Examination of the chronic mild stress-induced behavioral changes in the rat

abstracts of a Ph. D. Thesis

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Mood disorders and hence depression have long been in the focus of research due to their complex appearance and large prevalence in the population. These efforts, however, have been hindered by the difficulty to create appropriate animal models that mimic the human disorders and are suitable for testing newly developed drugs as well. Most of the animal models are based on the assumption that stress is one of the precipitators of depression. Many patients exhibit both anxiety and depressive symptoms leading some authors to the concept of a mixed anxiety-depression disorder. Another explanation of the comorbidity of anxiety and depression is a hypothesis of a sequential relationship, high anxiety being a risk factor for the occurrence of (other) mood disorders (Anisman 1982, Brown 1993).

Chronic exposure to a variety of mild unpredictable stressors (CMS) causes subsensitivity to rewards in rats, reported as a gradual decrease in consumption and preference for a palatable sucrose solution (Willner 1987). CMS also caused impairments in other measures of hedonic reactivity, such as preference conditioning (Benelli 1999, D’Aquila 1997, Valverde 1997), while it had no effect on aversive place conditioning (Papp 1996). This subsensitivity to rewards is claimed to be an analogous state of anhedonia, a core symptom of human depressive disorders (Willner 1987). Since stress is implicated in the etiology of depressive disorders (Anisman 1982, Lloyd 1980), the CMS paradigm has a reasonable construct validity. A number of physiological and behavioral alterations accompany the exposure to CMS. Both hypoactivity (Gorka 1996, Grippo 2003) and hyperlocomotion (Harris 1997, Gronli 2005, Mineur 2006) were observed in the open-field. Several research groups have used the degradation of the state of the fur of mice subjected to CMS, as a measure of depression-related behavior and demonstrated a reversal by clinically effective antidepressants (Griebel 2002, Pothion 2004). Sleep abnormalities, such as increased fragmentation and longer REM periods were also detected after exposure to CMS (Gronli 2004). A decrease in sexual activity and aggressive behaviors of mothers and males were reported (Pardon 2000, Gambarana 2001). Phase shifts in the circadian rhythms are also characteristic of the model (Gorka 1996).

In depressed patients, low mood may be associated with cognitive disturbances, especially in elderly patients, who are more sensitive to stressful events. Learning and memory impairments due to chronic mild stress were also reported in some recent studies (Song 2006). Numerous neurochemical changes were reported in both rats and mice (Bekris 2005, Song 2006, Gronli 2007). These results underpin the face validity of the chronic mild stress model.
Most of the behavioral and neurochemical changes induced by chronic mild stress can be reversed by chronic, but not acute treatment of clinically effective antidepressants of different classes (Moreau 1993, Papp 1996, Sluzewska 1996, Millan 2001), illustrating a high degree of predictive validity. Drugs known to be ineffective as antidepressants are not active in the model (Willner 2005).

Several research groups used behavioral assays involving reactivity to environmentally induced challenges to evaluate anxiety-like behaviors, an important element in depression. However, the messages of these studies examining anxiety-related behavior are rather contradictory. Some groups report anxiety-like (Griebel 2002, Maslova 2002), while others anxiolytic-like behavior induced by CMS (D’Aquila 1994, Kopp 1999, Ducottet 2003).

**Aims of the study**

- In order to enhance the construct validity of the CMS model we introduced a few modifications. The modified procedure was validated by measuring sucrose intake and preference.
- The effects of CMS were examined in various behavioral tests.
- We examined if there was any behavioral measure which correlated with the changes of the hedonic reactivity due to CMS.
- We examined short- and long-term effects of CMS on behavioral phenomena relevant to both anxiety and depression, in order to characterize time-course of behavioural changes.
- We examined the effects of chronic fluoxetine treatment and acute diazepam treatment on the altered behaviors after CMS.
- We also examined if CMS modified visceral sensations.

**Materials and methods**

Adult male Wistar rats (190-200 or 240-260 g) were used in all experiments. The chronic mild stress protocol consisted of two to three different stressors per day. Nociceptive stimuli and food or water deprivation were left out purposefully. Hedonic reactivity was examined in a two-bottle free-drinking test after a short-term deprivation of water. Behavioral changes were measured in the elevated plus-maze test (based on the work of Pellow 1985), in the
forced swim test (Porsolt 1978), in the social avoidance test (Haller 2002) and in the grooming test. Testing was conducted at different times after the termination of the stress procedure.

For the examination of the visceral sensations we used the two-bottle preference test and were monitoring the drinking behavior of rats. During the stress period animals were trained to make an association between a special taste and the discomfort induced by satiety. This association was tested in both the two-bottle and the one-bottle free drinking situations.

**Results**

- CMS applied in our experiments resulted in a similar decrease of sugar intake and preference as the original method, demonstrating that this intervention could induce anhedonia, too.
- Rats reduced their consumption of the sucrose solution parallel with a slower rate of body weight gain.
- CMS resulted in a low-anxiety profile in the elevated plus-maze test.
- CMS reduced activity in the Porsolt test, which might indicate a depression-like state.
- CMS induced social withdrawal in the social avoidance test.
- CMS-treated animals showed anxiety-like behavior in the grooming test.
- There was a positive, statistically significant correlation between sucrose intake and the avoidance behavior observed in the social avoidance test but not between sucrose intake and other measures.
- The effect of CMS was long-lasting in the social avoidance test, where we could detect a difference between CMS and control animals even after 21 days of rehabilitation from stress. A difference was measured after 10 but not 21 days in the grooming test.
- The elevated plus-maze the and Porsolt tests seemed to be less relevant for the examination of the time-course of the effects of CMS, due to the greater variability observed in these models.
- Chronic fluoxetine treatment had an antidepressant effect in the CMS-treated group in the social avoidance and the Porsolt tests. Diazepam was also effective in the social avoidance and the grooming tests, demonstrating the anxiety-generating character of the CMS-procedure.
We could not confirm our previous data concerning the negative alliesthesia induced by satiety in either control or CMS-treated animals. However, analysis of the data of the anhedonic subgroup of the CMS-treated animals showed a shift in the hedonic value of the taste associated with satiety-induced discomfort.

**Conclusions**

- The modified CMS protocol can induce anhedonia similarly to the original version. Food and water deprivation are not necessary.
- Sucrose preference may be a more reliable measure of hedonic reactivity than sucrose intake.
- One week of mild stress may be sufficient to provoke a decrease of sucrose consumption, but we consider a 3-week stress period as optimal.
- To our knowledge, this is the first study showing a statistically significant positive correlation between hedonic deficits and social withdrawal induced by CMS. If CMS represents an animal model of depressive states, and reduced sucrose preference is an indicator of depression in the model, then it is reasonable to assume that the behavioural changes that correlate with sucrose preference may also indicate depressive states. The reduction of social interactions in the social avoidance test may be explained as a parallel of social withdrawal in humans.
- No such correlation was found between the changes in hedonic reactivity and changes in other behaviors measured in our experiments, although CMS affected both anxiety-related and depression-like behaviours. In the lack of statistical correlations, we can not prove (nor confute) that the changes of the other behavioral measures are signs of a depression-like state.
- The effects of CMS on anxiety-related behavior was not only test dependent, but also time dependent. The social avoidance and the grooming tests seem to be more reliable when measuring the time-course of behavioral changes induced by CMS than the elevated plus-maze or the Porsolt tests. We, therefore, suggest that social avoidance – together with the decrease of sugar consumption – is probably the most valid measure of CMS-induced (maybe also any other) depressive state in rats.
- The social avoidance test and the Porsolt test seem to have stronger predictive validity when examining the effects of antidepressant drug treatment after CMS than the other behavioral tests. However, much additional research is needed in this field.

- The satiety experiments showed a similar phenomenon in the anhedonic subgroup of the CMS-treated animals to the one described earlier in isolation stress-treated rats. This finding may be considered as another evidence of the link between social deficits and depression.

- A shift in visceral sensation was observable in the anhedonic subgroup of the CMS-treated animals. Based on this result it is conceivable that we might be able to develop an animal model to mimic the non-specific symptoms of depressive states.

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