rat, a procedure referred to as chronic 'rat stress' (Figure 2A).

Mice and rats could hear, see and smell each other, without physical contact (Grootendorst et al. 2001a; Grootendorst et al. 2001b). Acute and some long-term effects on neuroendocrine and behavioral responses are evident in the mice during chronic 'rat stress'. Using two distinct spatial learning tasks, we showed that chronic 'rat stress' impaired learning and memory performance in C57BL/6J mice. More specifically,

Figure 2



stressed mice used a different strategy to locate the escape platform (water maze; Figure 2B) or exit hole (circular hole board; Figure 2C) in either two tasks. In addition, immediately after the 9th rat exposure and one week after the last rat exposure, the plasma CORT concentration was increased. Interestingly, three months after cessation of the stressor, stressed mice displayed a different behavioral response after being placed in the dark compartment of the light-dark box. Stressed mice were more active in the light compartment. This is opposite to their natural preference namely, seeking shelter in the dark area of the environment (Grootendorst et al. 2001a; Grootendorst et al. 2001b).

The effects of chronic stress in animal models are mainly assessed in short-lasting test-situations involving additional novelty stress. Less is known about the consequences of stress for the daily organization of behavior in a familiar environment where the animal (and the human) spends most of its time: the home cage (at home for humans).

3.2. Home cage observations

For patients that suffer from depression, the negative effects extend to both novel and familiar environments (Volkers et al. 2002; Keller et al. 2006). Animal models of depression have predominately assessed behavioral alterations in novel environments. In addition, the tests are short-lasting, and limited in the readout of the behavioral patterns. The few studies that address changes in circadian activity in mouse models include “chronic mild stress” and electric shocks which decreased the amplitude of circadian locomotor activity and food-intake (Willner 1984; Desan et al. 1988; Stewart et al. 1990; Gorka et al. 1996; Meerlo et al. 1999). To our knowledge, long-lasting analysis of activity patterns in the familiar environment of the home cage before, during and after a psychological stressor have not been described in mice.

Previous studies have shown that long term automatic recordings of the mouse in its home cage, allows detailed observations on dynamic changes in locomotor activity over days, with minimal human intervention (de Visser et al. 2005; de Visser et al. 2006) see Figure 3 for apparatus).

In addition, subtle changes in spontaneous behaviors under baseline conditions may reveal themselves more easily in the home cage than under conditions where the animal is prompted to explore or face a strong challenge. **Chapter 6** of this thesis will describe the daily organization of behavior in the familiar environment of the mouse’s home cage before, during and after chronic ’rat stress’.

Figure 3

We studied the impact of our chronic stress paradigm on undisturbed and novelty induced emotional, behavioral, cognitive and neuroendocrine responses that could underlie the expression of anhedonia in mice (**Chapters 6** and **7**).

3.3. How to measure anhedonia in an animal model?

To measure a diminished interest or responsiveness to positive stimuli, several methodological tools are available. In this thesis, we will use three tools: the sucrose consumption and preference test (**Chapters 6** and **7**), analysis of exploration patterns (**Chapter 6, 7** and **8**), and reward modulating effects on memory (**Chapters 5** and **7**).

An alteration in reward sensitivity can be measured using the sucrose task, where the consumption and preference for a sweet solution is determined. Depending on the experimental design, rodents are food and/or water deprived before testing takes place. However, as deprivation can induce stress, our experimental design did not use deprivation. Instead, during testing two bottles were presented for 24h. Allowing ample time for the mice to get 'used' to this new situation and drink when preferred. One bottle contains water, the other contains a sweet solution (5% sucrose: see Pothion et al. 2004); the bottles are weighed before and after a fixed time. The weight difference is a measure for fluid intake of both solutions, and the preference for either solution is calculated.

Novel stimuli may signal danger, but also possible rewards. During novelty exploration, activation of the avoidance-approach system occurs. Depending on the dominance of either system, exploration of the environment will be more or less intense. The hippocampus detects novel stimuli and is critical for the memory formation

of the novel event or environment. The novelty signal is also a major input to the brain reward mechanism, involving the neurotransmitter dopamine (Wittmann et al. 2007). Human fMRI studies show that joint activation of hippocampus and brain reward regions is crucial for the development of long term memories (Schott et al. 2006b). Thus, exploration patterns of a novel environment might provide leads to the emotional state of the animal (File 2001; Kalueff et al. 2006). Exploration is considered as self-rewarding behavior, involving the expectation of potential rewards, e.g., food, mates, a hiding place. While 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).

Reward has been shown to affect the strength of memory (Huston and Mondadori 1977; Huston and Oitzl 1989; Messier 2004). We aimed to demonstrate that post-training access to sugar (the reward) will facilitate spatial memory of mice. This experimental set-up might allow to study whether exposure of mice to the chronic stress paradigm changes the perception of the emotional quality of the stimulus. The performance in the learning and memory task could reflect anhedonia. Consequently, we expect the loss of the memory facilitating effect of post-trial sugar administration in stressed mice.

The three tools that can measure the expression of anhedonia are part of the design of the experiments described in this thesis. We believe that multiple read-outs for loss of interest or pleasure, will underline the strength of our chronic stress model to induce anhedonia, and its relevance as an animal model of depression, a stress-related mood disorder.

4. Scope and outline of the thesis

4.1. Rationale and objectives

Chronic stress, defined as a hyper- or hypoactivity of the stress-system, in concordance with alterations in neuroendocrine-, emotional- and cognitive responses, are characteristics described for mood disorders like depression. To mimic these characteristics animal models are widely used. The overall aim of this thesis is to work towards a mouse model that expresses a wide range of signs and symptoms as seen with patients that suffer from depression, with special focus on the processes that underlie the expression of anhedonia.

The specific **aims** of the studies described in this thesis address methodological issues, home cage observations, activity patterns, emotional-, and learning and memory processes, with the objective to achieve translation of chronic stress effects in mice to humans by:

(i) Determining the circadian pattern of HPA axis activity and its molecular markers in the brain of naïve (non-stressed) mice at different ages.

(ii) Development of a stress-free method for oral drug delivery in mice, which allows to more specifically study the effect of the drug under study (i.e., CORT or mifepristone).

(iii) Characterization of learning and memory processes of mice in two distinct spatial learning tasks. The possibilities of either two tasks to measure a wide range of processes, will determine which spatial task will be used during subsequent behavioral testing.

(iv) Assessment of recurrent glucocorticoid receptor (GR) blockade effects on stress-system activity and behavior in novel environments, in naive mice.

(v) Characterization of the chronic 'rat stress' model by assessment of the neuroendocrine and behavioral responses in novel environments, i.e. learning tasks. In addition, investigation of the daily organization of behavior in the familiar environment of the home cage, before, during and following chronic 'rat stress'. The results will indicate whether anhedonia is expressed in our chronic stress model of depression.

(vi) Assessment of learning and memory in mice and humans with a history of chronic stress: translational study.

4.2. Experimental approach and outline

The experiments that are conducted can be divided in three categories addressing:

1. Methodological optimization: To design new, and optimize existing neuroendocrine and behavioral measurements to closely control the activation of the stress system, induced by the experimental procedures. Since timing, context and duration of a stressor determine the outcome of the experiments, interference by unintentional stressors has to be controlled and minimized (**Chapters 3** and **4**).

2. Longitudinal measurements: Home cage observations and novelty exposure are used to measure circadian behavioral and neuroendocrine activity patterns, as well as emotional responses and learning and memory performance. These measurements are combined with tests of anhedonia (**Chapters 2, 5, 6** and **7**).

3. Translational approach: Humans and mice that experience a period of chronic stress are subjected to comparable experimental designs which allow to test the use of distinct memory systems between the two species (**Chapter 8**).

For all experiments described in this thesis male mice from the C57BL/6J strain were used. There is abundant knowledge on the phenotype of C57BL/6J mice. Less is known about the circadian stress system activity in undisturbed conditions. In **Chapter 2**, the circadian activity of several HPA axis markers, with special focus on the 24h secretion pattern of CORT, is described for mice aged 3, 9 and 16 months. The results will be used as reference for comparison with the expected impact of chronic stress on circadian HPA axis activity (**Chapters 6** and **7**).

Next, we aim to optimize methodological procedures related to drug delivery and behavioral testing. Stress and CORT are known to affect memory processes. Experimental manipulation of mice, such as an injection, already induces stress-system activation, which most likely interferes with neuroendocrine and behavioral testing. In **Chapter 3** we set out to develop a non-invasive, stress free method of drug delivery via oats in mice. We will measure CORT in blood plasma in response to conventional drug delivery methods (*intraperitoneal* i.p., *per os* p.o.) and drug-delivery via oats. The latter method will allow close-context delivery of corticosteroids (and other drugs) prior to and directly after behavioral testing. Also, administration of CORT to mice in the undisturbed environment of the home cage, allows to study the effect of GR blockade on circadian HPA axis and behavioral activity (**Chapters 4** and **6**). We will investigate the effects of

single and repeated GR blockade (using RU38486/mifepristone) on circadian CORT patterns and behavioral responses in **Chapter 4.**

Learning and memory abilities can be assessed using a behavioral task. The choice of the task is based on task-inherent appetitive and aversive characteristics, amongst others. In **Chapter 5** we compare the behavior of mice tested in two spatial learning tasks that were originally designed for rats: the Morris water maze and the circular hole board (dry-land maze). Depending on the variability of the parameters that can be assessed by either behavioral task, further experiments described in this thesis would make use of one of the two spatial learning tasks to assess the impact of chronic stress on the novelty exposure and learning and memory processes (**Chapters 6, 7** and **8**). Memory can be modulated by positive and negative reinforcers delivered in close-context to the learning task. We will provide naïve mice with post-trial free access to sugar as positive reinforcer. This proof of concept is further used to determine whether mice exposed to our chronic stress paradigm display an alteration in learning and memory modulation by a reward (**Chapters 7** and **8**).

The effects of chronic stress in animal models are mainly assessed in short-lasting test-situations that have task-inherent features of novelty and sometimes even include exposure to a physical stressor. The experimental design described in **Chapter 6** is aimed to monitor in a longitudinal set-up, the daily organization of behavior in the familiar environment of the home cage before, during and following exposure to our chronic stress paradigm. Mice are repeatedly, and during unpredictable and uncontrollable times exposed to rats (without physical contact). In addition, we will test changes in the consumption of and preference for a sweet solution (sucrose = dissolved table sugar). The result could be indicative for changes in the reward system: loss of interest in pleasurable activities or diminished response to positive stimuli, also known as anhedonia.

In **Chapter 7** we combine methodologies as described in the previous chapters to reveal the effects of chronic 'rat stress' on learning and memory assessed in the circular hole board, and the memory modulating effects of a post-trial positive reinforcer. In addition, sucrose consumption and preference, exploration patterns during novel environment exposure, behavior in the light/dark box, and the pattern of CORT secretion is measured on several days after cessation of the stressor. The results from **Chapters 6** and **7** will additionally (together with **Chapter 5**) indicate whether our chronic stress paradigm induces the expression of anhedonia.

Multiple memory systems guide behavior. Acute stress modulates the contribution of memory systems to behavior in favor of caudate nucleus-dependent

stimulus response learning and memory, at the expense of hippocampus-dependent spatial learning and memory. In **Chapter 8** we examined whether chronic stress has similar consequences in mice and humans on the use of memory systems, as described for acute stress. The circular hole board task was modified to mimic the characteristics of the human task, allowing stimulus-response as well as spatial learning.

A general discussion of the findings is presented in **Chapter 9**, followed by a summary in **Chapter 10**.