Towards a mouse model of depression : a psychoneuroendocrine approach

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Chapter 10

Summary

Worldwide, depression is among the leading causes of disability. It is a mood disorder that leads to substantial impairments in an individual's ability and pleasure to take care of everyday responsibilities. Antidepressant medications and brief structured forms of psychotherapy are still ineffective for 20-40% of the affected individuals. Therefore, more effective fast acting medicines are therefore urgently needed. What hampers the progress in new drug discovery is the complex nature of depression which involves multiple brain processes. A promising perspective for new drug targets is that the etiology of depression has been linked to the inability of the affected individual to cope with chronic stress. The aim of the research described in this thesis was to develop an animal model which would express a wide range of emotional, behavioral and neuroendocrine signs and symptoms of depression, based on exposure of mice to a chronic stressor. Using this model, new drug targets could be revealed and current pharmacologic treatment tested on a wide range of processes.

The glucocorticoids cortisol and corticosterone (collectively called "CORT") are secreted by the adrenal glands after activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis in response to stress. This response occurs on top of its ultradian (hourly burst in secretion) and circadian (24h) rhythms. CORT and the other hormones of the HPA axis are powerful neuro-endocrine mediators of stressful environmental stimuli. They coordinate adaptive functions of brain and body via the mineralocorticoid- and glucocorticoid receptor (MR and GR).

Using our chronic stress mouse model we investigated how adaptation to stress can become impaired and how this impaired adaptation is capable to precipitate emotional and cognitive disturbances as characteristic features of depression. In this line of reasoning, chronic stress leads to an altered pattern of HPA axis activity which is considered causal to the pathogenesis of depression. The key symptom of depression studied in this thesis is *anhedonia*, which is defined as a decrease in the sensitivity for a reward, i.e. positive stimuli.

We have tested the hypothesis that chronic stress alters glucocorticoid signaling thereby disturbing the appraisal processes that underlie the expression of anhedonia. We monitored the expression of reduced responsiveness to positive stimuli by assessing learning and memory performance, emotionality and endocrine response patterns during and after cessation of the chronic stress.

Before we assessed the effects of chronic stress on psychoneuroendocrine parameters in our mouse model, we characterized the basal 24h circadian activity patterns of selected

HPA axis markers in male 3, 9, and 16 months old C57BL/6J mice. In **Chapter 2** the results were described for the 9 and 16 month old mice in comparison to 3 month old mice.

Whereas 9 month old mice expressed a relative hypercorticism (high CORT level), 16 month old mice displayed a relative hypocorticism (low CORT level). Mineralocorticoid- (MR) and glucocorticoid receptor (GR) mRNA expression in the hippocampus were significantly decreased in 9 month old mice, whereas in 16 month old mice, the expression of both MR and GR was similar to that observed in young animals. The parvocellular hypothalamic paraventricular nucleus (PVN) expressed very high vasopressin mRNA in 16 month old mice, which was subject to circadian variation in 3 and 9 months old mice.

In conclusion, basal 24h-circadian HPA axis activity and expression of some of its central regulatory markers are age-dependent in mice. It is showing an inverted U-shape pattern with highest activity at 9 months of age at least with respect to CORT. For the remainder of the studies we continued using male 3 months old mice as to avoid interference of the basal endogenous and stress induced CORT secretion by age, induced by our chronic stress model.

Conventional drug delivery methods (e.g., subcutaneous, intraperitoneal, *per os*) are intrusive and consequently, can evoke a stress response. This additional stress response can interfere with the pharmacological action of the drug and behavior being studied. Because we wanted to study the effect of GR antagonist RU38486 (i.e. mifepristone – MIF) in naïve and stressed mice, we devised a novel non-invasive, stress-free method of drug delivery via oats in mice in, as described in **Chapter 3**. We measured CORT in blood plasma in response to conventional drug delivery methods and following drug delivery via oats.

Oat consumption induced a small increase in CORT concentrations after 15 min (< 50ng/ml) that returned to the initial low resting levels after 30 min (< 10ng/ml). Gavage and intraperitoneal vehicle injections resulted in long-lasting CORT elevations (> 100ng/ml and ~ 50ng/ml after 30 min and at 60 min respectively). To determine whether it would be possible to produce a pulse with exogenous CORT, three different CORT doses were added to the oats. These doses were offered to adrenalectomized mice as to eliminate the contribution of endogenous CORT. Adding CORT to oats resulted in a 3-fold higher plasma CORT concentration in the 15.0mg/kg-group (± 250ng/ml) compared to the 4.5mg/kg-group at t=30 and t=90 min. Interestingly, the administration of mifepristone (MIF -200mg/kg) via oats elevated plasma CORT for at least eight hours in non-stressed mice.

Concluding, oat delivery is a good, practical and useful non-invasive method for the delivery of glucocorticoid ligands. The method of administration induces a very low stress-CORT level, and allows also to mimic a CORT pulse. This method was applied in **Chapter 4**.

In **Chapter 4** the effects of single and repeated GR blockade using MIF on circadian CORT patterns and stress-induced neuroendocrine and behavioral responses were described. We designed a study to mimic the protocol which has proven successful in the treatment of patients that suffer from psychotic major depression. Naïve male C57BL/6J mice were offered MIF (200mg/kg) *per os* by oats, either once (1xMIF) or once per day on 7 consecutive days (7xMIF) or vehicle (VEH).

Whereas single administration of this very high dose of the GR antagonist resulted in very high CORT concentrations, repeated GR antagonism progressively downregulated HPA axis activity towards a normal CORT output. To explain this unexpected phenomenon we reasoned that in fact the very high CORT level remained elevated beyond the actual presence of the GR blockade, and hence were capable to exert a strong feedback signal suppressing HPA axis activity. This GR-mediated CORT feedback signal persisted because of its genomic nature, long after return of CORT to even below baseline levels as observed at 32h after the first administration. However, 24h after MIF administration, the mice were still capable to show a rapid stress-induced increase in CORT following exposure for 5 min to the circular hole board. After 7 cycles of MIF, the CORT feedback has proceeded to such an extent that neither MIF nor stress is capable to activate the HPA axis.

The contribution of the brain CORT receptors was also determined. While brain and pituitary GR were subsequently blocked by MIF and activated by endogenous CORT over several circadian cycles, the brain MR is freely accessible by circulating CORT under any condition. In response, the hippocampal MR expression was initially lower during high levels of CORT, but increased upon repeated GR antagonist exposure. Particularly within the hippocampal CA2 region at the time CORT exposure was back to baseline. The patterns of MR and GR activation during the course of daily repeated GR antagonism was also expressed in the choice of search strategy employed. Whereas 1xMIF mice were hyperactive, the 7xMIF mice showed relatively more serial search patterns than 1xMIF and VEH treated animals. This suggests an increased role of MR-mediated limbic function.

In conclusion, our data revealed that the recurrent daily blockade of GR by the very high dose of MIF did not produce the expected lasting hypercorticism. Instead,

it led to downregulation of basal and stress-induced HPA axis activity. Possibly this downregulation is caused in part by the long lasting CORT feedback activity hat becomes prevalent during timepoints when the GR antagonist dissociates from GR. This recurrent blockade and activation of the GR is thought to proceed in cooperation with a limbic MR mediated mechanism that may account for the reported (Wulsin et al. 2010) MIF-induced suppression of excitatory, and enhancement of inhibitory inputs to the HPA axis.

To determine which behavioral task would be most suitable to study a variety of behavioral responses that are indicative for chronic stress-induced improvement or impairment of learning and memory processes (see **Chapter 6, 7** and **8**), we compared two commonly used behavioral paradigms for (non-) spatial learning and memory: the circular hole board and the water maze. Additionally, we studied the modulation of spatial memory by reward as a post-training positive reinforcer as described in **Chapter 5**. Free access to sugar was chosen as a post-training reinforcer and was provided immediately (0h-sugar) after training, or with a delay of 4h (4h-sugar), while the controls did not receive sugar.

In both tasks, '0h-sugar mice' showed superior performance as indicated by shorter latencies and distances to the trained spatial location. The memory facilitating effect of sugar became detectable at distinct times during training: on the circular hole board from the first trial onwards, whilst in the water maze on training days 4 and 5. Both the '0h and 4h-sugar'-rewarded mice kept their superior performance during the free exploration/swim trial as expressed by their more persistent search strategies for locating the exit hole or platform. We showed that a sugar reward given immediately after the training trials each day (0h-sugar) reinforced memory processes via enhancement of consolidation.

These findings support the integrative theory of reinforcement and memory advanced by Huston & Oitzl (1989). This is in particular the case for the circular hole board procedure, which provides a broader range of behavioral responses that can be studied. The experimental set-up of the circular hole board allows differentiation of learning and memory processes as well as detection of alterations in reward processes. Accordingly, we have used the circular hole board in the experiments described in **Chapters 6, 7** and **8** designed to determine the consequences of the sucrose award for behavioral performance tested our animal model of depression.

The effects of chronic stress studied in a variety of animal models are mainly assessed in short-lasting test-situations that have task-inherent features of novelty. Sometimes these situations even include exposure to physical stressors. In **Chapter 6** we reported the

impact of chronic stress on the daily organization of behavior in the familiar environment of the home cage, during and after cessation of the stressor in our animal model (i.e., the repeated and unpredictable exposure of mice to rats without physical contact). In addition, exploration of a novel environment was determined.

Continuous longitudinal observation revealed that 'rat stress' decreased exploratory and foraging activity as characterized by increased time spent in the shelter and less time spent in the open area. The brain reward mechanism was affected as indicated by reduced sucrose consumption and inhibition in sucrose preference development. Stressed mice used a more perservative strategy during exploration of a novel environment, whilst general locomotor activity was unaffected. Interestingly, already the control procedure, that includes spending the same amount of time in another cage without rat exposure, disrupted the organization of behavioral activity patterns, albeit to a lesser degree. In some aspects this was different than observed in rat-stressed mice.

The results support our notion that mice repeatedly exposed to rats might serve as a model of (human) chronic stress. Distinct behavioral changes in explorative and foraging activities, as well as the reduced response to a rewarding stimulus, suggest that negative changes in the reward system have occurred during chronic 'rat stress', in the context of changes in circadian CORT secretion. The loss of interest in pleasurable activities is known as anhedonia which is a hallmark, not only in individuals suffering from chronic stress exposure, but also of depression.

In **Chapter 7** we reported a combination of methodologies as described in the previous chapters, to determine whether the expression of anhedonia in our 'rat-stress' paradigm would be measurable using additional read-out parameters. Following cessation of the chronic stressor we assessed: learning and memory performance, facilitation of memory by reward, reward sensitivity, the emotional response and CORT levels.

It appeared that chronic 'rat stress' induced alterations in three domains of reward processing as indicated by (1) suppression of behavioral reactivity to novelty; (2) enhanced memory processes in response to sugar reward: spatial performance improved in control mice, whereas sugar reward "ameliorated" the impaired performance of stressed mice to the level of non-stressed controls without sugar; (3) increased sucrose and water intake: stressed mice that had received sugar post-training preferred to drink water at the location of prior sucrose consumption. Finally, the total CORT secretion during the light period of the day increased from day 1 to day 7, following the first week after 'rat stress'.

Taken together, chronic 'rat stress' altered the circadian CORT secretion pattern over time, impaired spatial memory and increased caloric intake. These alterations show, in addition to the previously observed diminished response to positive stimuli, that mice exposed to our chronic stress paradigm express anhedonia, which is supported by the changes in the three read-out parameters used. Sugar offered in the context of spatial learning partially rescued stress-induced, emotional and cognitive impairments. Collectively, these findings suggest that reward can ameliorate part of the negative consequences of chronic stress on memory processes.

Acute stress has been shown to modulate different memory systems to guide behavior in favor of caudate nucleus-dependent stimulus-response learning and memory at the expense of hippocampus-dependent spatial learning. In **Chapter 8,** a translational study was described where we examined in mice and humans, whether chronic stress has similar consequences as acute stress for the use of either one or both memory systems.

In our animal test, male C57BL/6J mice exposed to chronic 'rat stress' more often used a stimulus-response strategy than control mice for locating the exit hole on the circular hole board task. Thirty three percent of the stressed mice altered their strategy to stimulus-response or habit learning, while none of the control mice did; the controls all adhered to the spatial strategy. In the human test, forty healthy young men and women were divided into a "high chronic stress" and a "low chronic stress" group based on their answers posed in a questionnaire (the "Trier Inventory of Chronic Stress"-TICS) to identify symptoms of chronic stress. The subjects were trained in a 2D task where they had to remember the location of an object. We found that 94% of the participants of the "high chronic stress" group more often used the stimulus-response strategy, while this was the case only for 52% of the "low chronic stress" participants.

Chronic stress seemed to affect the quality of learning. This means that chronic stress affects *which* memory system is involved in the process of learning and *how* an individual learns. The induced shift towards a more rigid habit of stimulus-response learning strategy appears to be one of the consequences of chronic stress that can make an individual more vulnerable to the negative consequences, when exposed to additional stressors in the future.

As discussed in **Chapter 9** the following conclusions were reached

1) Our chronic stress model of *repeated and unpredictable* exposure of mice to rats proves to fulfill criteria of construct and face validity for depression.

2) The long lasting decrease in responsiveness to positive stimuli, which is considered indicative of anhedonia, served as presumed symptom of depression in our chronic stress model.

3) A history of chronic stress produces in both mice and men a shift towards a more rigid habit of stimulus-response learning.

4) Rigidity i.e., behavioral inhibition and habit learning, appears to be one of the consequences of chronic stress that can make an individual more vulnerable to the negative consequences of subsequent periods of stress.

5) The new methodology to reduce stress by either administration of the "anti-stress'' drug mifepristone or by providing positive and rewarding stimuli during behavioral testing, increases the well-being of the animals and may - in translational perspective - protect against depression.

6) The daily recurrent blockade of the GR with a very high dose of mifepristone may downregulate HPA axis activity because of the rebound surge of endogenous CORT. This subsequently mediates a negative feedback action in the face of a persistent limbic MR activation with concomitant changes in explorative behavior

7) Modification of the MR-GR balance may provide an interesting lead towards a novel class of antidepressants and/or antipsychotics.