BEHAVIOURAL AND PHARMACOLOGICAL VALIDATION OF CHRONIC SOCIAL STRESS AS A MODEL OF DEPRESSIVE-LIKE SYMPTOMS IN RATS

Dissertation

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Contents

Abbreviations

5-HT	5 hydroxytryptamine, Serotonin
Ach	Acetylcholine
ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
BSA	Bovine Serum Albumin
CIT	Citalopram
CMS	Chronic Mild Stress
CNS	Central Nervous System
CPP	Conditioned Place Preference
CRF	Corticotrophin releasing factor
D	Dopamine
DA	Dopaminergic
DEX	Dexamethasone
DST	Dexamethasone Suppression Test
DZP	Diazepam
EDTA	Ethylenediaminetetraacetic acid
FLX	Fluoxetine
FST	Forced Swim Test
GABA	Gamma Amino Butyric Acid
HAL	Haloperidol
HPA	Hypothalamus Pituitary Adrenal axis
HPG	Hypothalamus Pituitary Gonadal axis
HPLC	High Performance Liquid Chromatography
hr	hour(s)
ICSS	Intra Cranial Self Stimulation
i.p.	intra peritoneal
LA	Locomotor Activity
LH	Learned Helplessness
L.C.	Locus Coeruleus
MD	Major Depression
MDD	Major Depression Disorder
min	minute(s)
NE	Noradrenaline

OB	Olfactory Bulbectomy
PBS	Phosphate Buffered Saline
PTSD	Post Traumatic Stress Disorder
RBX	Reboxetine
REM	Rapid Eye Movement
SSRI	Selective Serotonin Reuptake Inhibitor
SNRI	Selective Noradrenaline Reuptake Inhibitor
S.E.M.	Standard Error of Mean
UV	Ultra Violet
VTA	Ventral Tegmental Area

1 Introduction

Ever since the introduction of the concept of the "animal model" into pre-clinical research, various experimental models of stress-induced depressive symptoms have been used for the investigation of neurobiological mechanisms of psychopathologies and for the screening of new antidepressant drugs (Willner and Mitchell 2002). A great variety of stressors were used in these models; however, surprisingly little attention has been paid to the psycho- or socio-genic factors that are presumed to play an important role in many cases of human depressive disorders (Kessler, Price et al. 1985; Kessler 1997; Gilbert, Allan et al. 2002). Converging lines of evidence suggest that, in research aimed at the analysis of stress-related biomedical or psychopathological phenomena, the use of naturalistic psychosocial stressors may represent an advantageous research strategy (Kessler, Price et al. 1985; Gilbert and Allan 1998; Gilbert, Allan et al. 2002). The social stressors are more likely to elicit stress response patterns similar to those that in humans result from stressful events of everyday life (Bjorkqvist 2001). The present studies evaluated the effects of psychosocial stress on various behaviours in rats being behavioural correlates of depressive symptoms in human. The study focused on behaviours that may be reflective of motivational deficits and anhedonia as the former is one of the two fundamental characteristics of depression (the other being depressed mood), and is common to all subtypes of the disorder. Any model however needs to be evaluated in terms of its ability to simulate the human condition. Therefore an extensive pharmacological validation has been carried out.

The studies which have been here described were performed within the Deutsche Forschungsgemeinschaft (DFG) Center for Molecular Physiology of the Brain (CMPB); project "Behavioural and Pharmacological Effects of Antidepressant Drugs". The project was carried out in collaboration between Department of Psychiatry and Psychotherapy (U. Havemann-Reinecke, E. Rüther) at University of Göttingen and Clinical Neurobiology Laboratory (G. Flügge, E Fuchs) at German Primate Centre.

Before embarking on an analysis of the effects of psychosocial stress on hedonic and motivational processes in rats, it would seem opportune to revisit

the state of art in depression research and some of the difficulties encountered in the development of an animal model of a complex human disorder.

1.1 Depression

Mood disorders are among the most prevalent forms of mental illness. Severe forms of depression affect 2- 5% of population and up to 20% of the population suffer from milder forms of the illness. Depression is almost twice as common in females as males. Another roughly 1%-2% are affected by bipolar disorder (also known as maniac-depressive illness), which affects females and males equally. Mood disorders are recurrent, life threatening (due to the risk for suicide) and a major cause of morbidity worldwide (Blazer 2000).

Depression has been described by mankind for several millennia. The term melancholia (black bile in Greek) was first used by Hippocrates around 400 BC. Since the 1960s, depression has been diagnosed as "major depressive disorder" (MDD) based on symptomatic criteria set forth in Diagnostic and Statistical Manual (recent DSM-IV, 2000) see Table 1.

Diagnostic Criteria for Major Depression
Depressed mood
Irritability
Low self Esteem
Feelings of hopelessness, worthlessness, and guilt
Decreased ability to concentrate and think
Decreased or increased appetite
Weight loss or weight gain
Insomnia or hypersomnia
Low energy, fatigue or increased agitation
Decreased interest in pleasurable stimuli (e.g. sex, food, social interactions)
Recurrent thoughts of death and suicide
A diagnosis of major depression is made when a certain number of the above symptoms are reported for longer than a 2 week period of time, and when the symptoms disrupt normal social and occupational functioning (see DSM-IV, 2000)

Table 1. Diagnostic criteria for major depression.

Epidemiological studies show that roughly 40-50% of the risk for depression is genetic (Fava and Kendler 2000) However, non-genetic factors such as viral infections (e.g. Borna virus), stochastic processes during brain development and stress and emotional trauma, have been also strongly

implicated in the aetiology of depression (Akiskal, Bourgeois et al. 2000; Fava and Kendler 2000). The role of stress demands particular attention.

1.1.1 Role of stress in depression

Stressful life events have been reported to favour the evolution of depressive illness (Billings and Moos 1985; Brown, Bifulco et al. 1987; Cui and Vaillant 1996; Paykel 2001), and among dysthemic patients stressors may precipitate the emergence of major depressive episode (Griffiths, Ravindran et al. 2000). The impact of the stressors is dependent on upon characteristics of the stressor itself (e.g. severity, chronicity, predictability), coping ability and individuals stressor history (including early life trauma) (Anisman, Kelly et al. 2000; Kendler, Thornton et al. 2000; Paykel 2001). While depression is often precipitated by severe events (Paykel 2001), particularly psychosocial stressors (Monroe, Rohde et al. 1999) antecedents may also comprise a series of slight stressors (daily hassles) (Kanner, Coyne et al. 1981).

While it is commonly accepted that stressful events may either provoke depressive symptoms or exacerbate an already existent depression, debate continues as to whether affective illness stems from the neurochemical disturbances imparted by stressors, cognitive processes that may be set in motion, or a combination of the two. What is certain however is that there is a great number of factors that influence how the stressor will affect well being, and numerous variables that affect the degree to which a particular pathology will be engendered .

The constellation of factors will determine whether stressor favour the development of depressive illness. These can generally be characterised as those related to stressor itself (or individuals' interaction with the stressor), personality variables and orgasmic variables. To gain an appreciation of the impact of stressors, each of these needs to be considered.

A substantial body of evidence using state-of-art interview measures of episodic life events has found higher levels of significant stressors prior to the onset of major depressive episodes compared to controls (Mazure, Bruce et al. 2000). Mazure noted that stressors were 2.5 times more likely in depressed patients compared to controls and that in community samples 80% of depressed cases were preceded by major life events. Significant associations between prior stressors and depression have been confirmed by the genetic studies in twin pairs (Kendler, Gardner et al. 1999) and "natural" experiments

that occur when exposure to events is random and independent of depressive outcomes- such as widowhood and exposure to natural disaster (Kessler 1997). Overall, therefore the recent evidence is based on sound methods of stress assessment and novel designs strongly suggest that most episodes of major depression are preceded by stressful life events (although most people do not become depressed even if they experience a negative life events).

1.1.2 Neurobiology of depression

While many brain regions have been implicated in regulating emotions, we still have a very rudimentary understanding of the neural circuitry underlying normal mood and the abnormalities in mood that are the hallmark of depression. It is likely that many brain regions mediate the diverse symptoms of depression. This is supported by human brain imaging studies, which have demonstrated changes in blood flow or related measures in several brain areas, including regions of prefrontal and cingulated cortex, hippocampus, striatum, amygdala and thalamus to name a few (Liotti and Mayberg 2001). Anatomic studies of the brains of depressed patients have reported abnormalities in many of these same brain regions (Zhu, Klimek et al. 1999; Rajkowska 2000; Manji, Drevets et al. 2001). Knowledge of the function of these brain regions under normal conditions suggests the aspects of depression to which they may contribute. Neocortex and hippocampus may mediate cognitive aspects of depression such as memory impairments and feelings of worthlessness, hopelessness, guilt, doom and suicidality. The striatum (particularly the ventral striatum and nucleus accumbens) and amygdala and related brain areas are important in emotional memory, and could as a result mediate the anhedonia (decreased drive and reward for pleasurable activities), anxiety and reduced motivation that predominate in many patients. Given the prominence of so called neurovegetative symptoms of depression, including hyper or hyposomnia, changes in appetite as well as a loss of interest in sex and other pleasurable activities, a role of hypothalamus has also been speculated. Of course, these various brain regions operate in a series of highly interacting parallel circuits, which perhaps formulates a neural circuitry involved in depression (Figure 1).

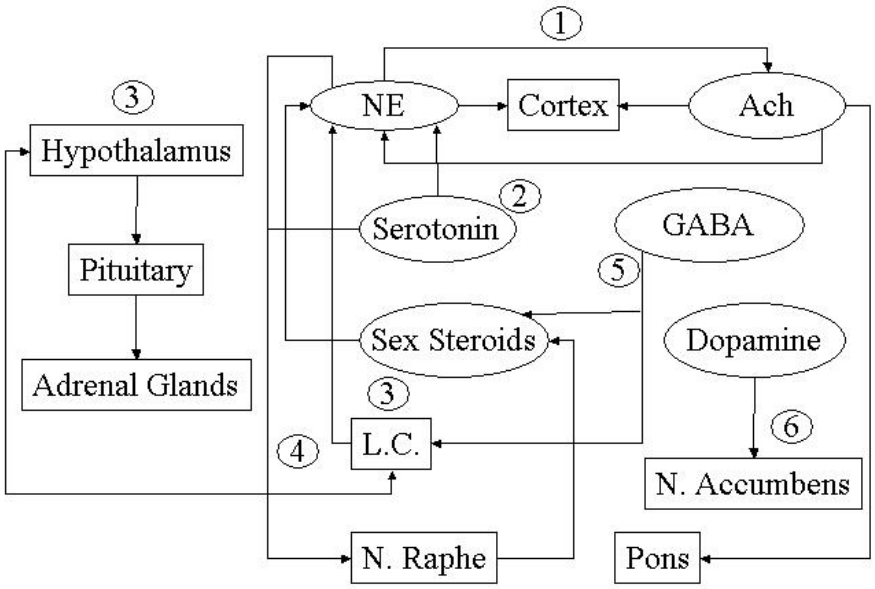

Figure 1. Neurotransmitter abnormalities and brain regions implicated in depression (numbers indicate important interactions).

1. Abnormalities in reciprocal activity of noradrenaline (NE) and acetylcholine (Ach) cause short REM latency and negative mood.
2. 2. Low levels of serotonin or lack of sensitivity to serotonin receptors linked to depression
3. Stress activates locus coeruleus (L.C.) and hypothalamus-pituitary-adrenal (HPA) axis causing release of epinephrine and ultimately depletion of NA
4. Serotonin and NE activity modulate activity of nucleus raphe, which in turn alters release of sex steroids possibly contributing factor in observed sex differences in depression.
5. Increase in GABA receptors generates a cascade of noradrenergic activity through L.C.
6. Reduction of dopamine activation of neurons in nucleus accumbens is related to the appearance of anhedonic behaviours.

There is also significant evidence for an enhanced activity of the hypothalamus- pituitary- adrenal (HPA) axis in MDD. This enhanced activity has been associated with a greater frequency of episodic release of cortisol, marked reductions in bone mineral density compared to matched controls and increased adrenal glands volumes. Evidence has also emerged that corticosteroid receptor function is impaired in many patients with major depression and in man healthy individuals at increased genetic risk for a depressive disorder (Holsboer 1999). Furthermore, clinical and pre-clinical data suggest that unrestrained secretion of corticotrophin releasing factor

(CRF) in CNS produces several signs and symptoms of depression through continuous activation of CRF receptors (Zobel, Nickel et al. 2000).

Monoamines have been the primary focus of the earlier etiological theories of MDD. Although the monoamine depletion hypothesis now seems to be oversimplified view of pathophysiology of MDD, one should acknowledge the therapeutic significance of the hypothesis. The putative role of serotonin (5-HT) in MDD has been extensively studied, partly because of the broad therapeutic effects in depression of drugs such as selective serotonin reuptake inhibitors (SSRI). Some, but not all studies have shown reduced endocrine responses to indirect or direct serotonin agonists. Post-mortem studies have shown both an increase in the density of 5-HT_2 receptor binding sites, and a decreased number of serotonin transporter binding sites (Owens and Nemeroff 1994) as well as an increase in the serotonin 5-HT_{1A} autoreceptors in the dorsal raphe of suicide victims with MD (Stockmeier, Shapiro et al. 1998). This evidence is supported by imaging studies which have evidenced widespread reductions in serotonin $5HT_{1A}$ autoreceptor binding (Sargent, Kjaer et al. 2000) and reduced density of serotonin transporter binding sites (Malison, Price et al. 1998).

Other neurotransmitter systems have also been investigated. Post-mortem studies have shown a selective increase in the high affinity conformation of the brain α_{2A}-adrenoceptors as well as decreased binding to norepinephrine transporters in locus coeruleus of depressed patients (Klimek, Stockmeier et al. 1997). The latter finding was interpreted as suggesting a compensatory down regulation of this transporter protein in response to an insufficient availability of norepinephrine at the synaptic level (Klimek, Stockmeier et al. 1997).

Janowsky and colleagues have suggested that the regulation of acetylcholine may also play a role in MDD. Cholinergic agonists cholinesterase inhibitors and acetylcholine precursors have all been shown to worsen mood in MDD (Janowsky, Risch et al. 1983; Duberstein, Conwell et al. 1993). Moreover depressed patients show a heightened response to muscarinic cholinergic agonists as evidenced by worsening of mood, anergia, papillary construction sleep and β endorphin release (Janowsky, el-Yousef et al. 1972; Dilsaver and Coffman 1989). Furthermore, abnormalities in levels of cortical choline an

acetylcholine precursor have been reported in several imaging studies (Charles, Lazeyras et al. 1994; Steingard, Yurgelun-Todd et al. 2000).

Despite the fact that the role of dopamine in depression has been studied extensively over the past several decades, the evidence of its involvement in MD is still discussed. In vivo receptor labelling studies have shown increased dopamine D2 binding in the right striatum of MDD patients, although a recent study has found higher striatal dopamine transporter density in major depression. The evidence aroused from animal models has been described in discussion section.

1.1.3 Treatment of depression

In contrast to our limited understanding of depression, there are many effective treatments. The large majority of people with depression show some improvements with any of several antidepressant medications and in case of lack of responsiveness, with electro convulsive seizures. In addition, several forms of psychotherapy (cognitive and behavioural) can be effective for patients with mild to moderate cases, and the combination of medication and psychotherapy can exert synergistic effect. The treatment of depression was revolutionised about 50 years ago, when two classes of agents were discovered- entirely by serendipity- to be effective antidepressants: the tricyclic antidepressants and the monoamine oxidase inhibitors. The discovery that depression may be treated with these medications provided one of the first clues into the types of chemical changes in the brain that regulate depressive symptoms. Indeed, much depression research over the last half century was based on the notion that understanding how these treatments work would reveal new insights into the causes of depression. The acute mechanisms of action of antidepressant medications were identified: inhibition of serotonin or norepinephrine reuptake by the tricyclic antidepressants and inhibition of monoamine oxidase by (a major catabolic enzyme for monoamine transmitters) by monoamine oxidase inhibitors (Frazer 1997) These discoveries led to the development of numerous second generation medications such as selective serotonin reuptake inhibitors (SSRI), and selective noradrenaline reuptake inhibitors (SNRI) which are widely used today. The mechanism of action of antidepressant medications is far more complex that their acute mechanisms might suggest. Inhibition of serotonin or noradrenaline reuptake or catabolism would be expected to result in enhanced

actions of these transmitters. However all available antidepressants exert their mood elevating effects only after prolonged administration (1-3 weeks or even longer) which means that enhanced serotonergic or noradrenergic neurotransmission per se is not responsible for the clinical actions of these drugs. Rather some gradually developing adaptations to this enhanced neurotransmission would appear to mediate drug action. Moreover there is still no fully convincing evidence that depression is primarily caused by abnormalities in the brain's serotonin or norepinephrine systems.

1.2 Behavioural paradigms for investigation of depressive symptoms in animals and screening of antidepressant activity.

The creation or discovery of animal models of psychiatric disorders, such as major depressive disorder, is fraught with many problems which are not encountered in the development of models in other areas of medicine. Some of these problems are the result of the apparent nature and complexity of the symptoms presented by the psychiatric patient. However other problems reflect philosophical positions from those based on the premise that primary depression does not fit the medical model and therefore is not a "disease" that can be modelled, to those holding that the emotional state of depression is so uniquely human that subhuman organisms cannot be used in its analysis.

In considering an animal model of depression, or any other malady seen in humans, it is critical to be clear of the goals of the model (McKinney 2001). The best animal model of disease is theory driven. In the case of depression, one could replicate in laboratory animals the etiological factors that cause depression in humans and consequently, many of the symptoms as well. A related approach is to model a disease mechanism in a laboratory animal and recreate particular features of the disorder. Both of these approaches have been used in recent years with considerable success for creating animal models of depression and to explore possible new medications. However many of the core symptoms of depression involve higher brain functions that we do not yet know with certainty how to model in animal (e.g. suicidal thoughts).

An alternative approach is to reproduce in laboratory animals particular symptoms of depression. These models can then be used to study the biological mechanisms that underlie those symptoms and to develop new

treatments that alleviate the symptoms. Most of the animal models being in widespread use today fall into this category. They induce in animals by use of a variety of stressful conditions, certain symptoms that are inferred to be "depression-like". The main limitation of these models is that they may poorly reflect mechanisms involved in the human situation. As a result, the biological basis of the animal symptoms may be different from the biological basis of the human symptoms and drugs that treat the former may not treat the later. While it is recognized that animal models of affective disorders may not be entirely congruent with the human condition, it is generally agreed that there are minimal criteria that must be met for animal model to be considered valid. These have been iterated in numerous reviews (Nemeroff, Kinkead et al. 2002; Newport, Stowe et al. 2002; Willner and Mitchell 2002) and include: (a) similarity in the symptoms profile (face validity), (b) amelioration or attenuation by treatments effective in treating the human condition, and conversely not be affected by those treatments that are ineffective in attenuating the human disorder (predictive validity), (c) provocation by events thought to be important in eliciting the human disorder (construct validity). At first sight, these fundamental criteria appear to be relatively straightforward. However fulfilling of these criteria may in fact meet multiple obstacles. Some of these are related to defining the syndrome that is being simulated, while others are aligned with the individual difference factors that influence the evolution of clinical symptoms.

Even though there are several intrinsic limitations, a number of animal models have been developed for depression and they are summarized in following paragraphs.

1.2.1 Forced Swim Test

The forced swim test, also known as Porsolt's test, is the most widely used animal paradigm in depression research, more specifically as a screen for antidepressant treatments (Lucki 1997). The test involves placing a rat or mouse in a tank filled with water and measuring the amount of time the animal is immobile- when stops struggling and swimming and begins to float, or the latency of become immobile. Acute or short term treatment with most antidepressants increases the latency of immobility and decreases the amount of immobility time (Porsolt, Anton et al. 1978). Although used mostly as an empirical test, one interpretation is that antidepressants may increase active

coping responses to swim stress. Indeed, an ongoing controversy is whether the forced swim test produces depression like symptoms in the animals or rather is merely a relatively acute testing protocol for detecting agents with antidepressant-like activity. False negatives in that test include drugs that are stimulants (and hence decrease immobility) but are not antidepressants. From the time of its introduction procedural iterations have improved the detection of pharmacologically diverse compounds and distinction of false positives. For example, measurement of other behaviours in the tank may enable more specific identification of antidepressants (e.g. SNRI may increase climbing behaviour whereas SSRI increase swimming) (Lucki 1997). A variant of the forced swim test used in mice is the tail suspension test (Cryan, Mombereau et al. 2005). Here mice are suspended by their tails and the time it takes an animal to become immobile (to hang passively upside down) is measured. Acute administration of most antidepressants decreases immobility. The major advantage of the FST is its relatively high throughput and ease of use. The test also provides insights to study the neurobiological and genetic mechanisms underlying stress and antidepressant responses (Porsolt 2000; Lucki 2001). There are disadvantages however. Antidepressants decrease immobility in the test even after single doses, despite the fact that clinical effects of these agents require administration for several weeks at least. Thus the test is sensitive to immediate effects of these agents and may not be picking up the true "mood-elevating" changes per se that these medication produce in the brain.

1.2.2 Learned helplessness

Learned helplessness (LH) is one of a relatively large number of tests that involve an animal's response to stress. In this paradigm some animals that are exposed to inescapable shock subsequently fail to escape from a situation in which escape is possible (Weiss and Simson 1988; Hitzemann 2000) Depending on the laboratory, learned helplessness is induced in 1 day or over several days of repeated exposures. Learned helpless animals show several neuro-vegetative changes that are reminiscent of depression such as REM sleep alterations, reduced body weight, diminished sexual behaviour and elevated CRF and corticosterone levels (Overmier 2002). Repeated administration of antidepressants reduces the latency to escape and decreases the number of animals that show learned helplessness. Antidepressant

treatment is also reported to reduce the various neurovegetative concomitants seen in these animals (Overmier 2002). The attractiveness of learned helplessness is that it is based on plausible theory that links cognitive function to visceral sequelae. Nevertheless the learned helplessness paradigm has shortcomings. It remains unclear to what extend learned helplessness is a better model of post traumatic stress disorder (PTSD) and other conditions in which stress is a clear etiological factor than of depression. A related point is that LH has typically involved relatively extreme regimens of stress which are outlawed in several countries. Although the use of extreme stresses may have been due to the need to obtain reliable and robust data, it may be counterproductive in terms of modelling depressive disorders.

1.2.3 Early life stress

Several models involving manipulation of early life environment have been used including prenatal stress, early postnatal handling and maternal separation (Caldji, Diorio et al. 2000; Ladd, Huot et al. 2000; Meaney, Diorio et al. 2000). The early life stress models produce neuroendocrine and behavioural changes in rats and mice that persist into adulthood. For example animals subjected to early stress show a hyperactive HPA axis as indicated by elevated CRF and glucocorticoid levels in response to stress. They also exhibit increased locomotor responses to novelty and, in some studies greater vulnerability to learned helplessness and drug self-administration. The models are generally good in terms of replicability and have been successfully used with a variety of species, from rodents to nonhuman primates. In addition many of the resulting abnormalities may be reversed by antidepressant treatments although negative reports have also appeared. On the other hand abnormalities of cognitive performance that persist into adulthood have been less reliable. Also despite the fact that early social stress produces robust social abnormalities in nonhuman primates, abnormalities in social behaviour including aggression have not been adequately examined in rodent models.

1.2.4 Chronic stress and chronic mild stress

The theoretical rationale for chronic mild stress model is that this procedure stimulates anhedonia, a loss of responsiveness to pleasant events, which is one of the core symptoms of depression and the defining feature of melancholia (DSM-IV) The fundamental finding on which the models are

based, and against which therapeutic effects of various drugs have been assessed, is that animals subjected to a variety of stressors show a decreased intake of palatable sucrose solution. The starting point was a series of studies by Katz and colleagues, published in early 1980s, in which rats were exposed sequentially to a variety of severe stressors. In most of these studies, the effects of stress were assessed b changes in open field behaviour, which were reversed specifically by chronic treatment with antidepressant drugs, but not by non-antidepressants (Katz and Hersh 1981; Katz, Roth et al. 1981; Katz 1982). In one of these studies it was observed that animals exposed to the chronic stress failed to increase their fluid consumption when saccharin or sucrose were added to their drinking water, and it was postulated that this might reflect a decrease in the hedonic impact of the sweetener (Katz 1982). This hypothesis was supported by Anisman and colleagues that uncontrollable foot shock can lead to impairments of behaviour maintained by brain stimulation reward. (Zacharko, Bowers et al. 1983; Zacharko, Bowers et al. 1984). In the chronic mild stress (CMS) model two major changes to the procedure described by Katz have been introduced: the severity of the stressors employed was greatly reduced and hedonic measures were made the primary focus of the model. In typical experiment rats (Willner, Muscat et al. 1992; Monleon, D'Aquila et al. 1995) or mice (Monleon, D'Aquila et al. 1995) are exposed sequently to a variety of mild stressors (e.g. overnight illumination, periods of water and/or food deprivation, cage tilt, change of cage mate) which change every few hours over a period of weeks or months. The effectiveness of this procedure is usually monitored by tracking, over repeated tests, a decrease in the consumption and/or preference for a palatable, weak (1-2%) sucrose solution. Also other behavioural endpoints have been studied including brain stimulation reward threshold and conditioned place preference (CPP) as well as variety of measures not directly related to reward sensitivity. The major disadvantage of CMS is its poor reproducibility. Both the behavioural abnormalities produced by chronic stress and palliative effects of antidepressants have been difficult to replicate across laboratories.

1.3 Social stress

As described in previous paragraphs, the impact of stressful events on the development of psychopathologies has been thoroughly investigated in

pre-clinical animal studies. These show that the kind of stressor, its duration, predictability and intensity produce different stress responses (Puglisi-Allegra, Kempf et al. 1991; Cabib and Puglisi-Allegra 1996). The most common stressors in man are of a psychological or social nature (Kessler, Price et al. 1985; Kessler 1997; Bjorkqvist 2001), and therefore using social conflict between members of the same species to generate stress has an obvious advantage over animal models that require aversive physical stimuli such as electric foot shock, restraint, water or food deprivation, or cold exposure. A number of studies have shown that temporal loss of social control (social defeat) is important factor that may lead to psychopathological changes (Bjorkqvist 2001; Fuchs and Flugge 2002). Social defeat in rats can be obtained in the resident-intruder paradigm (Tornatzky and Miczek 1994; Koolhaas, De Boer et al. 1997). In this paradigm, an adult male (the intruder) is introduced into the home cage of an unfamiliar, aggressive individual (the resident). The animals interact rapidly, fight and the intruder usually loses the encounter, see Figure 2.

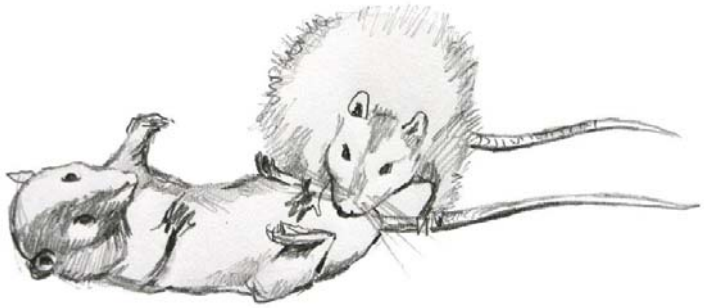

Figure 2. Social defeat in rats. Supine posture of the defeated animal is a typical submissive behaviour.

The experimenter terminates the interaction as soon as the intruder shows signs of submissive behaviour. This procedure minimizes injury while emphasizing the psychosocial component of the stress. In rats, social defeat by an aggressive male is a natural stressor, producing a variety of molecular, physiological and behavioural changes that are sometimes long lasting. These include decreased locomotor and exploratory activity (Meerlo, Overkamp et al. 1996; Koolhaas, De Boer et al. 1997); reduced aggression and sexual behaviour (McGrady 1984); increased submissive behaviour; and anxiety

(Ruis, te Brake et al. 1999). Moreover, social defeat alters the animal's sensitivity to subsequent challenges of other kinds of stress, impairs anticipatory behaviour (Von Frijtag, Reijmers et al. 2000) and induces cross sensitisation to psychostimulants (Kabbaj, Norton et al. 2001). Physiologically, the defeated animals show increased ACTH and glucocorticoid activity (Buwalda, de Boer et al. 1999; Buwalda, Felszeghy et al. 2001) altered circadian rhythms in heart rate, blood pressure and core temperature (Meerlo, De Boer et al. 1996; Sgoifo, Koolhaas et al. 1999), impaired immunological function and reduced resistance to diseases (Stefanski and Engler 1998; Engler, Dawils et al. 2004). Social defeat produces a variety of changes in neurotransmitter systems, including altered dopamine turnover in different brain areas (Isovich, Engelmann et al. 2001), change in GABA A (Miller, Thompson et al. 1987), glutamate (Krugers, Koolhaas et al. 1993) and 5-HT receptor binding (McKittrick, Magarinos et al. 2000), and has been shown to affect the opioid system (Miczek 1991; Coventry, D'Aquila et al. 1997).

Despite these findings, information is still very meagre about the effects in rodents, particularly rats, of chronic social defeat. Until now, the main effort has been devoted to the analysis of the effects of brief social stress, rather than chronic stress, as a main factor leading to stress related pathologies.

1.4 Aim of the study

The aim of present study, therefore, was to obtain information on the behavioural and physiological changes in rats evoked by chronic social stress.

The objectives of this thesis are:

-Development of the chronic social stress paradigm in rats

-Evaluation of the behavioural effects of chronic social stress in rats.

-Assessment of predictive validity of the chronic social stress paradigm in rats as a model of depression.

The working hypothesis stated that the chronic psychosocial stress evokes in rats behavioural changes that may be considered as behavioural correlates of human' depressive symptoms and that some/all of these changes may be alleviated specifically by antidepressant treatment. In other words, that chronic psychosocial stress paradigm in rats is a valid model for studying depressive like symptoms with construct, face and predictive validity.

The hypothesis was tested by asking the following questions:

Does the chronic social stress in rats evoke behavioural changes that may be considered as depressive-like symptoms?

Do treatments with different classes (SSRI, SNRI) of antidepressant drugs reverse behavioural changes induced by chronic social stress in rats?

Does the treatment with an anxiolytic drug reverse the stress-induced changes?

Does the treatment with a neuroleptic drug reverse the stress induced changes?

The aim of the first study, therefore, was to develop the chronic social stress paradigm in rats and to obtain information on the behavioural and physiological changes in rats evoked by chronic social stress. The rats were subjected to social defeat on a daily basis for five weeks and, in parallel, evaluated using a battery of behavioural tests to reveal changes evoked by stress-induced, psychological overload. The choice of behavioural tests (forced swimming, sucrose preference, open field) was prompted by the growing body of evidence (Koolhaas J.M. 1990; Von Frijtag, Van den Bos et al. 2002) that social stress can induce depressive-like symptoms in rats similar to those seen in humans.

The following studies were designed for the pharmacological validation of chronic psychosocial stress as a model of depression. For this, rats were subjected to chronic social defeat, as described previously (Rygula, Abumaria et al. 2005) and in parallel, treated for a period of four weeks with the antidepressant drugs; citalopram (CIT), fluoxetine (FLX) and reboxetine (RBX). These drugs, being highly selective serotonin and noradrenaline reuptake inhibitors, are broadly used for the treatment of depressive symptoms in humans (Montgomery and Djarv 1996) and has been shown already to be effective in the animal models of human psychopathologies (Sanchez and Meier 1997; Page 2003). The drugs were given in orally in drinking water to minimise physical stress factors that might be induced by daily injections. A pilot drug monitoring study was performed to determine the doses of CIT and RBX that lead to plasma concentrations of the drugs themselves and their metabolites similar to those in human patients receiving clinically effective doses. The effects of chronic antidepressant treatment were investigated in behavioural paradigms aimed at revealing antidepressant

activity, such as the sucrose preference test, forced swimming test and open field test.

Two additional studies were performed to validate the model by checking the specificity of antidepressant treatment in reversing stress induced changes. In one the animals were treated chronically with neuroleptic drug haloperidol (HAL). Neuroleptics, broadly used in the treatment of schizophrenia are sometimes used in the management of depression, but their efficacy is mainly established in delusional depression (Nelson-Gray 2003). In fact, depression as a side effect of the therapy with typical neuroleptics (Randrup and Munkvad 1975; Siris, Bermanzohn et al. 1991) and antidepressant effects on withdrawal of neuroleptics (Randrup and Munkvad 1975; Del Zompo, Bocchetta et al. 1986) are both well documented. The optimal dose for oral administration in rats has been determined previously in studies by (Schmitt, Dahmen et al. 1999). Several reports from clinical studies indicated also that depression is often associated by elevated levels of anxiety. Therefore in the last study the animals were treated with anxiolytic drug diazepam (DZP) in order to investigate the fear components of the chronic social stress model. Diazepam due to its sedative and addictive properties and in order to avoid tolerance was administered acutely.

2 Experimental procedures and methods

2.1 Animals

Male *Wistar* rats from (Harlan-Winkelmann, Brochen, Germany) weighing 180-200g at the time of arrival were used as intruders. They were housed individually in macrolon cages (type III) with rat chow and water available *ad libitum*. The colony room was maintained at a temperature of 21±1 °C and on a reversed 12h: 12h light/dark cycle (lights on at 22:00). After arrival, animals were habituated to the housing conditions for a period of 2 weeks and handled daily. All experimental manipulations were conducted during the dark phase of the light/dark cycle under dim red light. *Lister Hooded* male rats, weighing 300-350g (Harlan-Winkelmann, Brochen, Germany) were used as residents. Rats were pair housed with age-matched sterilized females in plastic cages (60 x 40 x 40 cm = l x w x h) located in a separate room. Housing conditions were the same as for the *Wistar* rats. Animal experiments were conducted in accordance with the European Council Directive of November 24, 1986 (86/609/ECC) and were approved by the local authority for laboratory animal care and use (Government of Lower Saxony, Germany). The minimum number of animals required to obtain consistent data was used.

2.2 Induction of social stress

A modified resident-intruder confrontation procedure was used to induce daily social stress in male rats. In brief, as described recently (Miczek 1991; Rygula, Abumaria et al. 2005), before the start of the social defeat procedure, the female resident rats were removed from the cages. Male confrontation started in the middle of the active (dark) period by transferring each intruder male *Wistar* rat into the cage containing an unfamiliar male *Lister Hooded* resident rat. Usually within first 1-3 min, the intruder was attacked by the resident and defeated. As soon as the intruder showed clear freezing behaviour and submissive postures he was separated from the resident, transferred into a small wire-mesh compartment (25 x 15 x 15 cm) and kept in the resident's cage. Thus, the intruder was protected from direct physical contact, but remained in olfactory, visual and auditory contact with the resident. After one hour intruders were returned to their home cages. Animals from the stressed groups were subjected to social defeat daily for five

weeks. Using a daily rotation system, intruders were exposed every day to one of 18 different resident males. Control animals were handled daily throughout the entire experiment. Handling comprised picking up each rat, transferring to experimental room and returning it to its home cage. Experimental males were randomly assigned to intruder (stress) or control groups.

2.3 Experimental designs

2.3.1 Experiments with social stress

After arrival, animals were habituated to the housing conditions for a period of 2 weeks and handled daily. Handling comprised picking up each rat, transferring it to the experimental room and returning it to its home cage. First behavioural tests (baseline) were performed at the end of this period. After this pre-stress (baseline) period the animals were divided into two groups; control and stressed. The animals from stressed groups were subjected to social stress daily for a period of 5 weeks. The animals from control groups were handled daily throughout the entire experiment. All experimental manipulations were conducted during the dark phase of the light/dark cycle under a dim red light. The experimental design is demonstrated in Figure 3 and described in the following sections.

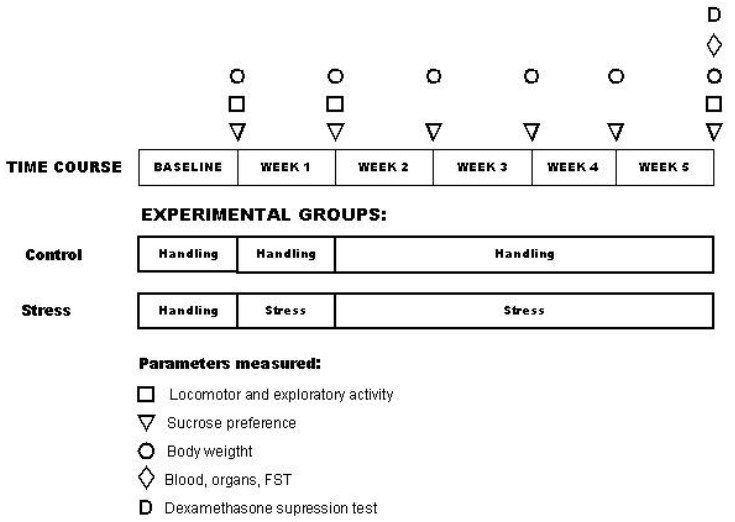

Figure 3. Experimental design and behavioural parameters measured during the experiments. The tests were performed at the end of each week. Forced Swim Test (FST) was performed 24 h after the last defeat. In experiment with dexamethasone, the drug was applied at the end of the stress phase (after week 5)

2.3.2 Experiments with pharmacological treatments

After arrival, animals were habituated to the housing conditions for a period of 2 weeks and handled daily. Handling comprised picking up each rat, transferring it to the experimental room and returning it to its home cage. First behavioural tests (baseline) were performed at the end of this period. After this pre-stress (baseline) period the animals were divided into four groups; control, control drug treated, stressed and stress drug treated. The animals from stressed groups were subjected to social stress daily for a period of 5 weeks. The animals from control groups were handled daily throughout the entire experiment. The animals from drug treated groups (CIT, RBX and HAL) were given the drug in their drinking water or via forced oral ingestion (FLX) for a period of four weeks. The treatment started after one week of stress (week 1) and was maintained till the end of the experiment (week 5). The animals treated with DZP received the drug only once; before the behavioural tests at the end of the experiment via i.p. injection. All experimental manipulations were conducted during the dark phase of the light/dark cycle under a dim red light. The experimental design is demonstrated in Figure 4 and described in the following sections.

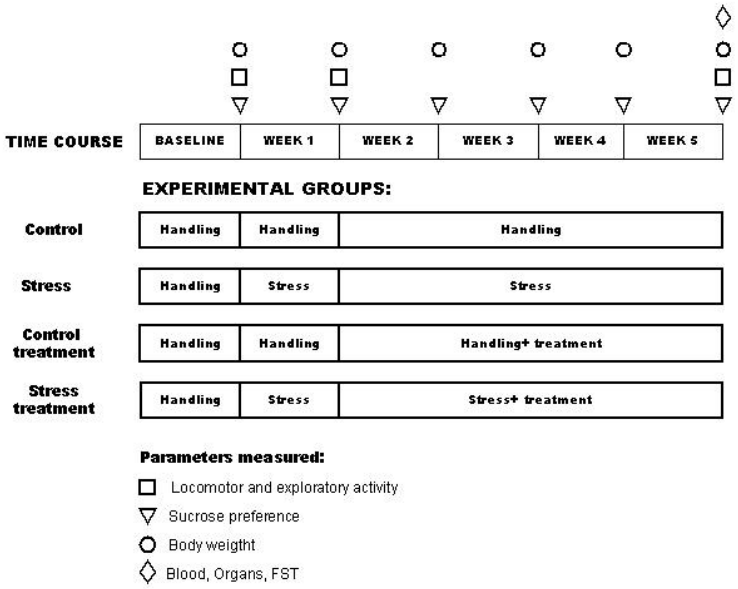

Figure 4. Experimental design and behavioural parameters measured during the experiments. The tests were performed at the end of each week. Forced Swim Tests (FSTs) were performed 24 h after the last defeat. In experiment with DZP, the drug was applied acutely at the end of the stress phase (week 5)

2.4 Physiological parameters

2.4.1 Body weight

Body weight was measured regularly at 12:00, first on at the end of control phase (baseline) then at weekly intervals (weeks 1, 2, 3, 4, and 5) during the stress phase. Body weight gain was calculated as a percentage of individual, baseline body weight at the beginning of experiment. Baseline values were transformed to the percentages of mean value from this time point.

2.4.2 Organ weights

At the end of the experiment, animals were sacrificed, the adrenals, and testicles were dissected, cleaned, weighed and the organ weights calculated as a percentage of body weight.

2.4.3 Dexamethasone suppression test

As shown in Table 2, male *Wistar* rats were divided into 2 groups: control and stressed. The stressed group of animals was subjected to 5 weeks of daily social defeat. Control animals were handled daily throughout the entire experiment. Handling comprised picking up each rat, transferring to experimental room and returning it to its home cage. At the end of the stress phase all animals were divided into 6 subgroups: control baseline, control after 3 hr, control after 6hr and stressed baseline, stressed after 3hr, stressed after 6hr (N=4 each). Samples for basal corticosterone levels were taken just before the onset of the dark phase of D/L cycle (09:00) from control baseline and stressed baseline groups. Dexamethasone (0,05 mg/kg (s.c.) (Cole, Kim et al. 2000) challenge was performed 21 hrs later. Blood samples were collected by decapitation, 3 (control 3hr, stress 3hr) and 6 hrs (control 6hr and stress 6hr) after the injection of DEX (Cole, Kim et al. 2000; Groenink, Dirks et al. 2002; Boyle, Brewer et al. 2005). Trunk blood samples of 300 µl were collected in EDTA-coated capillary system tubes and centrifuged. The plasma is stored at −20°C until corticosterone levels were assayed.

		Experimental Groups (N=4 each)	
Time course	**Procedure**	**Control group**	**Stress group**
Day 1, time: 0900	Baseline blood sample	Control baseline	Stressed baseline
Day 2, time: 0600	DEX injection	Control 3hr and 6hr	Stressed 3hr and 6hr
Day 2, time: 0900	Sample 1	Control 3hr	Stressed 6hr
Day 2, time: 1200	Sample 2	Control 3hr	Stressed 6hr

Table 2. Experimental design for dexamethasone suppression test. The test was performed after 5 weeks of chronic social stress.

2.4.4 Corticosterone

For determination of corticosterone, serum samples were directly assayed without extraction (Vahl et al., 2005). Specifically, 25µl of serum were diluted 1:40 with assay buffer (PBS, containing 0.1% BSA, pH 7.0) and duplicate 50µl aliquots of diluted samples were assayed by enzymeimmunoassay, as described by Goymann et al. (Goymann, Mostl et al. 1999) and (Steinmetz, Kaumanns et al. 2006). Intra- and interassay coefficients of variation for high and low value quality controls were 7.2% and Y% (high) and 8.5% and Y% (low), respectively.

2.4.5 Testosterone

For determination of testosterone, 100 µl serum was two times extracted with 1 ml of diethylether by vortexing for 10 min. Following extraction, the combined ether phases were evaporated under a stream of N_2 and dried extracts reconstituted in 250 µl assay buffer (PBS, containing 0.1% BSA, pH 7.0). Duplicate 50µl aliquots of reconstituted extracts were then measured for concentrations of testosterone by enzymeimmunoassay as described in detail by Kraus et al. (Kraus, Heistermann et al. 1999). Intra- and interassay coefficients of variation for high and low value quality controls were 6.4% and Y% (high) and 8.1% and Y% (low), respectively.

2.5 Behavioural tests

2.5.1 Open field test

Automated recording of locomotor activity was performed with the Opto-Varimex-3 Activity Meter (Columbus Instruments, Ohio, USA) equipped with a standard open, plexiglas arena (40 x 40 x 20 cm). Animal movement was recorded with infrared sensors positioned 3 cm above the floor, as described previously (Magnus-Ellenbroek and Havemann-Reinecke 1993). Each animal was placed in the centre of the experimental apparatus immediately before the test and allowed to explore it for 10 min. During this time, locomotor activity was automatically recorded and elements of exploratory activity (rearing, sniffing) scored by observation and counted. These two parameters were defined as follows: rearing, standing on hind legs with paws pressed against the wall of the arena; sniffing, continuous sniffing for at least 2 sec, as described previously (Rygula, Abumaria et al. 2005). The arena was cleaned between each test. In order to investigate the effects of sub-chronic and chronic stress and the effects of pharmacological compounds, rats were tested at the end of the control phase (baseline), after one week of stress (week 1) and at the end of the experiment (week 5).

2.5.2 Elevated plus maze test

The elevated plus-maze is a widely used and extensively validated animal model of anxiety based on the natural aversion of rodents for open spaces and on the elevation of the maze (Handley and Mithani 1984; Pellow, Chopin et al. 1985).The measurement of anxiety behaviour was performed using an automated elevated plus maze system (TSE Systems, Bad Homburg, Germany) The elevated plus-maze consisted of two open arms (425 × 145 mm) and two enclosed arms (425 × 145 × 225 mm), which all extend from a common central platform (12 × 12 cm). The configuration formed the shape of a plus sign, with similar arms arranged opposite to each another, and the apparatus was elevated 60 cm above the floor on a central pedestal. The maze was made from black Plexiglas. The investigation room was brightly illuminated. For each rat a 5-min trial was performed, and the maze was cleaned between subjects. The automated recordings were performed by use of light beams in the walls of the apparatus and PC with TSE software.

2.5.3 Sucrose preference test

The sucrose preference test was performed in one week intervals (starting from baseline) throughout the entire experiment. During this test, rats were given a free choice between two bottles for 24 hrs, one with 0.8% sucrose solution and another with tap water. To prevent the possible effects of side preference in drinking, the position of the bottles was switched after 12 hrs. No water or food deprivation was applied before the test. The consumption of water and sucrose solution was measured by weighing the bottles. The preference for sucrose was calculated as the percentage of consumed sucrose solution of the total amount of liquid intake. During the tests, drugs were dissolved in both water and sucrose containing bottles.

2.5.4 Forced swimming test

The forced swimming test follows the method described by Porsolt (Porsolt, Anton et al. 1978). Twenty-four hours after the final social defeat, the animals were individually placed into glass cylinders (40 cm height; 18 cm diameter) containing 18 cm of water at 23 °C. After 15 min, they were transferred to a 30 °C drying environment for 30 min (pre-test). The animals were returned to the cylinder 24 hrs later for 5 min (test), and this session was recorded with a video camera. Fresh water was used for each rat and the cylinder cleaned. Experiments were performed between 12:00 and 16:00. An experimenter observed the videotapes, unaware of the treatment received by the animals and immobility time was measured. A rat was considered immobile when floating and making only the necessary movements to keep its nostrils above the water surface.

2.5.5 Drugs

Citalopram hydrochloride (Lundbeck A/S, Copenhagen, Denmark) was dissolved in water so as to result in three doses 10, 20 and 40 mg/kg/day for the drug monitoring study and 40 mg/kg/day for the stress experiment. Reboxetine (Edronax®, Pharmacia GmbH, Erlangen, Germany) was dissolved in water so as to result in doses 20, 40 and 80 mg/kg/day for the drug monitoring study and 40 mg/kg/day for the stress experiment. Haloperidol (Haldol ratiopharm®, Ratiopharm GmbH, Ulm, Germany) was dissolved in water so as to result in the dose of 2 mg/kg/day. The drugs were given in drinking water, with the controls receiving tap water. Fluid intake was

monitored for 7 days before prior to and throughout the entire experiment. Bottles were weighed always at the same time of the day (12:00). Since for a 250g rat the average water consumption during the initial period was approximately 30 ml/day, the drugs were dissolved in water at concentrations of 0.0166, 0.166, 0.332 and 0,664 mg/ml to approach the target doses of 2, 20 40 and 80 mg/kg respectively. Body weight was recorded in one week intervals for the dose adjustments.

Animals treated with fluoxetine received the compound (10 mg/kg body weight per day) orally between 15:00 and 16:00 hr. The drug (Fluoxetin ratiopharm®, Ratiopharm GmbH, Ulm, Germany) was administered via a bulb-headed cannula into the bucal cavity and the animals were allowed to swallow the solution. This dose has been demonstrated to be effective as antidepressant by previous studies(Kirby and Lucki 1997; Page, Detke et al. 1999). Control animals were treated with vehicle only.

Diazepam (Diazepam ratiopharm®, Ratiopharm GmbH, Ulm, Germany) due to its sedative and addictive properties and in order to mimic clinical situation, was administered acutely in the dose 1 mg/kg of body weight. The drug was injected i.p., 30 minutes before the behavioural tests after 5 weeks of stress. In case of sucrose preference test, DZP was injected 3 times, in 4 hr intervals, during the first 12 hr of the test performed after 5 weeks of stress.

2.5.6 Pilot study- drug monitoring

For CIT and RBX, drug monitoring was performed in a pilot study using separate groups of animals. In the first study male Wistar rats (N=15) were divided into 3 groups. Each group (N=5) received CIT for 5 days in doses 10, 20 and 40 mg/kg/day, respectively. In case of the study with RBX the doses of the drug were: 20, 40 and 80 mg/kg. The drugs were administered via drinking water, as described above. The amount of consumed water was measured daily by weighing the bottles, for 3 days before the start of the treatment, and then continued for the following 5 days. This procedure allowed adjusting the applied dose of dissolved drugs to the individual water consumption and body weight of each animal. Animals were weighed daily. On the fifth day, at 12:00, animals were decapitated, and trunk blood samples taken. Blood samples were stored in heparinized standard laboratory tubes. Blood was centrifuged for preparation of plasma which was stored frozen (-20^{o}

C) until assayed for drug concentrations. Additionally, plasma levels of all the drugs except DZP were measured after chronic treatment at the end of the main experiments:

The doses of the remaining drugs had been chosen upon available literature data (Schmitt, Dahmen et al. 1999) (Schmitt and Hiemke 1998)

2.5.7 Analysis of the drugs and their metabolites

Citalopram and its N-demethylated metabolite dCIT as well as FLX and its metabolite norFLX were determined in blood plasma by high performance liquid chromatography (HPLC) with column switching and spectrophotometric detection as described previously for the antipsychotic drug amisulpride (Sachse, Hartter et al. 2003) with slight modifications. Plasma (0.1 ml) was injected into the HPLC system. For on-line sample clean-up on a column (10 x 4.0 mm i.d.) filled with LiChrospher CN material of 20 µm particle size (MZ-Analysentechnik, Mainz, Germany) the column was washed with deionised water containing 8% (V/V) acetonitrile to remove proteins and other interfering compounds. Drugs were eluted and separated on LiChrospher CN material (5 µm; column size 250 x 4.6 mm i.d., MZ-Analysentechnik) using 50% (V/V) acetonitrile and phosphate buffer (8mM, pH 6.4) and quantified by ultraviolet (UV) spectroscopy at 210 nm. HPLC analysis of a single sample was completed within 20 min. Each analytical series included at least two control samples containing a low or a high concentration of CIT and demethylCIT or FLX and norFLX respectively. There was linear correlation between drug concentration and UV signal from 5 to at least 250 ng/ml. The limit of quantification was 3 ng/ml. The intra- and inter-assay reproducibility of quality control samples was below 10%.

A fully automated method including column-switching and isocratic high-performance liquid chromatography (HPLC) was applied for quantitative analysis of RBX. After serum injection into the HPLC system and on-line sample clean-up on a silica C8 (10x4.0 mm I.D.) clean-up column with an eluent consisting of 2.5% acetonitrile in deionized water, the chromatographic separation was performed on an analytical column (Lichrospher CN; 250x4.6 mm I.D.) with an eluent of acetonitrile-aqueous potassium phosphate buffer (0.008 M, pH 6.4) (50:50). The UV detector was set at 273 or 226 nm. The limit of quantification was about 15 ng/ml at 273 nm and about 4 ng/ml at

226 nm. The day-to-day relative standard deviation ranged between 2.7 and 6.7% with recovery rates > or = 90%.

Serum concentrations of HAL were determined by radioligand binding assay following the method described by (Browning, Harrington et al. 1985).

2.6 Statistical analysis

The data was analysed using Graph Pad Prism version 4.0. Immobility time in the forced swimming test and weight of the adrenals were analysed using an independent sample's t-test or analysis of variances (ANOVA) in case of 4 groups experimental design. Sucrose tests and body weight gain were analysed using two factorial (stress vs. control) x (baseline, 1, 2, 3, 4, 5) ANOVA for repeated measurements. One factorial ANOVA was used for analysis of sucrose preference test in experiment with DZP. Motility and exploratory activity were analysed using two factorial (stress vs. control) x (baseline, week 1, week 5) repeated measures analysis of variances (ANOVA). To detect significant differences among the experimental groups and days, ANOVAs were supported by the Bonferroni post-hoc tests.

3 Results

3.1 Effects of chronic psychosocial stress

3.1.1 Locomotor and exploratory activity

Stressed rats had significantly less locomotor activity (counts/10 min) after one ($p < 0.001$ compared to baseline and $p < 0.05$ compared to controls) and five weeks of stress ($p < 0.001$ compared to baseline and $p < 0.05$ compared to controls. A significant ($p < 0.01$ compared to baseline) decrease in locomotor activity was observed also in control animals after five weeks of experiment (Figure 5A). Two-way ANOVA revealed significant effects of stress [$F(1,28)= 10.19$, $p < 0.01$], time [$F(2,28)= 34.66$, $p < 0.001$] and significant stress x time interaction [$F(2,28)= 7.54$, $p < 0.01$].

Stressed rats had significantly decreased frequency of rearing (counts/10 min) after one ($p < 0,001$ compared to baseline and $p < 0.05$ compared to controls) and five weeks of stress ($p < 0,001$ compared to baseline and $p < 0.01$ compared to controls). No significant differences were observed in control animals (Figure 5B). Two-way ANOVA revealed significant effects of stress [$F(1,28)= 15.54$, $p < 0.01$], time [$F(2,28)= 6.56$, $p < 0.01$] and significant stress x time interaction [$F(2,28)= 9.68$, $p < 0.001$].

Stressed rats had significantly decreased frequency of sniffing (counts/10 min) after one ($p < 0,001$ compared to baseline and $p < 0.05$ compared to controls) and five ($p < 0,001$ compared to baseline and $p < 0,001$ compared to controls) weeks of stress. No significant differences were observed in control animals (Figure 5C). Two-way ANOVA revealed significant effects of stress [$F(1,28)= 16.45$, $p < 0.01$], time [$F(2,28)= 28.05$, $p < 0.001$] and significant stress x time interaction [$F(2,28)= 19.76$, $p < 0.001$].

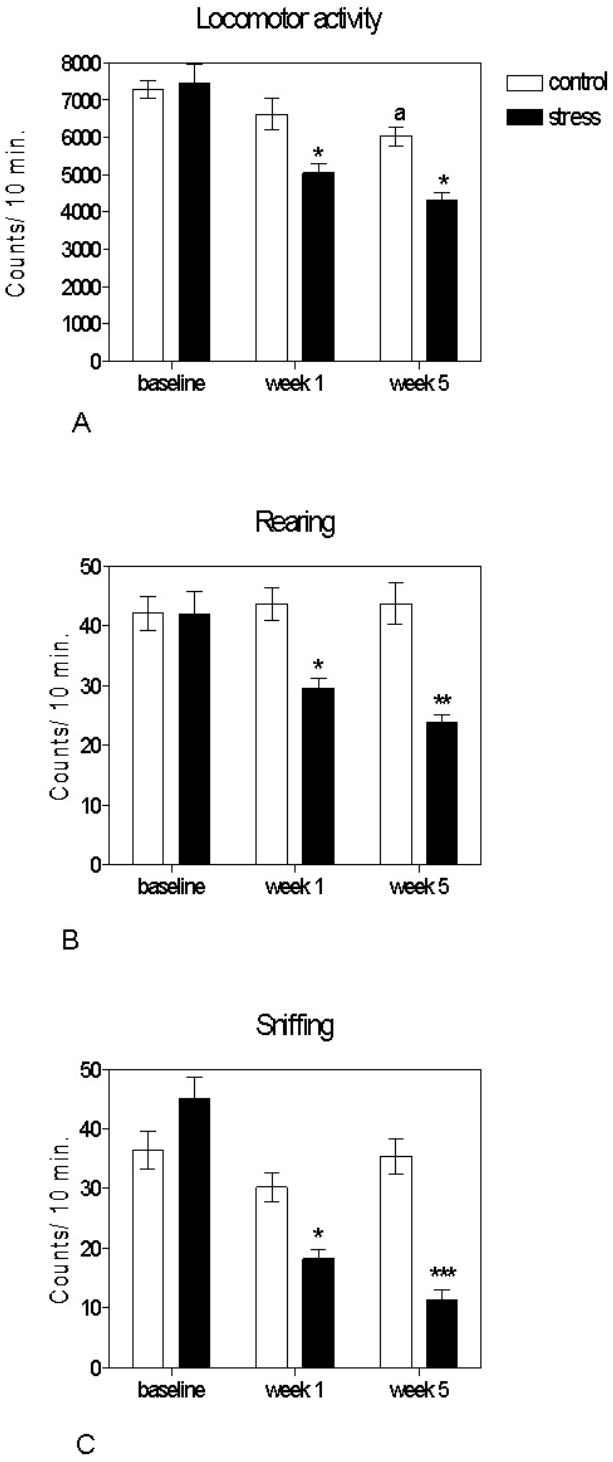

Figure 5. Effects of one and five weeks of social stress on locomotor and exploratory activity; (A) Locomotor activity. Data represent the mean values ± S.E.M. (counts of light beams interruption per 10 min); (B) Frequency of rearing behaviour. Data represent the mean values ± S.E.M. (counts of rearing per 10 min); (C) Frequency of sniffing behaviour. Data represent the mean values ± S.E.M. (sniffing counts per 10 min). All data from control (n= 8) and stressed (n= 8) animals; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. control group, $^{a}p < 0.05$ vs. baseline (Bonferroni test).

3.1.2 Sucrose preference test

Following the control phase (baseline) and after one week of stress, both groups of animals (stress and control) had a similar preference for sucrose solution (Figure 6). Two weeks of stress reduced this preference in stressed animals, which reached statistical significance after three weeks of stress, $p < 0.01$) and persisted until the end of the experiment (week 4, $p < 0.05$ and week 5, $p < 0.05$). Two-way ANOVA revealed significant effects of stress [$F(1,70)= 29.91$, $p < 0.0001$], time [$F(5,70)= 3.42$, $p < 0.01$] and significant stress x time interaction [$F(5,70)= 5.66$, $p < 0.001$].

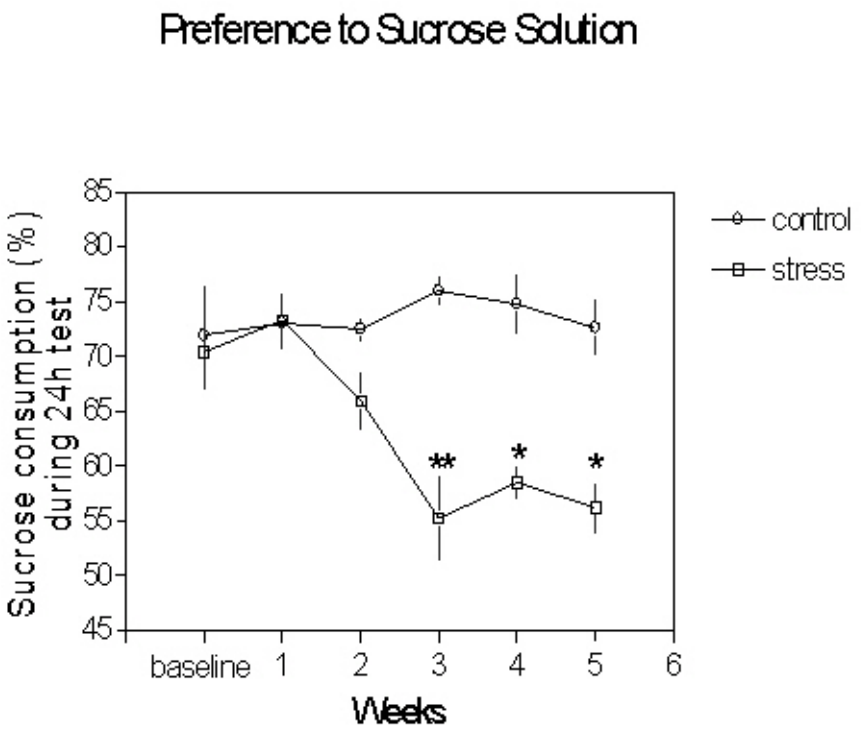

Figure 6. Sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 hrs. Data represent the mean values ± S.E.M. from control (n= 8) and stressed (n= 8) animals; *p < 0.05 and **p < 0.01 (Bonferroni test).

3.1.3 Forced swimming test

As shown in Figure 7, socially stressed rats spent a significantly longer time immobile (211.9 ± 8.2 s) than did the control rats (149.3 ± 3.8s), [t(14)=6.908, p < 0.001].

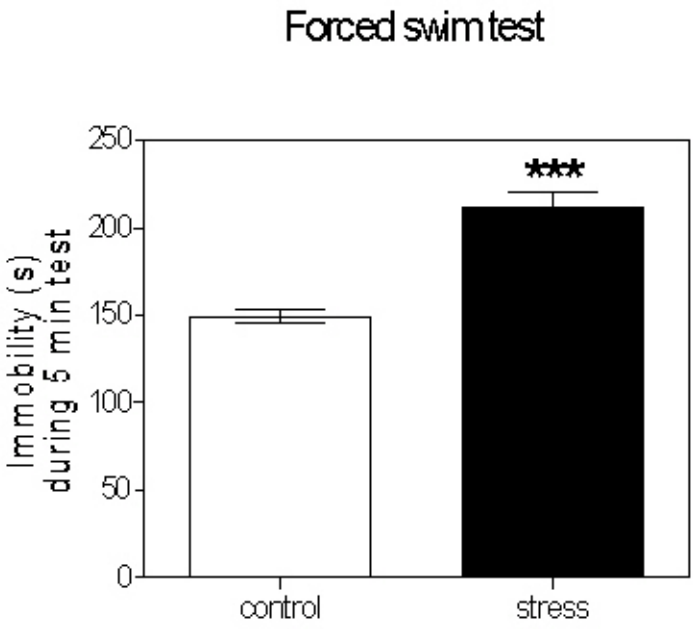

Figure 7. Effect of chronic social stress determined in the forced swimming test: Immobility time (s). Data represent the mean values ± S.E.M. from control (n= 8) and stressed (n= 8) animals; ***p < 0.001 (t-test). Animals were tested at the end of the experiment. Data are shown as a time spent immobile during 5 min test.

3.1.4 Body weight, organs and hormones

Stressed rats gained less body weight than did the control rats (Figure 8). Statistical analyses revealed a significant effect of the stress [F(1,70)= 7.86, p < 0.05] and significant stress x time interaction [F(5,70)= 9.92, p < 0.001]. Subsequent Bonferroni post hoc tests confirmed significant (p < 0.01) reduction in body weight gain in stressed animals after 3, 4 and 5 weeks of social stress, as compared with the control group.

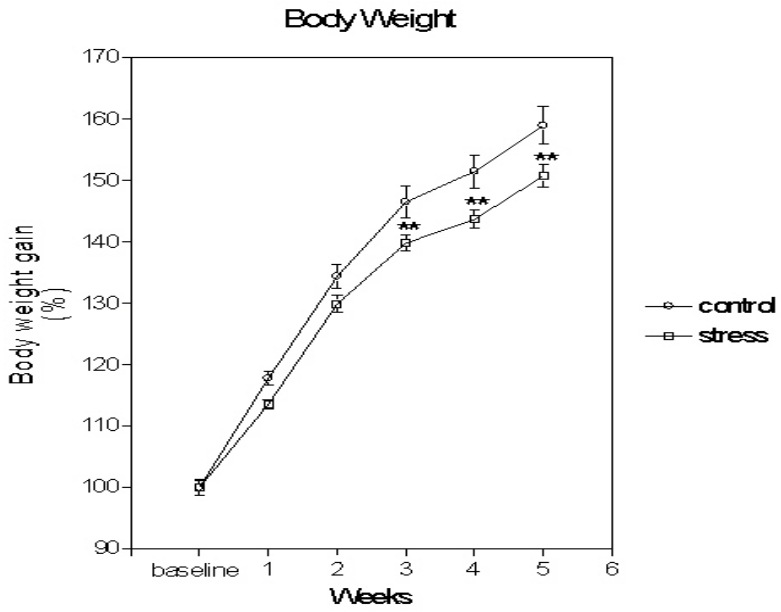

Figure 8. Effects of social stress on body weight gain. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 8) and stressed (n= 8) animals; **p < 0.01 vs control group (Bonferroni test).

As shown in Table 3, chronic social stress resulted in significantly increased adrenal weight. The plasma corticosterone levels were not significantly changed after 5 weeks of social defeat however slight increase in stressed animals was noticeable. Stress had no significant effects on weight of testicles. Statistical analysis followed by Bonferroni post hoc tests confirmed the significant effects of stress [t(14)=2,562; P < 0.05] on adrenal weight.

	Control	**Stress**
adrenal glands (% of body weight)	0.01100 ± 0.00049	0.01343 ± 0.00081* ↑
Corticosterone (ng/ml)	11.96 ± 3.15	17.70 ± 3.55
Testicles (% of body weight)	0.6885 ± 0.0456	0.8091 ± 0.0380
Testosterone (ng/ml)	not measured	not measured

Table 3. Effects of social stress on plasma corticosterone levels, adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 8) and stressed (n= 8) animals; *p < 0.05 (t-test).

3.1.5 Dexamethasone suppression test

As shown in Figure 9, five weeks of social defeat had no significant effects on basal corticosterone levels. Injection of dexamethasone to control animals caused significant reduction in circulating, blood corticosterone level after 3 and 6 hrs. Injection of DEX to stressed animals had no significant effects on plasma corticosterone levels after 3 hrs and significantly decreased plasma corticosterone level after 6 hrs. The plasma level of corticosterone in stressed animals 3 hrs after DEX injection was significantly higher than that observed in control animals.

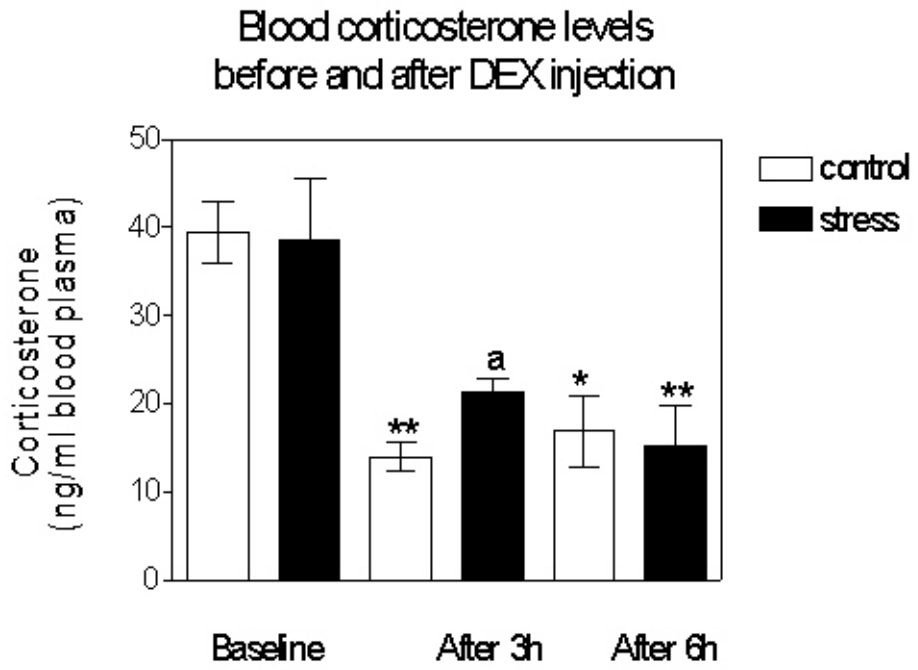

Figure 9. Effects of social stress on response to dexamethasone challenge. The blood samples for analysis of corticosterone were taken just before (baseline) and 3 or 6hr after the DEX injections. Data represent the mean values ± S.E.M. from control (n= 12) and stressed (n= 12) animals; *p < 0.05; **p < 0.01 vs. baseline and ^{a}p < 0.05 vs. control group (Bonferroni test).

3.2 Effects of citalopram

3.2.1 Drug monitoring- pilot study

Five days of treatment with three doses of CIT (pilot study) resulted in the following plasma concentrations of parent drug (CIT) and its metabolite desmethylcitalopram (DCIT): For the 10 mg/kg/day dose of citalopram, CIT was not detectable (< 3 ng/ml) and DCIT 13 ± 6 ng/ml; for the 20 mg/kg/day dose, CIT was 2 ± 2 ng/ml and DCIT 29 ± 6 ng/ml; for the 40 mg/kg/day dose, CIT was 56 ± 9 ng/ml and DCIT 178 ± 31 ng/ml (N = 5, mean ± SEM).

3.2.2 Drug monitoring- chronic treatment

Rats given chronic (four weeks) CIT treatment consumed an average of 20.7 ml/rat/day (stressed) and 22.5 ml/rat/day (control) of CIT solution. This was equivalent to an average CIT dose of 27.6 mg/kg (stressed) and 29.9 mg/kg (control) per day. Repeated measures ANOVA revealed that this was a significantly lower fluid intake than the average 31.8 ml/rat/day (stressed) and 28.7 ml/rat/day (control) consumed by rats receiving water without the drug over the same period [$F(3,140) = 17.46$, $p < 0.001$). There were no significant differences in fluid intake between the groups of animals that did not receive CIT.

Four weeks of CIT treatment resulted in the following concentrations of the parent drug and its metabolites in the blood of the treated rats: control group, CIT 140 ± 22 ng/ml and DCIT 312 ± 21 ng/ml (N = 8, mean ± SEM); stressed group, CIT 151 ± 26 ng/ml and DCIT 486 ± 63 ng/ml (N = 8, mean ± SEM).

3.2.3 Locomotor and exploratory activity

Both groups of stressed rats significantly ($p < 0.05$) decreased locomotor activity (counts/10min) after one week of stress, compared to the control group (Figure 10A). Stressed but untreated animals also showed a significantly ($p < 0.05$) decreased locomotor activity after five weeks of stress (week 5). Four weeks of CIT treatment abolished this effect. The stressed animals chronically treated with CIT showed a significant ($p < 0.05$) increase in locomotor activity compared to stressed but untreated rats. Locomotor activity of stressed animals after four weeks of CIT treatment did not differ significantly from that observed in controls. CIT treatment did not significantly

influence the locomotor activity of unstressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 2.94$, $p < 0.001$], time [$F(2,56) = 37.84$, $p < 0.05$] and significant treatment $\times$ time interaction [$F(6,56) = 4.08$, $p < 0.05$].

Both groups of stressed rats significantly ($p < 0.05$ and $p < 0.01$) decreased the frequency of rearing (counts/10min) after one week of stress compared to the control group (Figure 10B). Stressed but untreated animals also showed significantly ($p < 0.001$) decreased frequency of rearing after five weeks of stress (week 5). Four weeks of CIT treatment abolished this effect. The stressed animals chronically treated with CIT showed a significant ($p < 0.05$) increase in rearing frequency compared to stressed but untreated rats. Frequency of rearing in stressed animals after four weeks of CIT treatment did not differ significantly from that observed in controls. CIT treatment did not significantly influence the rearing behaviour of unstressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 8.17$, $p < 0.001$], time [$F(2,56) = 8.29$, $p < 0.001$] and significant treatment $\times$ time interaction [$F(6,56) = 4.78$, $p < 0.001$].

Both groups of stressed rats significantly ($p < 0.01$ and $p < 0.05$) decreased sniffing frequency (counts/10min) after one week of stress compared to the control group (Figure 10C). Stressed but untreated animals also showed significantly ($p < 0.001$) decreased sniffing frequency after five weeks of stress (week 5). Four weeks of CIT treatment abolished this effect. The stressed animals chronically treated with CIT showed a significant ($p < 0.01$) increase in the frequency of sniffing compared to stressed but untreated rats; however, there was still significantly ($p < 0.05$) less rearing than in control animals. CIT treatment did not significantly influence the sniffing behaviour of unstressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 8.17$, $p < 0.001$], time [$F(2,56) = 8.29$, $p < 0.001$] and significant treatment $\times$ time interaction [$F(6,56) = 4.78$, $p < 0.001$].

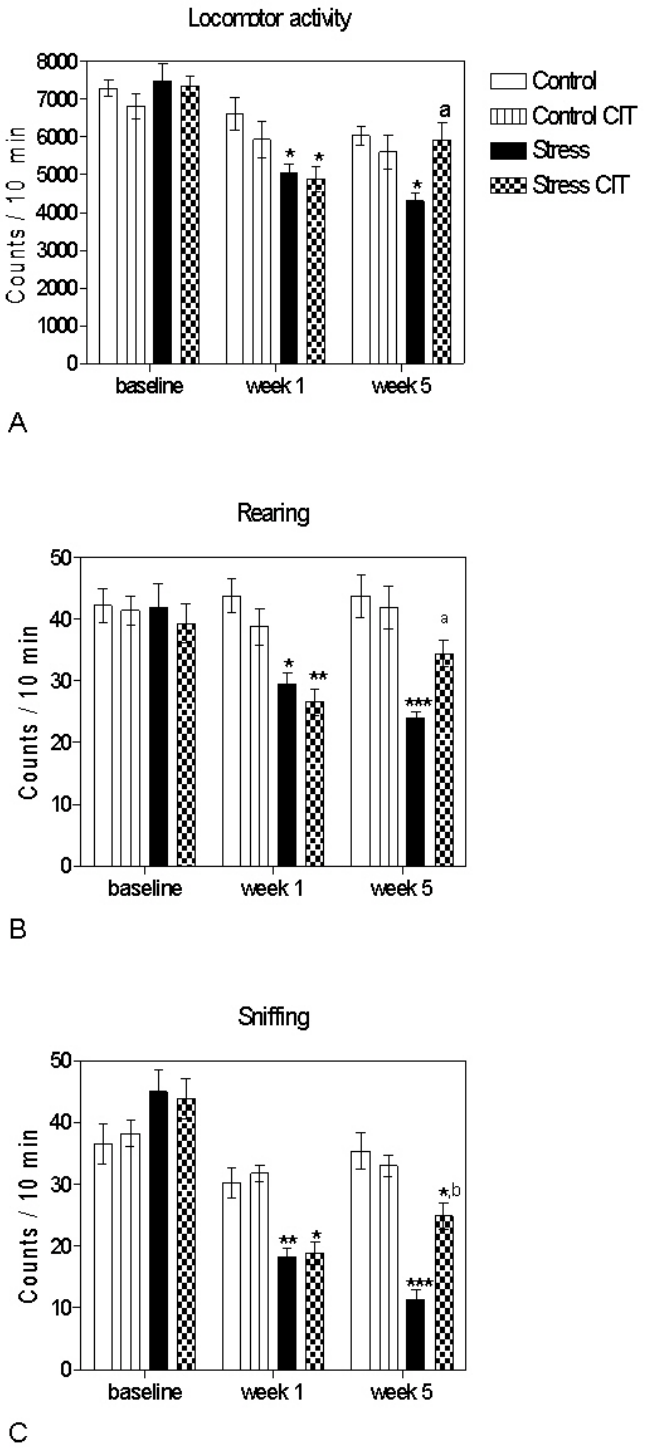

Figure 10. Effects of chronic CIT treatment on locomotor and exploratory activity in control, control CIT-treated, stressed and stressed CIT-treated rats. A) Locomotor activity. Data represent mean ± SEM (counts of light beams interruption per 10 min). B) Frequency of rearing behaviour. Data represent mean ± SEM (counts of rearing per 10 min). C) Frequency of sniffing behaviour. Data represent mean ± SEM (sniffing counts per 10 min). All data from control, control treated, stressed and stressed treated animals (N = 8 for each group); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group, ^{a}p < 0.05 and ^{b}p < 0.01 vs. stressed group (Bonferroni test).

3.2.4 Sucrose preference test

Following the control phase (baseline) and after one week of stress, all groups of animals had a similar preference for sucrose solution (Figure 11). Two weeks of stress reduced this preference in stressed animals. The reduction in sucrose preference reached statistical significance after three weeks ($p < 0.001$) and persisted until the end of the experiment (week 4: $p < 0.05$; week 5: $p < 0.01$). This effect was partially abolished in stressed animals after two and three weeks of CIT treatment (weeks 3 and 4) and was significantly ($p < 0.05$) reversed after four weeks of the drug administration (week 5) compared to stressed but untreated animals. CIT administration caused a slight, statistically not significant, reduction in sucrose preference in control animals throughout the entire treatment phase compared to the control untreated rats. Two-way ANOVA revealed significant effects of stress [$F(3,140) = 10.65$, $p < 0.001$], time [$F(5,140) = 3.93$, $p < 0.01$] and significant stress × time interaction [$F(15,140) = 1.97$, $p < 0.05$].

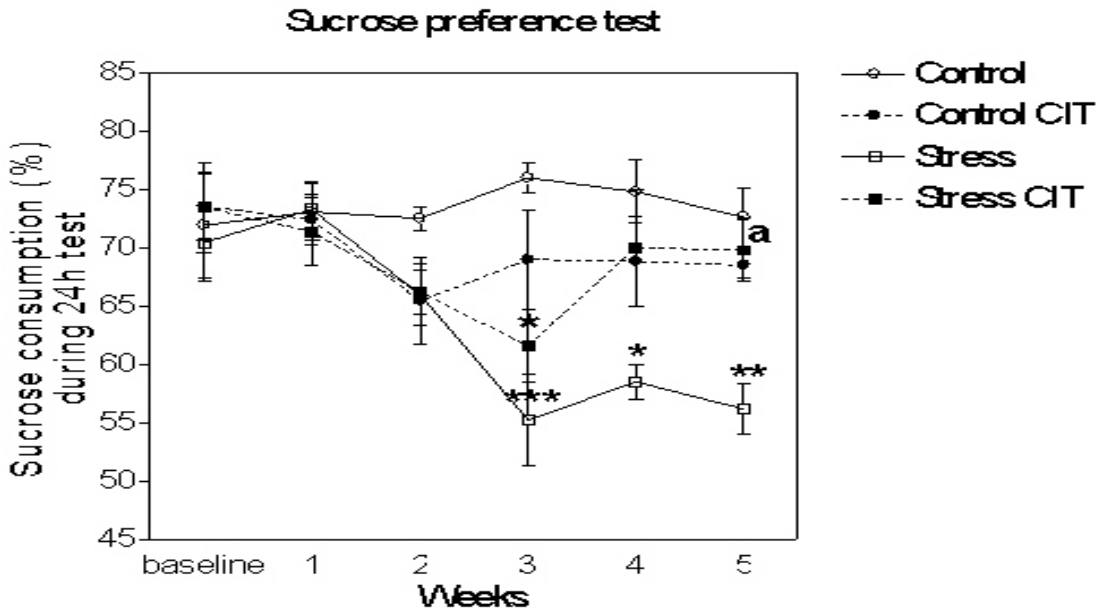

Figure 11. Effects of chronic CIT treatment on sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 h. Data represent mean ± SEM from control, control CIT-treated, stressed and stressed CIT-treated animals (N = 8 for each group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control group and $^{a}p < 0.05$ vs. stressed group (Bonferroni test).

3.2.5 Forced swimming test

As shown in Figure 12, psychosocially stressed rats spent significantly ($p < 0.001$) longer time immobile than the control rats. The immobility time of stressed animals treated with CIT did not differ significantly from control animals and was significantly ($p < 0.001$) shortened compared to stressed but untreated animals. CIT treatment had no effect on immobility time in control animals. Two-way ANOVA revealed significant effects of stress [$F(1,28) = 10.49$, $p < 0.01$], treatment [$F(1,28) = 9.65$, $p < 0.01$] and significant stress $\times$ time interaction [$F(1,28) = 18.37$, $p < 0.001$].

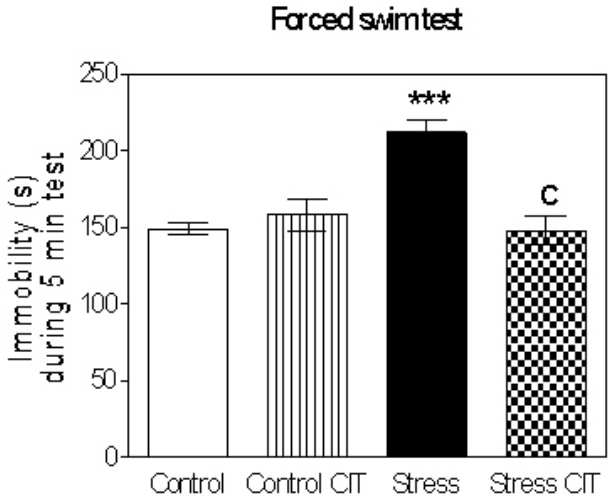

Figure 12. Effects of chronic CIT treatment in control and socially stressed rats on immobility time (s) in the forced swimming test. Data represent mean ± SEM from control, control CIT-treated, stressed and stressed CIT-treated animals (N = 8 for each group); ***$p < 0.001$ vs. control group and $^{C}p < 0.001$ vs. stressed group (Bonferroni test). Animals were tested at the end of the stress phase (week 5). Data are shown as a time (seconds) spent immobile during 5 min test.

3.2.6 Body weight, organs and hormones

Both chronic CIT treatment and chronic social stress had noticeable effects on body weight gain in experimental animals. Stressed animals gained significantly less weight after three ($p < 0.05$), four ($p < 0.05$) and five ($p < 0.001$) weeks of daily social defeat compared to controls. CIT caused significant reduction in body weight gain in treated animals after two ($p < 0.01$), three ($p < 0.05$) and four ($p < 0.001$) weeks of treatment (experimental weeks 3, 4 and 5 respectively) as compared to control animals. Two-way ANOVA revealed significant effects of CIT treatment [$F(3,140) = 1076.90$, $p < 0.001$] and stress [$F(5,140) = 13.74$, $p < 0.001$] on body weight gain (Figure 13).

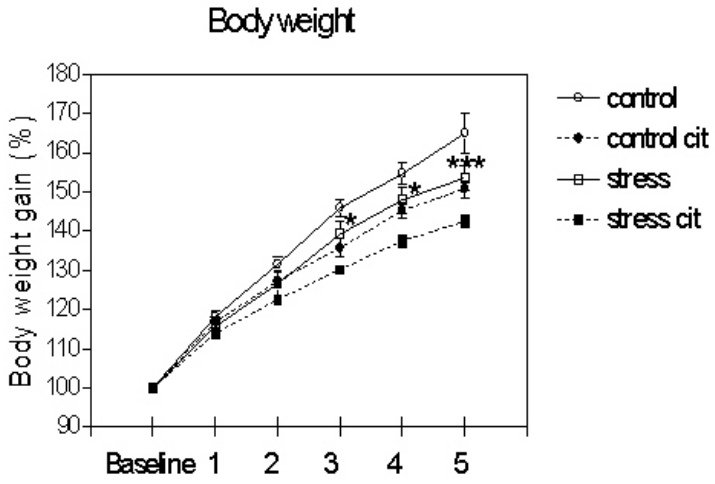

Figure 13. Effects of social stress and CIT treatment on body weight gain. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 8), control CIT (n= 8), stressed (n= 8) and stressed CIT (n= 8) animals. *p < 0.05; ***p < 0.001 vs. control group (Bonferroni test).

As shown in Table 4, chronic social stress resulted in significantly ($p < 0.05$) increased adrenal weight. Also testicles were significantly ($p < 0.05$) enlarged in both groups of treated animals. The plasma corticosterone levels were not significantly changed after 5 weeks of social defeat, however slight increase in stressed animals was noticeable. The stressed animals chronically treated with CIT showed significantly ($p < 0.05$) increased plasma level of corticosterone. Statistical analysis followed by Bonferroni post hoc test confirmed the significant differences in adrenal weight, testicles weight and plasma corticosterone levels among experimental groups.

	Control	Control CIT	Stress	Stress CIT
adrenal glands (% of body weight)	0.01209 ± 0.00103	0.01254 ± 0.00080	0.01509 ± 0.00059 * ↑	0.01536 ± 0.00091
Corticosterone (ng/ml)	19.87 ± 2.79	22.13 ± 2.42	24.31 ± 2.77	30.50 ± 1.93 * ↑
Testicles (% of body weight)	0.7405 ± 0.0799	0.8920 ± 0.0227	0.9195 ± 0.01658 * ↑	0.9767± 0.0265 * ↑
Testosterone (ng/ml)	not measured	not measured	not measured	not measured

Table 4. Effects of social stress and chronic CIT treatment on plasma corticosterone and testosterone levels, adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 8), control CIT (n= 8) stressed (n= 8) and stressed CIT (n= 8) animals; *p < 0.05 (Bonferroni test).

3.3 Effects of fluoxetine

3.3.1 Drug monitoring- chronic treatment

After four weeks of daily oral FLX application (10 mg/kg) and 24 hours after the last application the concentrations of FLX and its major metabolite nor-FLX in the blood plasma were determined. In the Control FLX group the parent compound FLX was not detectable. However, norFLX was in the range of 138.5 ± 20.83 ng/ml. The Stress FLX group revealed a similar picture with no detectable FLX and norFLX in the range 134.2 ± 20.76 ng/ml. There were no significant differences between Control FLX and Stress FLX groups of animals.

3.3.2 Locomotor and exploratory activity

Before the onset of drug treatment both groups of stressed rats significantly ($p < 0.05$) decreased locomotor (counts/10min) activity after one week of stress compared to the control group (Figure 14A). Stressed but untreated animals showed- compared to their baseline condition - a highly significantly ($p < 0.001$) decreased locomotor activity after 5 weeks of stress (Stressed, week 5). Four weeks of FLX treatment counteracted this effect (Stress FLX group). Stressed animals chronically treated with FLX showed a significant ($p < 0.05$) increase in locomotor activity compared to stressed but untreated rats (Stress group). Importantly, after four weeks of FLX treatment locomotor activity of animals from the Stress FLX group normalized and was comparable to controls. Obviously FLX is effective in stressed animals only since it did not influence the locomotor activity of unstressed animals (Control FLX*)*. Two-way ANOVA revealed significant effects of treatment [$F(3,40)= 5.29$, $p < 0.01$], time [$F(2,40)= 62.20$, $p < 0.001$] and significant treatment x time interaction [$F(6,40)= 5.58$, $p < 0.001$].

One week of daily social defeat decreased the frequency of rearing (counts/10min), (Figure 14B) however the difference was not statistically significant. After 5 weeks of social stress we observed a significant decrease of rearing behaviour ($p < 0.05$; week 5). Four weeks of FLX treatment abolished this effect. Animals from the Stress FLX group showed a significant ($p < 0.05$) increase in frequency of rearing compared to animals from the Stress group. Moreover, the effect of FLX is revealed by the finding that the frequency of rearing in animals from the Stress FLX group did not differ significantly from controls. This improvement is not a sole drug effect since FLX did not influence

rearing behaviour in animals from the Control FLX group. Two-way ANOVA revealed significant effects of time [$F(2,40)= 33.23$, $p < 0.01$] and significant treatment x time interaction [$F(6,40)= 3.90$, $p < 0.001$].

Frequency of sniffing, another component of exploratory behaviour was significantly ($p < 0.05$) reduced (counts/10min) after one week of daily social conflict (Figure 10C). This decrease in sniffing frequency continued until week 5 in animals from the Stress group. Daily FLX treatment of stressed animals counteracted this reduction in exploration yielding a higher sniffing frequency in animals from the Stress FLX group. Similar to the findings described above on the effect of FLX in control animals, the SSRI had no influence on sniffing behaviour on animals from the Control FLX group. Two-way ANOVA revealed significant effects of time [$F(2,40)= 33.23$, $p < 0.001$] and significant treatment x time interaction [$F(6,40)= 3.90$, $p < 0.01$].

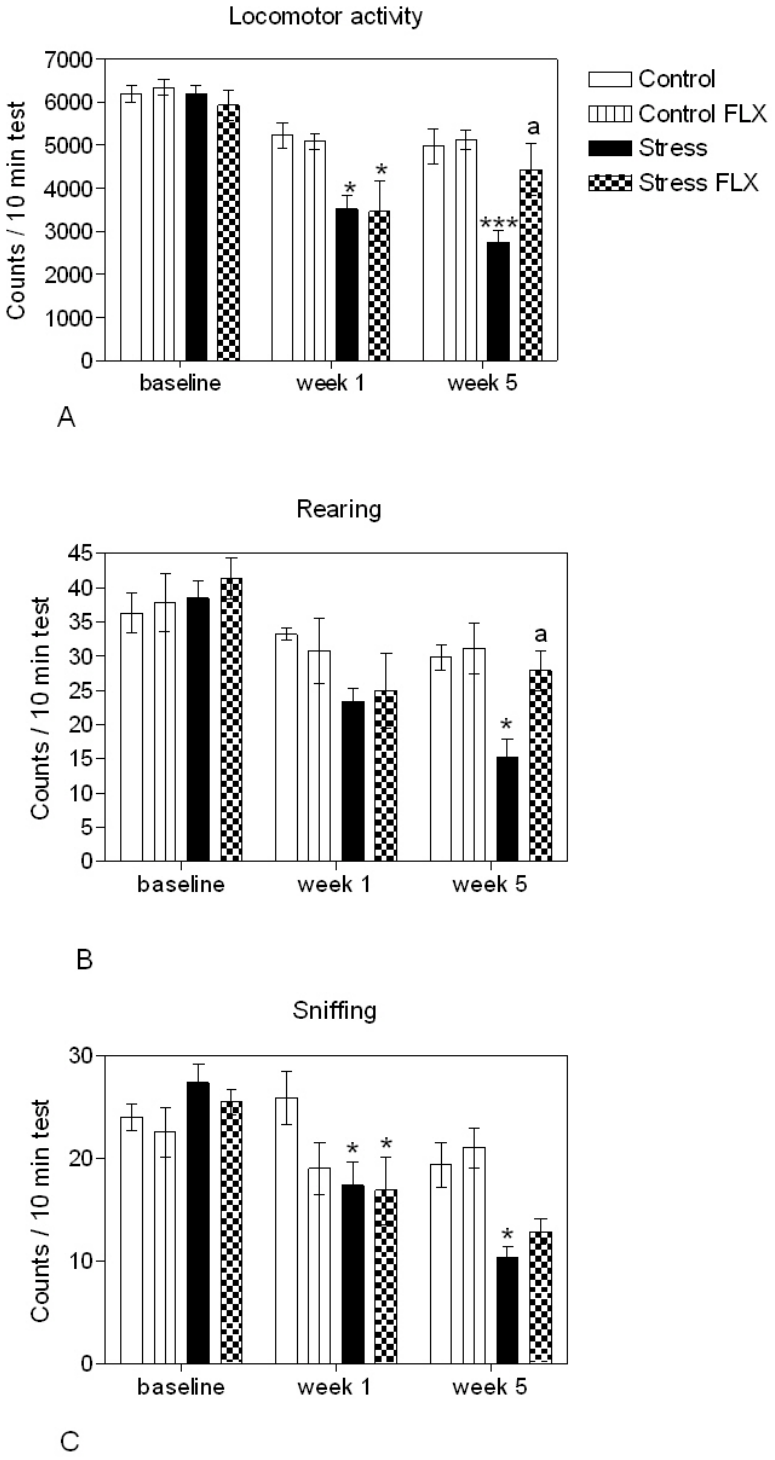

Figure 14. Effects of chronic FLX treatment on locomotor and exploratory activity in control, control FLX-treated, stressed and stressed FLX-treated rats. A) Locomotor activity. Data represent mean ± SEM (counts of light beams interruption per 10 min). B) Frequency of rearing behaviour. Data represent mean ± SEM (counts of rearing per 10 min). C) Frequency of sniffing behaviour. Data represent mean ± SEM (sniffing counts per 10 min). All data from control, control treated, stressed and stressed treated animals (N = 6 for each group); *$p < 0.05$, ***$p < 0.001$ vs. control group, $^{a}p < 0.05$ vs. stressed group (Bonferroni test).

3.3.3 Sucrose preference test

Following the control phase (baseline) and after one week of stress (week 1), the preference for sucrose solution in stressed animals was slightly reduced compared to controls (Figure 15). In animals from the Stress group sucrose intake continuously decreased and remained on this low level for the remaining two weeks of the experiment. This effect was partially, but not significantly abolished in animals of the Stress FLX group and sucrose intake was nearly comparable to control animals after four weeks of drug administration. Two-way ANOVA revealed significant effects of stress [$F(3,140)= 10.65$, $p < 0.001$], time [$F(5,140)= 3.93$, $p < 0.01$] and significant stress x time interaction [$F(15,140)= 1.97$, $p < 0.05$].

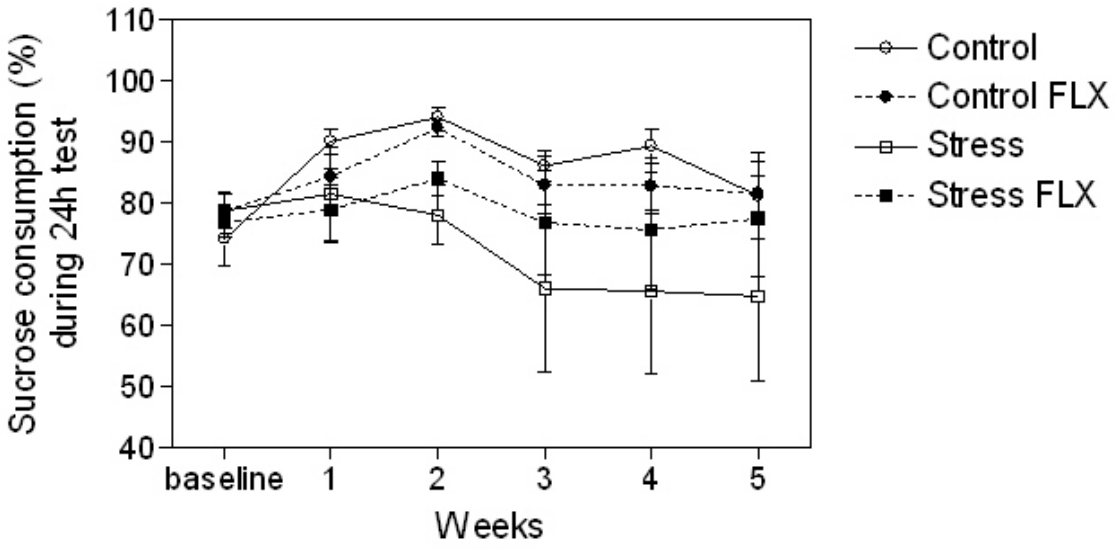

Figure 15. Effects of chronic FLX treatment on sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 h. Data represent mean ± SEM from control, control FLX-treated, stressed and stressed FLBX-treated animals (N = 6 for each group).

3.3.4 Body weight, organs and hormones

Stressed rats gained less body weight than did the control rats (Figure 16). Interestingly, FLX had no effect in control animals. In stressed animals a gentle positive effect of FLX on body occurred in week 5 Statistical analyses revealed a significant effect of treatment [$F(3,100)= 5.95$, $p < 0.05$] time [$F(5,100)= 339.26$, $p < 0.05$] and significant stress x time interaction [$F(15,100)= 4.12$, $p < 0.001$]. Subsequent Bonferroni post hoc tests confirmed significant reduction in body weight gain in stressed animals after 2 ($p < 0.05$), 3 ($p < 0.01$), 4 ($p < 0.001$) and 5 ($p < 0.001$) weeks of social stress, as compared with the control group. The effect of the drug was not significant.

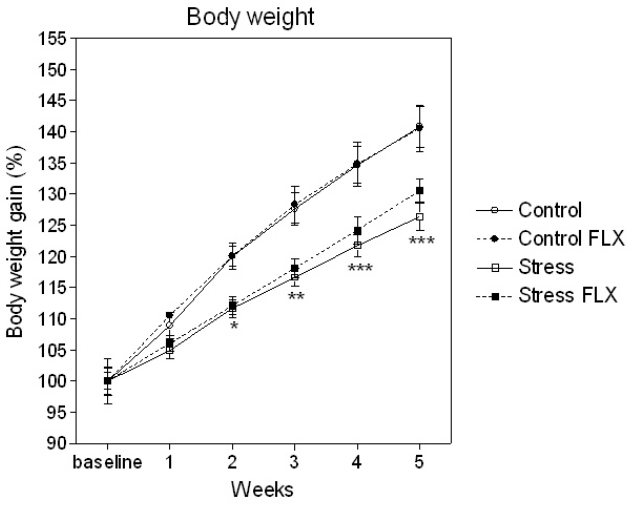

Figure 16. Effects of social stress and FLX treatment on body weight gain. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 6), control FLX (n= 6), stressed (n= 6) and stressed FLX (n= 6) animals. **p < 0.01; ***p < 0.001 vs. control group (Bonferroni test).

Daily social stress for 5 weeks resulted in increased adrenal weight (Table 5). When compared with controls the adrenals were heavier ($p < 0.05$ and $p < 0.01$) in both groups of stressed animals. Fluoxetine treatment caused no significant effects on adrenal weights. Plasma testosterone levels were significantly lower in both groups of stressed animals ($p < 0.05$ and $p < 0.01$). FLX treatment caused also significant ($p < 0.05$) decrease in plasma testosterone level in control group. There were no significant effects neither of stress nor of FLX treatment on relative weight of testicles. One way ANOVA

revealed significant differences among experimental groups with respect to adrenal weight [$F(3,20)=6.862$; $p < 0.05$] and plasma testosterone levels [$F(3,20)=7.066$; $p < 0.01$].

	Control	**Control FLX**	**Stress**	**Stress FLX**
adrenal glands (% of body weight)	0.01357± 0.00084	0.01358 ± 0.00062	0.01798 ± 0.00111 * ↑	0.01617 ± 0.00061 ** ↑
Corticosterone (ng/ml)	not measured	not measured	not measured	not measured
Testicles (% of body weight)	0.8152 ± 0.0361	0.8193 ± 0.0173	0.9084 ± 0.0218	0.8320 ± 0.0357
Testosterone (ng/ml)	2.06 ± 0.16	1.09 ± 0.22 * ↓	1.36 ± 0.24 * ↓	1.11 ± 0.17 ** ↓

Table 5. Effects of social stress and chronic FLX treatment on plasma corticosterone and testosterone levels, adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 6), control FLX (n= 6) stressed (n= 6) and stressed FLX (n= 6) animals; *p < 0.05; **p < 0.01 vs. control group (Bonferroni test).

3.4 Effects of reboxetine

3.4.1 Drug monitoring- pilot study

Five days of treatment with three doses of RBX (pilot study) resulted in the following plasma concentrations of RBX in blood plasma: For the 20 mg/kg/day dose, RBX was 5.7 ± 07 ng/ml; for the 40 mg/kg/day dose, RBX was 12.6 ± 4 ng/ml and for the 80 mg/kg/day dose, RBX was 48 ± 21 ng/ml (N = 5, mean ± SEM).

3.4.2 Drug monitoring- chronic treatment

Rats given chronic (four weeks) RBX treatment consumed an average of 23.9 ml/rat/day (stressed) and 26.0 ml/rat/day (control) of RBX solution. This was equivalent to an average RBX dose of 31.7 mg/kg (stressed) and 34.7 mg/kg (control) per day. Repeated measures ANOVA revealed that this was a significantly lower fluid intake than the average 34.4 ml/rat/day (stressed) and 35.1 ml/rat/day (control) consumed by rats receiving water without the drug over the same period [$F(3, 96) = 17.85$, $p < 0.001$). There were no significant differences in fluid intake between the groups of animals that did not receive RBX.

Four weeks of RBX treatment resulted in the following concentrations of the drug in the blood of the treated rats: control group, RBX 23.7 ± 12 ng/ml (N = 8, mean ± SEM); stressed group, RBX 22.0 ± 5 ng/ml (N = 8, mean ± SEM).

3.4.3 Locomotor and exploratory activity

Both groups of stressed rats decreased locomotor activity (counts/10min) after one week of stress, compared to the control group (Figure 17A). Stressed but untreated animals also showed a significantly ($p < 0.01$) decreased locomotor activity after five weeks of stress (week 5). Four weeks of RBX treatment abolished this effect. The stressed animals chronically treated with RBX showed a significant ($p < 0.05$) increase in locomotor activity compared to stressed but untreated rats. Locomotor activity of stressed animals after four weeks of RBX treatment did not differ significantly from that observed in controls. RBX treatment did not significantly influence the locomotor activity of unstressed animals although caused moderate increase in

that parameter. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 1.52$, $p < 0.01$] and time [$F(2,56) = 4.14$, $p < 0.05$]

Both groups of stressed rats decreased the frequency of rearing (counts/10min) after one week of stress compared to the control group (Figure 17B). Stressed but untreated animals also showed significantly ($p < 0.05$) decreased frequency of rearing after five weeks of stress. Four weeks of RBX treatment abolished this effect. Frequency of rearing in stressed animals after four weeks of RBX treatment did not differ significantly from that observed in controls. RBX treatment caused also slight increase in rearing behaviour in unstressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 4.12$, $p < 0.05$], time [$F(2,56) = 19.76$, $p < 0.001$] and significant treatment $\times$ time interaction [$F(6,56) = 4.24$, $p < 0.01$].

Both groups of stressed rats significantly ($p < 0.01$ and $p < 0.05$) decreased sniffing frequency (counts/10min) after one week of stress compared to the control group (Figure 17C). RBX treatment had no significant effects on sniffing behaviour neither in stressed nor control group of animals. After 5 weeks of stress both groups of stressed rats showed significant ($p < 0.01$ and $p < 0.05$) impairment of rearing behaviour comparing to control animals. RBX treatment did not significantly influence the sniffing behaviour of unstressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 6.12$, $p < 0.001$], and time [$F(2,56) = 11.39$, $p < 0.001$].

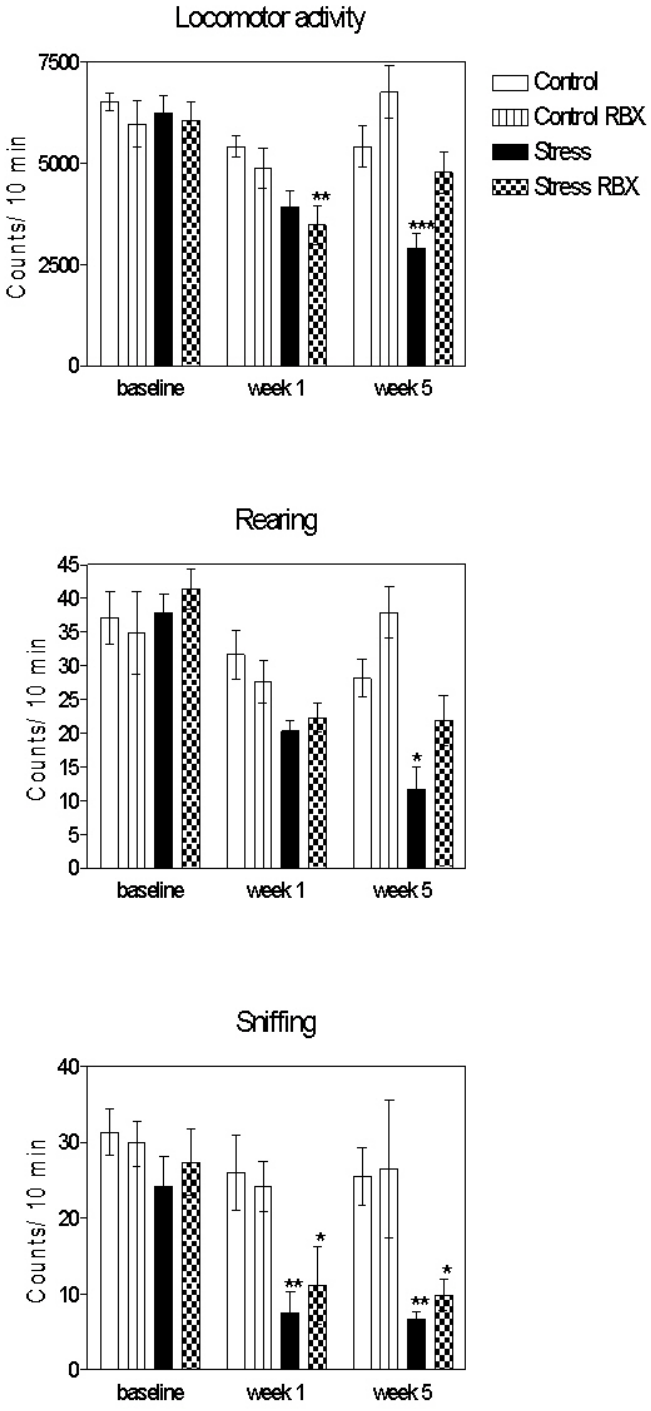

Figure 17. Effects of chronic RBX treatment on locomotor and exploratory activity in control, control RBX-treated, stressed and stressed RBX-treated rats. A) Locomotor activity. Data represent mean ± SEM (counts of light beams interruption per 10 min). B) Frequency of rearing behaviour. Data represent mean ± SEM (counts of rearing per 10 min). C) Frequency of sniffing behaviour. Data represent mean ± SEM (sniffing counts per 10 min). All data from control, control treated, stressed and stressed treated animals (N = 8 for each group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control group (Bonferroni test).

3.4.4 Sucrose preference test

Following the control phase (baseline) and after one week of stress, all groups of animals had a similar preference for sucrose solution (Figure 18). Three weeks of stress reduced this preference in stressed animals. The reduction in sucrose preference reached statistical significance after four weeks ($p < 0.05$) and persisted until the end of the experiment (week 5: $p < 0.05$). This effect was partially abolished in stressed animals after four weeks of the drug administration (week 5) compared to stressed but untreated animals. RBX administration caused a slight, statistically not significant, reduction in sucrose preference in control animals throughout the entire treatment phase compared to the control untreated rats. Two-way ANOVA revealed significant effects of stress [$F(3,140) = 5.32$, $p < 0.01$] and time [$F(5,140) = 2.30$, $p < 0.05$].

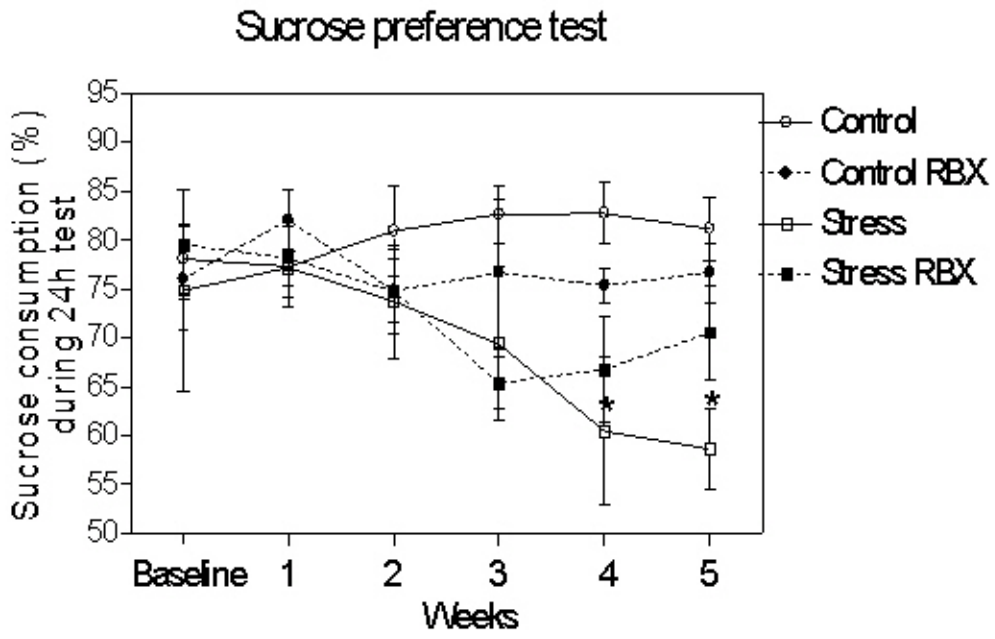

Figure 18. Effects of chronic RBX treatment on sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 h. Data represent mean ± SEM from control, control RBX-treated, stressed and stressed RBX-treated animals (N = 8 for each group); *$p < 0.05$ vs. control group (Bonferroni test).

3.4.5 Forced swim test

As shown in Figure 19, psychosocially stressed rats spent significantly ($p < 0.05$) longer time immobile than the control rats. RBX treatment

abolished this effect. The immobility time of stressed animals treated with RBX did not differ significantly from control animals. RBX treatment had no significant effects on immobility time in control animals. One-way ANOVA revealed significant differences among experimental groups [$F(3,28) = 7.182$, $p < 0.01$].

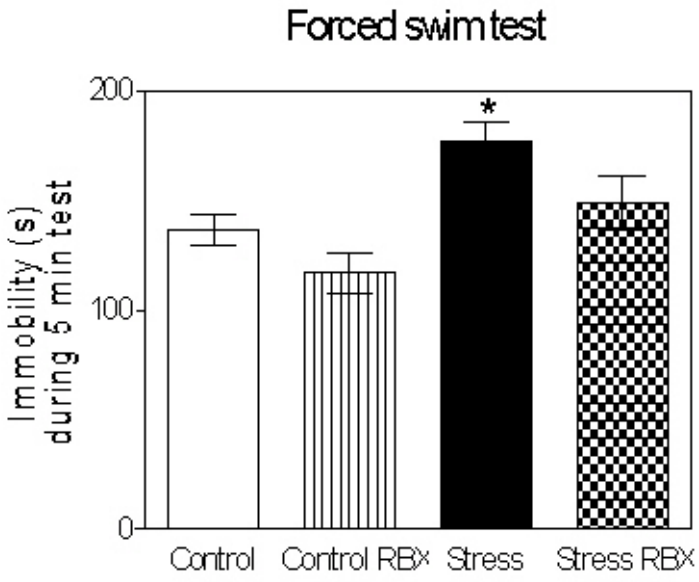

Figure 19. Effects of chronic RBX treatment in control and socially stressed rats on immobility time (s) in the forced swimming test. Data represent mean ± SEM from control, control RBX-treated, stressed and stressed RBX-treated animals (N = 8 for each group); *p < 0.05 vs. control group (Bonferroni test). Animals were tested at the end of the stress phase (week 5). Data are shown as a time (seconds) spent immobile during 5 min test.

3.4.6 Body weight, organs and hormones

Both chronic RBX treatment and chronic social stress had noticeable effects on body weight gain in experimental animals. Stressed animals gained less weight after 2, 3, 4 and 5 weeks of daily social defeat compared to controls. RBX treatment also caused reduction in body weight gain in treated animals along the entire treatment in both groups of treated animals as compared to controls. Two-way ANOVA revealed significant effects of RBX treatment [$F(3,140) = 2.96$, $p < 0.05$] and stress [$F(5,140) = 97.50$, $p < 0.001$] on body weight gain and significant treatment x stress interaction [$F(15,140) = 1.93$, $p < 0.05$] (Figure 20).

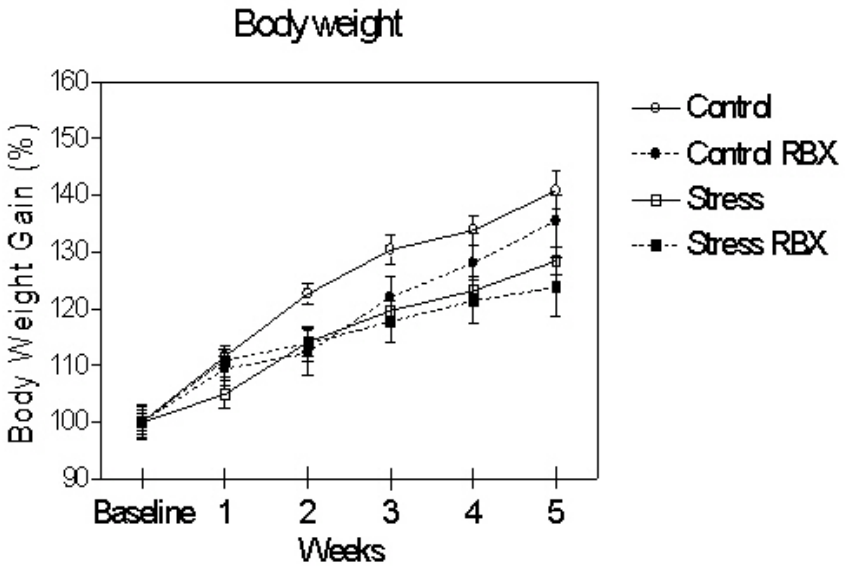

Figure 20. Effects of social stress and RBX treatment on body weight gain. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 8), control RBX (n= 8), stressed (n= 8) and stressed RBX (n= 8) animals.

As shown in Table 6, chronic social stress resulted in increased adrenal weight in both groups of stressed animals. Neither the testicle weight nor plasma levels of corticosterone or testosterone were significantly changed after five weeks of social defeat. Statistical analysis confirmed the significant differences in adrenal weight among experimental groups (ANOVA [$F(3,26) = 3.707$, $p < 0.05$]).

	Control	Control RBX	Stress	Stress RBX
adrenal glands (% of body weight)	0.01053 ± 0.00052	0.01194 ± 0.00079	0.01281 ± 0.00039	0.01326 ± 0.00061 * ↑
Corticosterone (ng/ml)	20.34 ± 1.73	23.12 ± 3.75	18.57 ± 2.66	19.08 ± 2.11
Testicles (% of body weight)	0.7972 ± 0.0210	0.8426 ± 0.0228	0.8383 ± 0.0257	0.8780 ± 0.0240
Testosterone (ng/ml)	3.40 ± 0.87	2.48 ± 0.57	1.94 ± 0.26	2.06 ± 0.43

Table 6. Effects of social stress and chronic RBX treatment on plasma corticosterone levels, adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 8), control RBX (n= 8) stressed (n= 8) and stressed RBX (n= 8) animals; *$p < 0.05$ (Bonferroni test).

3.5 Effects of haloperidol

3.5.1 Drug monitoring- chronic treatment

Rats given chronic (four weeks) HAL treatment consumed an average of 28.2 ml/rat/day (stressed) and 29.0 ml/rat/day (control) of HAL solution. This was equivalent to an average HAL dose of 1.88 mg/kg/day (stressed) and 1.93 mg/kg/day (control). Untreated groups of rats consumed water of an average of 33.2 (stressed) and 33.9 ml/rat/day (control). There were no significant differences in water consumption between treated and untreated animals. There were no significant differences in fluid intake between the groups of animals that did not receive HAL.

Four weeks of HAL treatment resulted in the following concentrations of the drug in the blood of the treated rats: control group, HAL 4.025 ± 2.024 ng/ml (N = 8, mean ± SEM); stressed group, HAL 4.563 ± 1.465 ng/ml (N = 8, mean ± SEM).

3.5.2 Locomotor and exploratory activity

Both groups of stressed rats decreased locomotor (counts/10min) activity after one week of stress, compared to the control group (Fig 21A). After 5 weeks of stress both groups of stressed rats showed significant ($p < 0.01$ and $p < 0.05$) impairment of locomotor activity comparing to control animals. HAL treatment had no significant effects on locomotor activity in stressed group of treated animals, caused however significant ($p < 0.01$) reduction in locomotor activity of unstressed animals as compared to controls. Two-way ANOVA revealed significant effects of stress [$F(2,56) = 49.56$, $p < 0.001$] and significant treatment $\times$ stress interaction [$F(6,56) = 7.94$, $p < 0.001$].

Both groups of stressed rats slightly decreased the frequency of rearing (counts/10min) after one week of stress compared to the control group (Figure 21B). After 5 weeks of stress both groups of stressed rats showed significant ($p < 0.001$ and $p < 0.05$) impairment of rearing behaviour comparing to control animals. HAL treatment significantly decreased the rearing behaviour in both groups of treated animals as compared to controls ($p < 0.001$) and stressed untreated animals ($p < 0.05$). Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 9.85$, $p < 0.001$], stress

[F(2,56) = 34.55, p < 0.001] and significant treatment ×stress interaction [F(6,56) = 5.40, p < 0.001].

One week of stress caused slight reduction (counts/10min) in sniffing behaviour (Fig 21C). After 5 weeks of stress both groups of stressed rats showed significant (p < 0.001 and p < 0.05) impairment of rearing behaviour comparing to control animals. HAL treatment significantly (p < 0.05) decreased the rearing behaviour of unstressed animals as compared to controls (p < 0.001). HAL treatment had no significant effects on stressed animals as compared to stressed untreated animals. Two-way ANOVA revealed significant effects of treatment [F(3,56) = 5.83, p < 0.01], time [F(2,56) = 18.34, p < 0.001] and significant treatment × time interaction [F(6,56) = 2.48, p < 0.05].

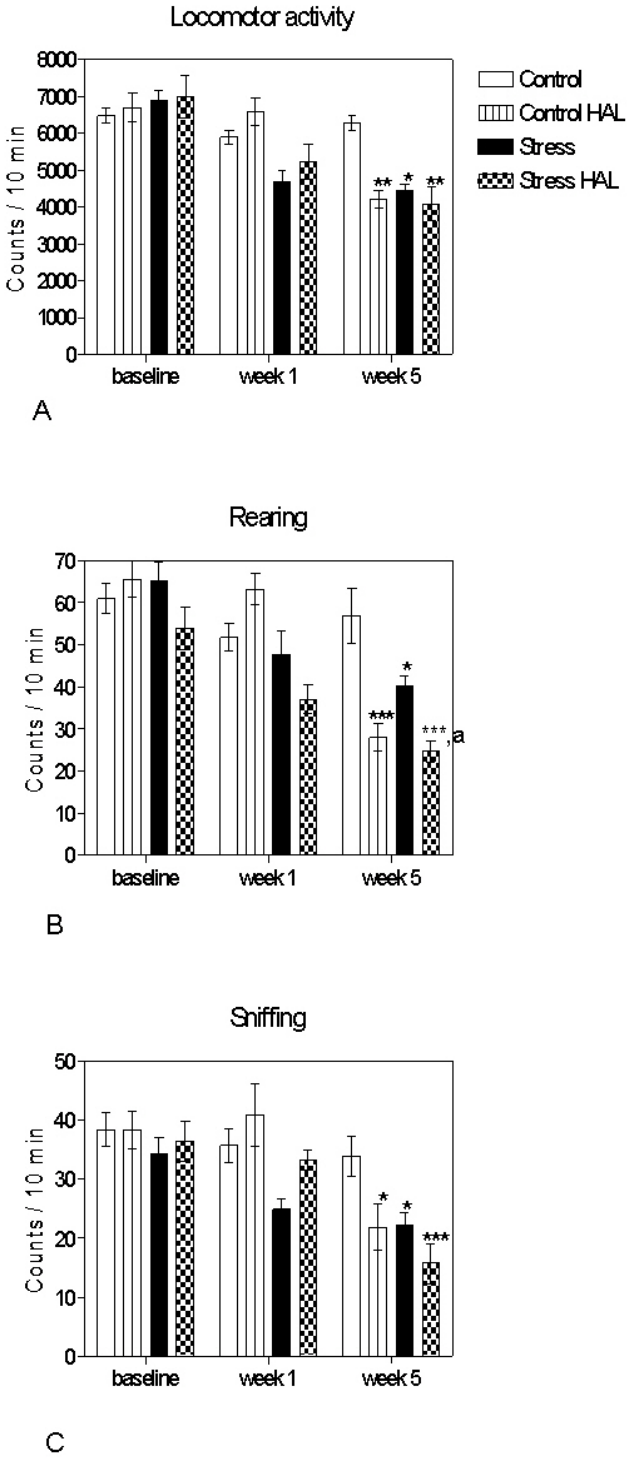

Figure 21. Effects of chronic HAL treatment on locomotor and exploratory activity in control, control HAL-treated, stressed and stressed HAL-treated rats. A) Locomotor activity. Data represent mean ± SEM (counts of light beams interruption per 10 min). B) Frequency of rearing behaviour. Data represent mean ± SEM (counts of rearing per 10 min). C) Frequency of sniffing behaviour. Data represent mean ± SEM (sniffing counts per 10 min). All data from control, control treated, stressed and stressed treated animals (N = 8 for each group); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group, [a]p < 0.05 vs. stressed group (Bonferroni test).

3.5.3 Sucrose preference test

Following the control phase (baseline) and after one week of stress, all groups of animals had a similar preference for sucrose solution (Figure 22). Two weeks of stress caused reduction in sucrose preference in stressed animals. The reduction in sucrose preference reached statistical significance after four weeks of stress (week 4: $p < 0.01$) and persisted until the end of the experiment (week 5: $p < 0.05$). This effect was reinforced during the treatment phase in the group of stressed and HAL treated animals. HAL treatment caused also a robust decrease in sucrose preference in the group of control treated animals. One week of treatment caused significant ($p < 0.05$) reduction in sucrose preference as compared to control animals and this effect persisted till the end of the treatment (week 3: $p < 0.01$, week 4: $p < 0.001$, week 5: $p < 0.001$). Two-way ANOVA revealed significant effects of treatment [$F(3,140) = 16.54$, $p < 0.001$], stress [$F(5,140) = 30.98$, $p < 0.001$] and significant treatment × stress interaction [$F(15,140) = 4.23$, $p < 0.001$].

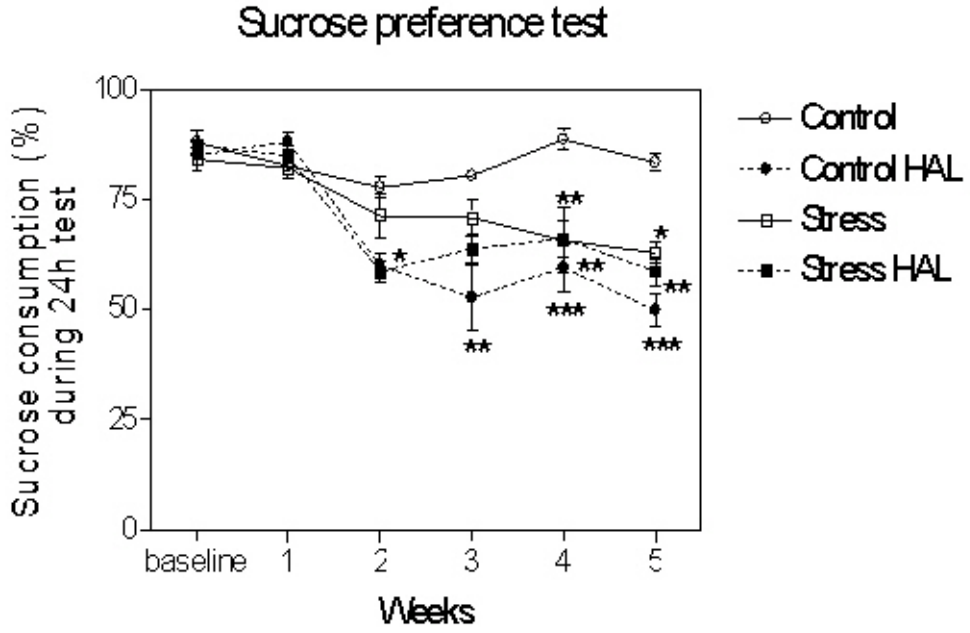

Figure 22. Effects of chronic HAL treatment on sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 h. Data represent mean ± SEM from control, control HAL-treated, stressed and stressed HAL-treated animals (N = 8 for each group); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group (Bonferroni test).

3.5.4 Forced swim test

As shown in Figure 23, psychosocially stressed rats spent significantly ($p < 0.05$) longer time immobile than the control rats. HAL treatment caused

robust prolongation of immobility time in both groups of treated animals as compared to controls (control treated vs. control: $p < 0.001$) and as compared to stressed untreated animals (stressed treated vs. stressed: $p < 0.05$). One way ANOVA revealed significant differences among experimental groups [$F(3,28) = 16.84$, $p < 0.001$].

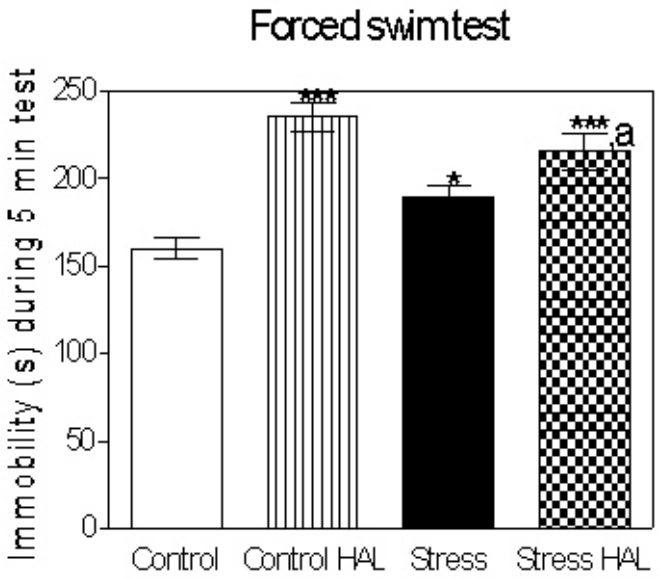

Figure 23. Effects of chronic HAL treatment in control and socially stressed rats on immobility time (s) in the forced swimming test. Data represent mean ± SEM from control, control HAL-treated, stressed and stressed HAL-treated animals (N = 8 for each group); *p < 0.05, ***p < 0.001 vs. control group and ^{a}p < 0.05 vs. stressed group (Bonferroni test). Animals were tested at the end of the stress phase (week 5). Data are shown as a time (seconds) spent immobile during 5 min test.

3.5.5 Body weight, organs and hormones

Chronic social stress had noticeable effects on body weight gain in experimental animals. Stressed animals gained significantly less weight after two ($p < 0.01$) three ($p < 0.01$), four ($p < 0.01$) and five ($p < 0.001$) weeks of daily social defeat compared to controls. HAL treatment had significant effects on body weight gain in neither group of treated animals. Two-way ANOVA revealed significant effects of HAL treatment [$F(3,140) = 7.41$, $p < 0.001$] and stress [$F(5,140) = 401.49$, $p < 0.001$] on body weight gain and significant treatment x stress interaction [$F(15,140) = 5.32$, $p < 0.001$] (Figure 24).

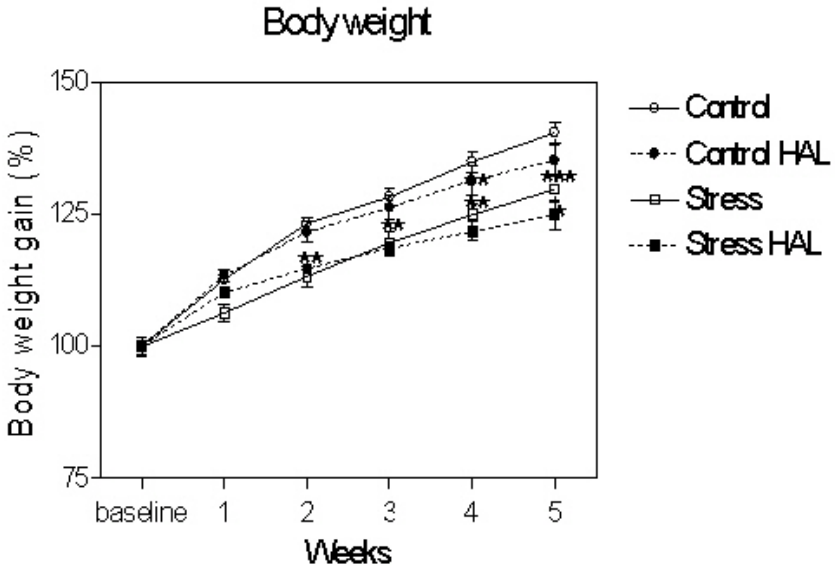

Figure 24. Effects of social stress and HAL treatment on body weight gain. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 8), control HAL (n= 8), stressed (n= 8) and stressed HAL (n= 8) animals. *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group (Bonferroni test).

As shown in Table 7, chronic social stress resulted in significantly (p < 0.05 and 0.01) increased adrenal weight in both groups of stressed animals. Neither testicle weight nor plasma corticosterone or testosterone levels were significantly changed. Statistical analysis followed by Bonferroni post hoc test confirmed the significant differences in adrenal weight among experimental groups (ANOVA [F(3,28) = 5.666, p < 0.01])

	Control	Control HAL	Stress	Stress HAL
adrenal glands (% of body weight)	0.01236 ± 0.00035	0.01307 ± 0.00048	0.01420 ± 0.00039 * ↑	0.01498 ± 0.00067 ** ↑
Corticosterone (ng/ml)	21.7 ± 3.5	18.2 ± 2.3	14.5 ± 1.5	20.7 ± 3.7
Testicles (% of body weight)	0.7967 ± 0.0189	0.8143 ± 0.0345	0.8461 ± 0.0274	0.8550 ± 0.0221
Testosterone (ng/ml)	0.86 ± 0.18	0.94 ± 0.16	1.135 ± 0.16	0.75 ± 0.15

Table 7. Effects of social stress and chronic HAL treatment on plasma corticosterone levels, adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 8), control HAL (n= 8) stressed (n= 8) and stressed HAL (n= 8) animals; *p < 0.05, **p < 0.01 vs. control group (Bonferroni test).

3.6 Effects of diazepam

3.6.1 Locomotor and exploratory activity

Both groups of stressed rats decreased ($p < 0.01$ and $p < 0.05$) locomotor activity (counts/10min) after one week of stress, compared to the control group (Figure 25A). After 5 weeks of stress, animals showed significant ($p < 0.05$) impairment of locomotor activity comparing to control animals. Acute administration of DZP (i.p. 1 mg/kg) partially reversed this effect. Injection of DZP had no significant effects on locomotor activity of control animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 3.87$, $p < 0.05$] and stress [$F(2,56) = 28.18$, $p < 0.001$]

Both groups of stressed rats significantly ($p < 0.05$) decreased the frequency of rearing (counts/10min) after one week of stress comparing to the control group (Figure 25B). After 5 weeks of stress, animals showed significant ($p < 0.05$) impairment of rearing behaviour comparing to control animals. The acute DZP injection partially reversed this effect. DZP injection caused slight increase in frequency of rearing of both treated groups. Two-way ANOVA revealed significant effects stress [$F(2,56) = 14.52$, $p < 0.001$] and significant treatment $\times$stress interaction [$F(6,56) = 3.23$, $p < 0.01$].

Both groups of stressed rats significantly ($p < 0.01$ and $p < 0.05$) decreased sniffing frequency (counts/10min) after one ($p < 0.01$ and $p < 0.05$) and five ($p < 0.01$) weeks of stress compared to the control group (Fig 25C). Acute DZP injection had effects neither on control nor on stressed animals. Two-way ANOVA revealed significant effects of treatment [$F(3,56) = 5.63$, $p < 0.01$], stress [$F(2,56) = 18.77$, $p < 0.001$] and significant treatment $\times$ stress interaction [$F(6,56) = 3.18$, $p < 0.01$].

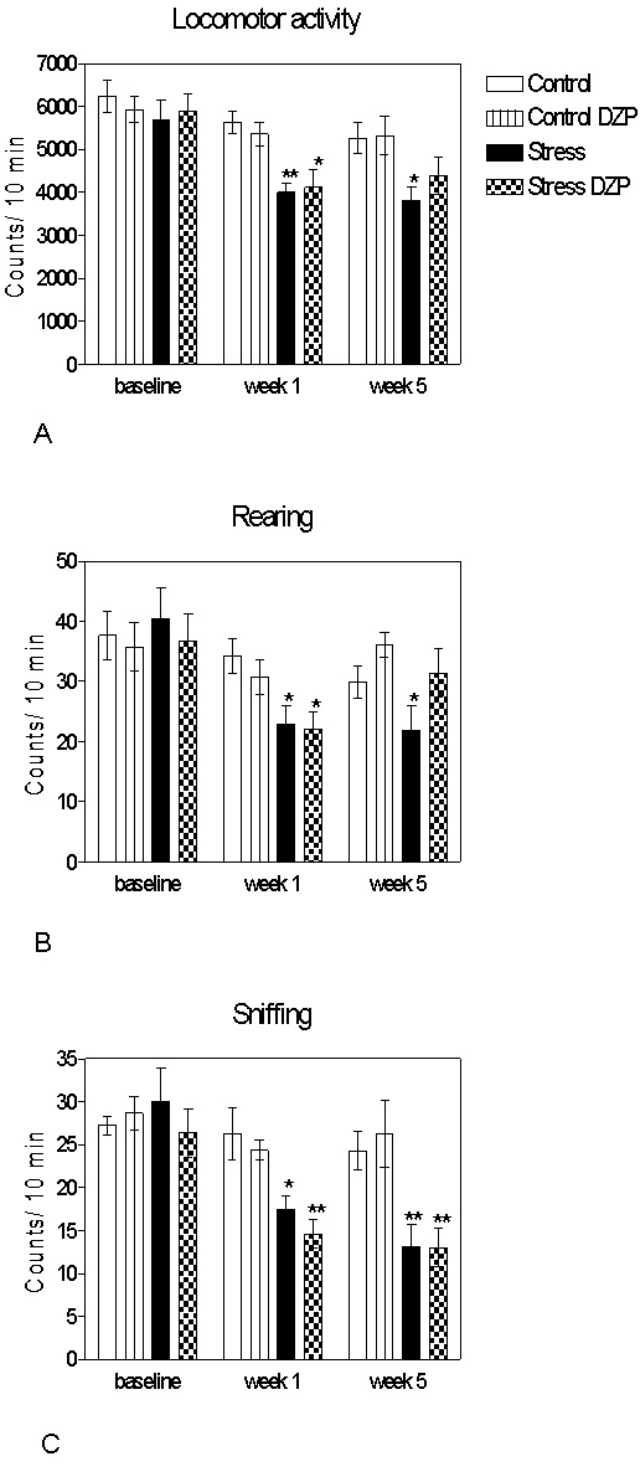

Figure 25. Effects of social stress and acute DZP treatment (i.p. 1 mg/kg) on locomotor and exploratory activity in control, control DZP-treated, stressed and stressed DZP-treated rats. A) Locomotor activity. Data represent mean ± SEM (counts of light beams interruption per 10 min). B) Frequency of rearing behaviour. Data represent mean ± SEM (counts of rearing per 10 min). C) Frequency of sniffing behaviour. Data represent mean ± SEM (sniffing counts per 10 min). All data from control, control treated, stressed and stressed treated animals (N = 8 for each group); *p < 0.05, **p < 0.01 vs. control group (Bonferroni test).

3.6.2 Sucrose preference test

Five weeks of chronic social stress caused significant ($p < 0.05$) reduction in sucrose preference in both groups of stressed animals. Acute injection of DZP (1 mg/kg i.p.) had no influence on sucrose preference in neither of experimental groups (Fig 26). One way ANOVA revealed significant differences among the experimental groups [$F(3,28) = 4.121$, $p < 0.05$].

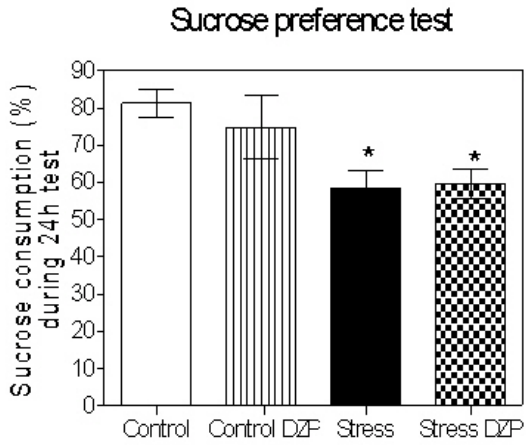

Figure 26. Effects of acute DZP treatment (1 mg/kg i.p.) on sucrose preference in control and socially stressed rats. Data were calculated as percentage of the total fluid intake during 24 h test performed after 5 weeks of chronic social stress. Data represent mean ± SEM from control, control DZP-treated, stressed and stressed DZP-treated animals (N = 8 for each group); *$p < 0.05$ vs. control group (Bonferroni test).

3.6.3 Forced swim test

As shown in Figure 27, psychosocially stressed rats spent significantly ($p < 0.01$) longer time immobile than the control rats. DZP injection (1 mg/kg i.p.) had no significant effects on immobility time in neither of experimental groups. One way ANOVA revealed significant differences among experimental groups [$F(3,28) = 7.421$, $p < 0.001$].

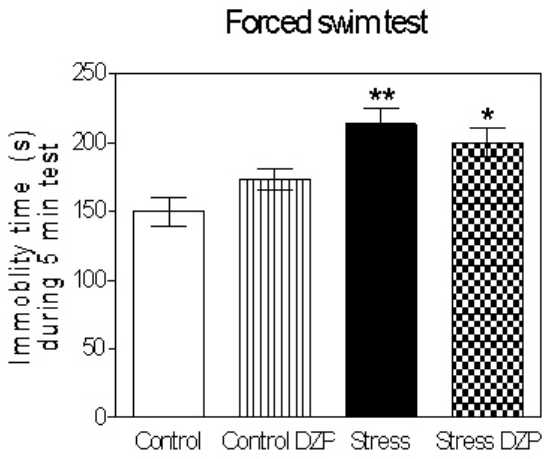

Figure 27. Effects of acute DZP treatment (1 mg/kg i.p.) in control and socially stressed rats on immobility time (s) in the forced swimming test. Data represent mean ± SEM from control, control DZP-treated, stressed and stressed DZP-treated animals (N = 8 for each group); *p < 0.05, **p < 0.01 vs. control group (Bonferroni test). Animals were tested at the end of the stress phase (week 5). Data are shown as a time (s) spent immobile during 5 min test.

3.6.4 Elevated plus maze

As shown in Figure 28, control animals treated with DZP (1 mg/kg i.p.) spent longer time in open arms of elevated plus maze than did control untreated animals ($p < 0.05$). DZP injection had no effect on stressed group of animals. One way ANOVA revealed significant differences among experimental groups [$F(3,24) = 3.48$, $p < 0.05$].

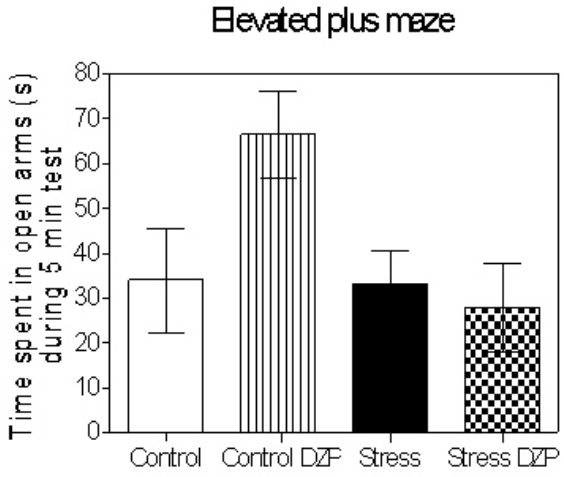

Figure 28. Effects of acute DZP treatment in control and socially stressed rats on the time (s) spent in the open arms of elevated plus maze. Data represent mean ± SEM from control, control DZP-treated, stressed and stressed DZP-treated animals (N = 8 for each group). Animals were tested at the end of the stress phase (week 5). Data are shown as a time (seconds) spent in open arms of the experimental apparatus during 5 min test.

3.6.5 Body weight, organs and hormones

Stressed animals gained significantly less weight after two ($p < 0.05$), three ($p < 0.05$), four ($p < 0.05$) and five ($p < 0.001$) weeks of daily social defeat compared to controls. Two-way ANOVA revealed significant effects of time [$F(1,90) = 72.16$, $p < 0.001$] and stress [$F(5,70) = 73.63$, $p < 0.001$] on body weight gain and significant time x stress interaction [$F(5,90) = 4.46$, $p < 0.01$] (Figure 29).

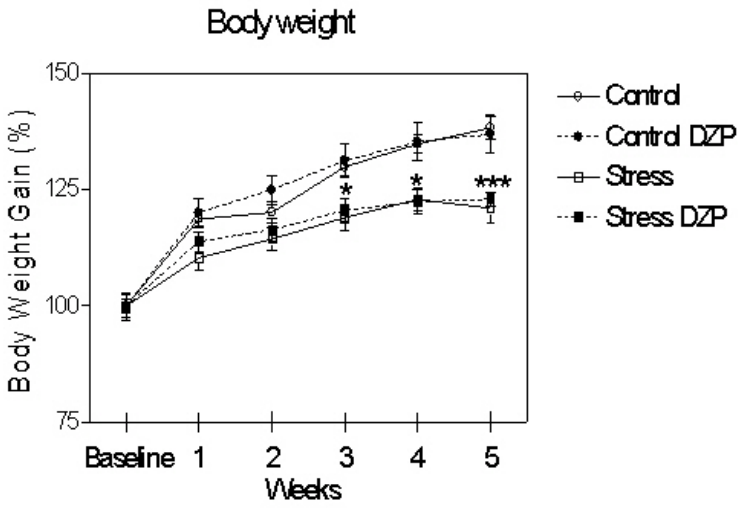

Figure 29. Effects of social stress on body weight gain in all experimental groups of animals. Body weight gain was calculated as the percentage of the initial (baseline) body weight. Data represent the mean values ± S.E.M. from control (n= 8), control DZP (n= 8), stressed (n= 8) and stressed DZP (n= 8) animals. *$p < 0.05$, ***$p < 0.001$ vs. control group (Bonferroni test).

As shown in Table 8, chronic social stress resulted in a moderate however statistically not significant increase in adrenal weight in stressed animals. Neither testicle weight nor plasma corticosterone or testosterone levels were significantly changed.

	Control	Control DZP	Stress	Stress DZP
adrenal glands (% of body weight)	0.01163 ± 0.00083	0.01211 ± 0.00080	0.01338 ± 0.00049	0.01136 ± 0.00059
Corticosterone (ng/ml)	17.37± 2.50	12.40 ± 1.81	13.39 ± 1.55	17.16 ± 2.94
Testicles (% of body weight)	0.7858 ± 0.0173	0.8472 ±0.0291	0.8666 ± 0.0268	0.9311 ± 0.0164
Testosterone (ng/ml)	1.09± 0.23	0.75 ± 0.17	1.61 ± 0.39	0.71 ± 0.12

Table 8. Effects of social stress and acute DZP treatment (1 mg/kg i.p.) on plasma corticosterone and testosterone levels. The table contains also adrenal weight and weight of testicles. Weight of organs was calculated as percentage of body weight at the end of the experiment. Data represent the mean values ± S.E.M. from control (n= 8), control HAL (n= 8) stressed (n= 8) and stressed HAL (n= 8) animals.

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4 Discussion

In order to develop a model of chronic social stress, in the first study the male *Wistar* rats were subjected to five weeks daily exposure to social defeat. This protocol resulted in a variety of behavioural changes in the rodents that might be regarded as behavioural correlates of depressive-like symptoms in humans. Based on the obvious 'face validity' and to investigate whether this model also has 'predictive validity' the question has been addressed how well stressed rats respond favourably to the different classes of antidepressant drugs such as SSRI (CIT and FLX) or SNRI (RBX). Four weeks of daily treatment with all of the tested antidepressants reversed the majority of adverse effects of chronic social stress at least on behavioural level. The antidepressant effects occurred with drug specific intensity. Importantly, antidepressant drugs did not influence the behaviour of control animals. In the last part of the study that was designed to evaluate the specificity of antidepressant treatment, the animals were treated with the neuroleptic drug haloperidol and anxiolytic diazepam. Neither treatment with HAL nor with DZP (apart slight effects on rearing behaviour) had beneficial effects on socially stressed individuals.

4.1 Effects of stress

Rats subjected to chronic social stress showed a decrease in sucrose preference in a time-dependent manner. Reduced preference for the sucrose solution in rats indicates a decreased sensitivity to reward and may be homologous to anhedonia (Willner, Muscat et al. 1992). Similar effects observed in the chronic, unpredictable mild stress model were associated with impairment in the conditioned place preference acquisition, decreased motor response to dopamine receptor agonists and to increased reward thresholds in a brain stimulation paradigm, for review see (Willner 1997). These findings suggest a link between the stress-induced anhedonic state and alterations in the mesolimbic dopamine system that is involved in the mediation of rewarding stimuli. It has been proposed that reward-related appetitive behaviours are mediated by dopamine systems, while consummatory behaviour is opioid dependent (Berridge 1996; Berridge and Robinson 1998). Social defeat has been reported to decrease place preference for morphine

(Berridge 1996; Coventry, D'Aquila et al. 1997; Berridge and Robinson 1998), to intensify response to morphine withdrawal (Vivian and Miczek 1991) and to induce tolerance to the analgesic effects of morphine (Miczek 1991). These findings imply socially induced changes in opioid systems. Since social defeat also impairs appetitive behaviour (Von Frijtag, Van den Bos et al. 2002), negative changes in both systems are likely to evoke a two-dimensional hedonic deficit. Interestingly, the adverse consequences of defeat, including impaired reward anticipatory behaviour and reduced density in striatal dopamine transporters, are counteracted by subsequent social housing (Ruis, te Brake et al. 1999; Von Frijtag, Reijmers et al. 2000; Isovich, Engelmann et al. 2001). It has also been reported that non-aggressive social interaction activates the endorphin system (File 1980). This may support the theory of the involvement of the opioid and dopamine systems in the adverse reaction to social stress, further emphasizing the role of social support in coping with social stresses.

The forced swimming test (Porsolt's test) is considered to be highly specific for the detection of antidepressant drugs (Porsolt, Anton et al. 1978; Lucki 1997). Since shortened immobility time in this test indicates anti-depressive activity, increase in this parameter, as observed in present study, *per analogiam*, might be considered the behavioural expression of a depressive-like symptom in rats. This finding appears to be important, suggesting that social stress experienced chronically can affect an animal's motivation and lead to the development of behavioural despair. Similar observations have been reported in rats (Koolhaas J.M. 1990) resulting from a single, but long lasting (four hours), social defeat session. Studies of mice exposed to chronic psychosocial stress report mixed results. Kudryawtseva demonstrated increased immobility of mice in Porsolt's test following chronic social defeat (Kudriavtseva, Bakshtanovskaia et al. 1992), while Keeney and Hogg reported no such effects (Keeney and Hogg 1999). Although the resident-intruder paradigm is standardized, variations in the intensity of social defeat might have affected previous results. In the present study, however, the development of behavioural despair was associated with hedonic and motivational deficits. These correlated effects are unlikely to be accidental and the proposed protocol (see Materials and Methods) allowed the elimination of possible variations in the intensity of the defeat experience. In spite of a great

deal of experimental evidence suggesting a role for different neurotransmitters in forced-swimming-induced behaviour, it appears that the increase in immobility may be the result of reduced sensitivity in the mesolimbic dopamine system (Imperato, Cabib et al. 1993; Cabib and Puglisi-Allegra 1996; D'Aquila, Panin et al. 2004). Short stressful experiences, including social defeat, increase activity in the mesocorticolimbic dopamine system. However, this initial activation may turn into inhibition if the situation does not produce an effective strategy for coping with stress (Puglisi-Allegra, Kempf et al. 1991; Kapur and Mann 1992; Imperato, Cabib et al. 1993). This hypothesis is also supported by studies demonstrating the role of dopamine in the anti-immobility effect of antidepressants (Gutierrez-Garcia, Contreras et al. 2003), and by studies which report increased immobility after prolonged antidepressant withdrawal leading to the desensitisation of the mesolimbic dopamine system (D'Aquila, Panin et al. 2004).

The initial activity of a rat placed in novel surroundings (e.g. an open field) can be taken as an indicator of its emotional and motivational state (Katz, Roth et al. 1981). It is assumed that an inescapable open field situation reflects both the stress and the rewarding component of novelty. In rats, decreased exploratory activity in a novel environment might reflect decreased motivation or drive, a behaviour representing "refractory loss of interest" (Roth and Katz 1979; Katz, Roth et al. 1981) and may also be related to hedonic deficit, since novelty is rewarding (Bevins and Bardo 1999; Bardo and Dwoskin 2004). Exploratory behaviour (defined as the time an animal spends investigating a novel environment) is sensitive to previous stress experience (D'Aquila, Peana et al. 2000) but the effects of physical stress are progressively reduced with repeated exposure to the stressor (Puglisi-Allegra, Kempf et al. 1991). In the present paradigm, we investigated the effects of both sub-chronic and chronic social stress upon open field activity. In both cases, social defeat consistently decreased activity as compared with non-stressed animals after one and five weeks of stress exposure. This important observation suggests that animals did not habituate to chronic social defeat but developed pathologies. A slight reduction in locomotor activity of control animals observed at the end of experiment can be explained by habituation learning which is typical after repeated open field exposure (Thiel, Muller et al. 1999). Habituation effects, however, were minor compared to the effects of

stress. The decreased exploration of a novel environment may be also related to elevated levels of anxiety in stressed animals. However, the results from elevated plus maze test did not confirm this hypothesis, moreover the behavioural despair observed in the forced swimming test as well as the decrease in preference for sucrose solutions suggest rather a motivational deficits. Furthermore, the open field set-up (darkness) was minimally anxiogenic. The unidirectional effects of social stress on locomotion, rearing and sniffing might suggest that the chosen parameters of exploratory activity were dependent on overall activity of animals. This would be in agreement with studies by Thiel et al. (Thiel, Muller et al. 1999) who have reported that intensity of rearing is correlated with locomotor activity. Because other studies reported lack of such correlation with regard to rearing (Pawlak and Schwarting 2002) and mainly to sniffing (Horvitz, Williams et al. 2001), in the present study all three parameters were analysed separately.

Other factors not studied that might affect present results should be mentioned. For example, single housing of control animals might evoke uncontrollable stress resulting from social isolation. It has not been checked whether the adverse effects of chronic social defeat are reversible by social housing, as has been shown after single defeat (Ruis, te Brake et al. 1999). Moreover, the daily exposure of stressed rats to novel environment (resident's cage) unlike the control animals could affect the results i.e. enhancing effects of habituation in the open field test. Although these two novel environments were extremely different effects of habituation can not be excluded. To assess locomotor and exploratory activity we used rather small size of open field set up. Finally the size could limit exploratory drive and fear response in experimental animals (Roth and Katz 1979).

In conclusion, present data showed that social defeat applied chronically in rats induces a broad spectrum of behavioural changes that are considered important analogues of depressive symptoms in humans. It has been proposed that reduced sensitivity to rewards in rodents might be homologous to human anhedonia (Willner, Muscat et al. 1992; Moreau 1997). According to DSM-IV criteria of the American Psychiatric Association (1994), human anhedonia occurs in patients suffering from major depression, opiate withdrawal and schizophrenia; it is characterized by the loss of interest and the inability to feel pleasure and joy (even in normally positive stimulating

situations). In present model, diminished sucrose preference indicates desensitisation of the brain reward mechanism. Increased immobility time in the forced swimming test represents behavioural despair resulting from defective motivational systems and is characteristic of depressive disorder. The reduced locomotor and exploratory activity represents the loss of interest in new stimulating situations and may imply the presence of motivational deficits. Since anhedonia is one of the core symptoms of depression, present findings suggested that the chronic social stress paradigm may be a reliable animal model for depressive like symptoms in humans; however pharmacological studies were necessary to determine its predictive validity.

4.2 Effects of citalopram

The mechanism of action of citalopram as an antidepressant is presumed to be linked to potentiation of serotonergic activity in the CNS resulting from its selective inhibition of neuronal reuptake of serotonin. In vitro and in vivo studies in animals suggest that citalopram is a highly selective serotonin reuptake inhibitor (SSRI) with minimal effects on NE and DA neuronal reuptake. Tolerance to the inhibition of 5-HT uptake is not induced by long-term (14- day) treatment of rats with citalopram. Citalopram is a racemic mixture (50/50), and the inhibition of 5-HT reuptake by citalopram is primarily due to the (S)-enantiomer. Citalopram has no or very low affinity for 5-HT_{1A}, 5-HT_{2A}, dopamine D1 and D2, α_1-, α_2-, and β-adrenergic, histamine H_1, gamma aminobutyric acid (GABA) and muscarinic cholinergic receptors.

The present study confirms the earlier observation that socially stressed rats display a number of behavioural changes that may be regarded as correlates of depressive-like symptoms in rodents (Rygula, Abumaria et al. 2005). Socially stressed animals showed impaired locomotor and exploratory activity, decreased preference for sweet sucrose solution and prolonged immobility in the forced swimming test. These changes suggest that chronic social stress causes hedonic and motivational deficits in experimental animals. Four weeks of oral treatment with the antidepressant CIT reversed these adverse effects and normalised behaviours related to motivation and reward sensitivity. Importantly, CIT did not influence the behaviour of control animals.

The drug treatment was successfully carried out orally, via drinking water, to avoid factors of physical stress derived from the prolonged injections.

Several studies indicated that drug injection comprises significant and measurable stress (Persico, Schindler et al. 1995; Cassano and D'Mello A 2001; Schramm, McDonald et al. 2001). Such a stressor may interfere with the experimental procedures and affect the outcome of the behavioural tests. Because our stress paradigm was based mainly on social components we decided to avoid injection stress and applied CIT orally via the drinking water. This way of administration has been successfully used previously in a number of pre-clinical studies with different psychoactive compounds (Dalterio and Bartke 1979; Shaldubina, Einat et al. 2002). Present results confirm that administration via the drinking water is a suitable way to chronically treat experimental animals non-invasively. Animals receiving CIT in their drinking water over several weeks consumed less fluid than rats receiving only water, which may have resulted from the presumptively aversive taste of the drug. Alternatively, it is possible that diminished fluid intake was due to minor gastrointestinal disturbances induced by the relatively high dose of the SSRI, which was slightly above the upper level recommended as optimal for the treatment of patients with CIT (Baumann, Hiemke et al. 2004). Such gastrointestinal disturbances might lead to reduced food intake and, because rats are prandial drinkers, also to lower water intake. Citalopram-treated rats gained significantly less weight than controls, an effect that was also observed in a previous study in rats (Kugelberg, Apelqvist et al. 2002) as well as in a long-lasting human study (Goldstein and Goodnick 1998).

In humans, at therapeutically effective doses, plasma concentrations of CIT are in the range of 30–130 ng/ml (Baumann, Hiemke et al. 2004). We determined the CIT dose necessary to reach similar plasma concentrations in rats in a pilot study. Because daily oral application of the target dose 40 mg/kg for five consecutive days resulted in the average CIT serum concentration of about 56 ng/ml, this dose was chosen to treat the experimental animals. Interestingly, drug monitoring performed at the end of the four weeks of treatment revealed average plasma levels of CIT to be more than double that found in the pilot experiment after five days of drug treatment (139.5 ± 22.10 ng/ml control group and 150.9 ± 25.93 ng/ml stressed group), suggesting time-dependent accumulation during long-term

treatment. However, when interpreting these data the short half-life-time of CIT in rodents of about 3 h (Hyttel, Overo et al. 1984) has to be taken into account. Hyttel and co-workers (1984) applied CIT to rats orally via the diet using similar doses as in the present study. Two hours after drug withdrawal the blood levels of CIT were 80 to 180 ng/ml, which is similar to the plasma concentration detected in the present study, whereas drug levels were not detectable 24 hours after drug withdrawal (Hyttel, Overo et al. 1984). However, the drinking pattern and the last time-point of water intake were not registered in the present study, and closer monitoring of drug intake and plasma levels would have been required to explain the unexpectedly high plasma concentrations of CIT at the end of the experiment. In spite of these shortcomings, the present data show that the animals were treated with sufficiently high doses of the drug to attain antidepressant effects.

In rats, reduced preference for the sucrose solution indicates a decreased sensitivity to rewards (Willner, Muscat et al. 1992). It has been proposed that reduced sensitivity to rewards in rodents might be homologous to human anhedonia (Papp, Willner et al. 1991). Anhedonia, a decreased ability to experience pleasure, is a core symptom of depression and other psychiatric disorders in humans. First Katz (Katz 1982), then others, showed that rats subjected to a variety of physical stressors decrease sucrose intake/preference in a time-dependent manner (Willner, Muscat et al. 1992; Moreau 1997). Stress-induced hedonic deficits have been also observed in reward-related paradigms such as Conditioned Place Preference and Intracranial Self-Stimulation (Willner 1997). We postulated that a psychosocially induced reduction in sucrose preference may be considered as a behavioural correlate of depressive symptoms, and results from the present study confirm this hypothesis (Rygula, Abumaria et al. 2005). The validity of animal models of depression depends not only on the similarity between behavioural alterations in the animals and depressive symptoms in humans (face validity), but also on the effectiveness of antidepressant treatments to reverse the experimentally induced behavioural deficits (predictive validity) (Willner 1995). In different models of chronic stress, such as the chronic mild stress model, the reduction in sucrose preference has been shown to be reversed by chronic administration of almost all classes of antidepressants, as well as by non-pharmacological treatments such as electroconvulsive

stimulation or sleep deprivation (Willner 1997). However, some of these models applied physical stressors in order to evoke depressive-like behavioural deficits, even though physical stressors such as electric shock or restraint are more or less artificial, and may be regarded as irrelevant for the situations and stressors that humans and animals may encounter in everyday life. In the present model of chronic social stress, decreased sucrose preference was reversed by an antidepressant drug, which provided first evidence for the predictive validity of the paradigm as a model of depression. The antidepressant drug treatment started after one week of stress, in the time window when the first behavioural consequences of social stress became detectable, such as reduction in locomotor and exploratory activity. The first effects of CIT on sucrose preference were observed after the second week of treatment while three weeks of the CIT administration caused reversal of stress-induced anhedonia. This time course of response is consistent with the time lag of antidepressant drug response in patients (Mischoulon 1997), which may be considered as another hint of predictive validity of the model.

Decreased sucrose preference is suggested to be a consequence of alterations in the mesolimbic dopaminergic system (Muscat, Papp et al. 1992). The sensitivity of the mesolimbic dopamine reward system is changed by stress experiences (Cabib and Puglisi-Allegra 1996), with both duration and nature of the stressor determining the degree of these changes leading to uncontrollability, chronicity and favouring desensitization. In present study, the alterations were reversed by CIT, one of the most selective SSRIs with minimal effects on noradrenaline reuptake. Serotonin has been shown to influence the mesolimbic dopamine system and neuroanatomical data point to an interaction between serotonergic and mesolimbic dopamine systems (Parent, Descarries et al. 1981; Steinbusch, Nieuwenhuys et al. 1981; Meltzer 1990). Moreover, there is evidence that sensitization of D2 dopamine receptors in the mesolimbic dopamine system may be central to the clinical action of SSRIs (Willner, Hale et al. 2005).

In the present study, we could not avoid some methodological issues regarding the performance of the sucrose preference test in rats being treated with CIT. For the tests, the drug was dissolved both in the sucrose solution and in the plain water, to keep the consistency of the treatment. Because of its presumptively aversive taste, CIT might have also resulted in slight

disturbances in palatability of the sucrose solution. Reduced palatability might be the cause for the slight reduction in sucrose preference observed in control animals treated with CIT, and reduced palatability due to the aversive taste of the drug could also explain the delayed recovery effect in stressed animals. However, after chronic CIT administration, both groups of animals (stressed and controls) showed similar sucrose preference, only slightly below the control level. To summarize, even if the taste of CIT impaired performance in the sucrose test to a minor degree, the antidepressant effects of this drug were apparent in the socially stressed animals.

The forced swimming test (FST) that was developed by Porsolt and colleagues in rats and subsequently used in mice (Porsolt 1979) is the most widely used tool for pre-clinical assessment of antidepressant activity. It is based on the observation that rats, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable cylinder of water. If they are reintroduced into the testing cylinder 24 hours later, they resume this posture faster. The immobility is thought to reflect either failure of persistence in escape-directed behaviour and/or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli. Shortened immobility time after antidepressant treatment reflects antidepressive activity of the compound. In the original test, when antidepressants were given between the two trials, the rats actively persisted engaging in escape-directed behaviours for a longer time than after vehicle treatment. In the present study, socially stressed rats showed increased immobility time in the FST compared to control animals, while immobility time was normalised in stressed animals chronically treated with CIT. In contrast, CIT had no effect on immobility in control animals. The interpretation of the test performed in present study is based on the assumption that prolongation of the immobility phase caused by psychosocial stress indicates depressive-like behaviour (D'Aquila, Panin et al. 2004; Gregus, Wintink et al. 2005) and may be interpreted as a symptom of motivational deficits and behavioural despair.

In the past, one of the major drawbacks of the forced swimming test was that SSRIs, which currently are the most widely prescribed antidepressants, did not reliably reduce immobility and were considered as a false negative in this test (Detke and Lucki 1996). A modified version of FST

was therefore proposed where serotonin-related compounds, such as SSRIs, increase the swimming behaviour instead of reducing immobility (Detke and Lucki 1996). The present results, however, indicate that the SSRI CIT can actually reduce immobility time, but that this effect is observed in stressed animals only. This observation may suggest that, for the screening of antidepressant activity of SSRIs in the FST, animals must be tested under conditions mimicking depressive-like states.

The other drawback of the classical FST is that acute antidepressant treatments are sufficient to increase the swimming behaviour, whereas chronic treatment is required for a clinical response. In the present study, the drug was administered for a period of four weeks. Thus, the observed anti-immobility effect might also have been triggered by the chronicity of the treatment. Detke and co-workers (Detke, Johnson et al. 1997) reported that low doses of chronic desimipramine and FLX in otherwise untreated rats reduced immobility time. Present data may suggest that stress conditions are necessary to reveal anti-immobility effects of CIT in the FST.

Although all experimental animals became less active over time (probably due to unavoidable habituation to repeated testing), socially stressed rats showed significantly lower locomotor and exploratory activity compared to unstressed controls. Citalopram treatment abolished these changes while having no effect in control animals. The latter observation is in line with previous reports indicating weak or no effects of CIT on open field activity in unstressed, naive animals. For example, neither single nor repeated administration of CIT affected locomotor activity in mice (Maj, Rogoz et al. 1983). Similarly, long-term administration of CIT had little effect on spontaneous exploratory activity of rats (Plaznik and Kostowski 1985; Kugelberg, Apelqvist et al. 2002). Only very few studies investigated the effects of CIT on open field activity in stressed animals, and these reported mixed results. Keeny and Hogg showed that chronic (three weeks; 20 mg/kg/day) CIT treatment reversed psychosocially induced deficits in exploration behaviour in mice (Keeney and Hogg 1999) while no effect of CIT was observed in a similar study (CIT 10 mg/kg twice daily (Kudriavtseva, Bakshtanovskaia et al. 1992). These divergent effects might be due to a different social stress procedure in the later study, characterized by a persistent traumatic situation resulting from the permanent presence of an

aggressor. Moreover, the use of a suboptimal dose of CIT in that study cannot be excluded.

4.3 Effects of fluoxetine

The antidepressant, antiobsessional, and antibulimic actions of fluoxetine are presumed to be linked to its ability to inhibit the neuronal reuptake of serotonin. Fluoxetine selectively inhibits the reuptake of serotonin into brain synaptosomes and platelets in rats and humans. In receptor binding studies, fluoxetine was shown to have only weak affinity for various receptor systems, namely opiate, dopaminergic, $\alpha_1$adrenergic, β-adrenergic, $\alpha_2$adrenergic, histaminergic, muscarinic and serotonergic 5-HT_1 and 5-HT_2 receptors. Unlike most clinically effective antidepressants, fluoxetine did not down-regulate β-adrenergic receptors; however, like all tested antidepressants, it caused up-regulation of GABA B receptors. Mixed effects have been observed on serotonergic receptor sensitivity

The following study was designed for further pharmacological validation of chronic social stress model as a model of depression in rats. The former studies revealed that repeated exposure of male rats to chronic social stress regime influences their sensitivity to rewards and causes motivational deficits and that these effects are reversible by CIT treatment. Based on the obvious 'face validity' and to further investigate the predictive validity of the model, present study addressed the question how well stressed rats respond favourably to the SSRI FLX. The results confirmed earlier observations (Rygula, Abumaria et al. 2005) that socially stressed animals show impaired locomotor and exploratory activity and decreased preference to sweet sucrose solution. Moreover these effects were associated with reduction of body weight gain, lowered plasma levels of testosterone and increased adrenal weight being physiological correlates of the stress response. To mimic a realistic situation of antidepressant intervention, we started treating the animals after stress-induced alterations had been established such as reduction in locomotor and exploratory activity. Four weeks of daily treatment with the FLX reversed the majority of these adverse effects on behavioural level. Importantly, FLX did not influence the behaviour of control animals. This is in agreement with the effects of the drug in healthy human and animal subjects. The drug

monitoring performed at the end of the experiment allowed to determine plasma levels of FLX and its metabolites at which the particular behavioural and physiological effects were observed. After four weeks of oral treatment the plasma concentrations of the norFLX were within the human therapeutic range (Hiemke, Dragicevic et al. 2004) although the parent drug was not detectable. The low plasma levels of the drug may explain limited though still apparent effects of fluoxetine on sucrose preference.

Stress in known to suppress the subsequent performance of rewarded behaviours. Chronic sequential exposure to unpredictable stressors in rats has been found to reduce consumption of and preference for highly palatable sweet solutions, to impair the conditioned place preference acquisition and to increased reward thresholds in a brain stimulation paradigm (for review see (Willner 1997)). The decreased sensitivity to reward observed in these experiments was claimed to be homologous to anhedonia, the inability to experience pleasures. Recently it was postulated that socially induced reduction in sucrose preference may be considered as a behavioural correlate of the depressive-like symptom (hedonia) (Rygula, Abumaria et al. 2005). The results of presents study confirm and extend this hypothesis. FLX – a SSRI drug being widely used for the treatment of depression in humans (Wong, Bymaster et al. 1995) significantly reduced the deficits in sucrose preference in socially stressed animals. Since the validity of animal models of depression depends not only on the similarity of behavioural alterations with depressive-like symptoms in humans (face validity) but also on effectiveness of antidepressant treatment to reverse the induced behavioural deficits (predictive validity) (Willner 1995) this observation provides support for validity of chronic social stress as a model of depressive-like symptoms in rats. Importantly, the time course of FLX effects resembled that observed in human patients since clinical use of FLX requires usually between 2 and 4 weeks prior to observing therapeutic response (Mischoulon 1997). The first therapeutic effects of FLX on stress-induced reduced sucrose intake (anhedonia) were observed after the 2nd week of the treatment and became more pronounced (however statistically not significant) after three weeks of application. In contrast, the socially stressed but untreated animals showed a significant reduction in sucrose preference indicating hedonic deficits.

It had been suggested that the decreased sucrose preference is a consequence of alterations mainly in the mesolimbic dopaminergic system. Based on the review by Cabib & Puglisi Allegra 1996 (Cabib and Puglisi-Allegra 1996) the sensitivity of mesolimbic dopamine reward system is changed by prior stress experiences. Moreover, duration and nature of the stressor determine the direction of these changes with uncontrollability and chronicity of the stress favouring desensitisation. Thus, chronic psychosocial stress being highly uncontrollable is very likely to impair mesolimbic dopaminergic sensitivity. This hypothesis is supported by previous findings (Rygula, Abumaria et al. 2005) and those of von Frijtag et al (Von Frijtag, Reijmers et al. 2000) suggesting defeat induced hedonic deficits. As shown in the present study, these alterations can be reversed by a drug having primary molecular mechanism of action selectively on the serotonergic neurons. Serotonergic neurotransmission is hypothesized to be involved in motivational processes and reward related behaviours (Miliaressis 1977; Hillegaart, Ahlenius et al. 1991). Nevertheless, the exact role of serotonin on reward processes is not clarified yet. One explanation of this phenomenon may implicate a direct interaction of the brain 5-HT systems with neuronal reward related mechanisms. Indeed, the neuroanatomical substrate for such an interaction between serotonergic and mesolimbic dopaminergic system has been confirmed in several studies. The ventral tegmental area and nucleus accumbens — both main structures of the brain reward system - receive inputs from 5-HT axon terminals originating in the raphe nuclei (Parent, Descarries et al. 1981; Steinbusch, Nieuwenhuys et al. 1981). Serotonergic neurons have been reported to directly terminate on dopaminergic cell bodies and to influence mesolimbic dopamine activity through 5-HT_2 receptors (Meltzer 1990). Moreover, microinfusions of 5-HT into the ventral tegmental area enhanced the release of dopamine in the nucleus accumbens (Fuller 1994). Another plausible explanation implies other than SSRI properties of FLX. Although studies on synaptosomal uptake of monoamines *in vitro* suggest that SSRIs selectively inhibit 5-HT uptake rather than that of other monoamines, microdialysis experiments *in vivo* show that SSRIs are not entirely selective for 5-HT (Perry and Fuller 1992). Systemic administration of 10 mg/kg FLX, a dose commonly used in behavioral and neurochemical experiments (Joly and Sanger 1986; Paez and Leibowitz 1993; Reneric and

Lucki 1998) significantly raised extracellular concentrations of serotonin, dopamine and noradrenalin in the prefrontal cortex of awake rats (Jordan, Kramer et al. 1994; Tanda, Carboni et al. 1994). Tanda et al also reported an increase of extra-cellular dopamine in the prefrontal cortex rats treated acutely with 10 mg/kg FLX (Tanda, Carboni et al. 1994). Although the effects of FLX in other dopamine - rich regions such as the striatum and nucleus accumbens are less clear because no change or a decrease has been reported (Perry and Fuller 1992; Tanda, Carboni et al. 1994; Clark, Ashby et al. 1996), FLX may also exert its effects directly on mesolimbic dopaminergic neurons within brain reward systems. Finally, since the antidepressant effects of FLX develop after two to three weeks of chronic treatment in humans (Wong, Bymaster et al. 1995), a transient increase in extracellular serotonin level may not be directly responsible for the therapeutic action of FLX. The delay in onset of antidepressant action may reflect adaptive changes in 5-HT neurotransmission induced by chronic FLX leading to neuroadaptations in brain reward system (Artigas 1993; Blier and de Montigny 1994; Markou, Kosten et al. 1998).

Following above hypothesis the antidepressant reversible reduction in locomotor and exploratory activity following chronic stress exposure may be also related to depressive-like hedonic deficit. Since it is assumed that an inescapable open field situation reflects both the stress and the rewarding component of novelty, reduced locomotor and exploratory activity of socially stressed rats in a novel environment might reflect decreased motivation or drive, behaviour representing "refractory loss of interest" (Roth and Katz 1979; Katz, Roth et al. 1981) since novelty is rewarding (Bevins and Bardo 1999; Bardo and Dwoskin 2004). As mentioned before the decreased exploration of a novel environment may be also related to elevated levels of anxiety in stressed animals however in present experimental design no changes in anxiety level were observed. Moreover behavioural despair in the forced swimming test observed previously (Rygula, Abumaria et al. 2005) as well as the decrease in preference for sucrose solution suggest rather motivational deficits than altered anxiety levels. On the other hand one has to consider that FLX reversed nearly completely the stress-induced reduction in rearing behaviour. Some studies reported rearing to be an anxiety-like behaviour and 5-HT agonists were reported to have anxiolytic properties

(Brocco, Dekeyne et al. 2002), this effect of FLX might reflect its anxiolytic profile. Indeed FLX pre-treatment was reported to diminish social defeat-induced anxiety in Lewis rats (Berton, Durand et al. 1999) In other studies, however, FLX did not yield anxiolysis in rats, as assessed by elevated plus maze and open field tests (Douglas, Varlinskaya et al. 2003)

In conclusion, the adverse behavioural effects of chronic social stress in male rats can be counteracted or at least reduced by chronic treatment with the SSRI FLX. Although the effects of FLX on depressive-like effects suggested predictive validity, the paradigm required further validation to become a valid model for research on the aetiology and pathophysiology of depression.

4.4 Effects of reboxetine

Reboxetine is one of the newer antidepressants, belonging to the category known as selective norepinephrine reuptake inhibitors (SNRI). Reboxetine selectively and potently inhibits norepinephrine reuptake in CNS. The drug has been shown to have negligible affinity for serotonin and dopamine uptake sites and for adrenergic and histaminergic receptors, and only weak affinity for muscarinic receptors.

Reboxetine profoundly ameliorated the effects social stress induced anhedonia having no effects on control animals. The slight decrease in preference to sucrose solution observed in control RBX treated animals may be explained by aversive taste of the drug being present during the tests. There exists almost no experimental evidence regarding the effects of RBX in reward related paradigms. Some studies showed that RBX treatment can diminish nicotine self-administration (Rauhut, Mullins et al. 2002). Noradrenergic neurons of the locus coeruleus send projections directly to the ventral tegmental area (Phillipson 1979) and to the shell region of the nucleus accumbens (Delfs, Zhu et al. 1998). Additionally, the locus coeruleus sends indirect projections to the ventral tegmental area via the hippocampus (Lindvall, Bjorklund et al. 1983). Stimulation of locus coeruleus neurons modulates the activity of ventral tegmental area dopamine neurons (Grenhoff, Nisell et al. 1993), suggesting that the noradrenergic system interacts with the mesolimbic dopamine system, potentially contributing to reward. Systemic administration of RBX has also been reported to increase norepinephrine

release in the hippocampus and frontal cortex (Sacchetti, Bernini et al. 1999). In a recent report, RBX was shown to increase the burst firing pattern, but not the average firing frequency, of ventral tegmental area dopamine neurons; however, systemic administration of RBX increased dopamine release in the prefrontal cortex but, paradoxically, not in nucleus accumbens (Linner, Endersz et al. 2001). Since increased dopamine release in the nucleus accumbens is generally considered critical for reward, these observations suggest that RBX would not serve as a reinforcer. However, if RBX exerts an inhibitory influence on accumbal dopamine release via a modulatory noradrenergic system, then this could be an indirect mechanism to explain the effects of RBX on sucrose preference observed in the present study.

A few studies before have examined the effects of RBX in the rat FST and those reported a robust antidepressant activity of the compound (Connor, Kelliher et al. 1999; Harkin, Kelly et al. 1999; Wong, Sonders et al. 2000; Cryan, Page et al. 2002). Like in these other studies, also in the present study RBX reduced immobility in the rat FST. Interestingly, in contrast to CIT the antidepressant effect was observed not only in the stressed animals but also in control rats. This is in agreement with previous studies reporting where RBX treatment (10 and 30 mg/kg) in naïve animals, significantly decreased immobility and defecation in the FST in dose dependent manner (Connor, Kelliher et al. 1999). In the same study, in addition to the behavioural activity, RBX was shown in a dose-dependent manner to attenuate swim stress-induced increases in serotonergic and dopaminergic activity occurring in a region specific manner.

The role of norepinephrine in mediating the actions of antidepressants has largely focused on locus ceruleus structure due to its extensive limbic innervations. Indeed electrophysiological studies have shown that antidepressants from various classes can impact on locus ceruleus discharge rates (Valentino, Curtis et al. 1990; Curtis and Valentino 1991). The role of norepinephrine arising from the other nuclei in mediating antidepressant action has largely been uninvestigated (for review see (Stanford 1995).

The effects of RBX on locomotor and exploratory activity are in agreement with reports describing its antidepressant like action in other animal models of depression (Connor, Kelliher et al. 1999; Harkin, Kelly et al.

1999; Wong, Sonders et al. 2000). Moreover they agree with those from olfactory bulbectomy (OB) model of depression in rats where chronic (14 days) RBX (10 mg/kg) treatment attenuated the OB-related behavioural hyperactivity in the open-field test (Harkin, Kelly et al. 1999). In that study, examination of the onset of the antidepressant effect in the open-field test demonstrated that RBX (10 mg/kg) reduced the behavioural hyperactivity after 14 days, following 3, 7 or 10 days of treatment (Harkin, Kelly et al. 1999). In other studies RBX did not significantly modify locomotor activity of mice exposed to a novel environment and at several doses locomotor activity was even significantly reduced (Brocco, Dekeyne et al. 2002). RBX reduced also locomotor activity (LA) in naïve rats placed in an activity chamber (Harkin, Kelly et al. 1999). At the highest doses it elicited modest ataxia in the rotarod test. Similarly several other SNRIs such as desipramine, maprotiline, nisoxetine and nortiptiline failed to elevate LA in mice exposed to novel environment and the latter actually reduced LA. Nortriptiline also elicited marked ataxia in rotarod procedure (Brocco, Dekeyne et al. 2002). The noradrenergic system is critically linked to stress and is, therefore, a likely substrate to target for therapeutic intervention. The noradrenergic system, especially that arising from the L.C., is activated by a wide variety of stressors (Haidkind, Eller et al. 2003; Jedema and Grace 2003; Featherby and Lawrence 2004). The neurons of the L.C. are sensitive to changes in the internal and external environment, especially when these cues are salient for the organism’s survival. All reports show that systemic administration of RBX results in an increase in extracellular NE and a smaller increase in extracellular DA with little or no effect on extra cellular 5-HT.

Since DA is also a substrate for reuptake by the NE transporter (Povlock and Schenk 1997) the transport of DA into the noradrenergic terminals may play a role in clearance of DA in regions such as mPFC, where dopaminergic and noradrenergic terminals overlap with comparable density (Cass and Gerhardt 1995; Levitt and Moore 1979). Indeed the NE transporter may be the primary mechanism of DA reuptake in frontal cortex (Moron, Brockington et al. 2002), and drugs that block NA transporter such as RBX, may also prevent the uptake of extracellular DA in such areas as PFC but not in the nucleus accumbens (Carboni, Tanda et al. 1990; Tanda, Carboni et al. 1994; Millan, Lejeune et al. 2000; Linner, Endersz et al. 2001). Chronic

administration of SNRI potentates behaviours typically associated with pharmacological activation of mesolimbic dopaminergic neurotransmission in nucleus accumbens (Bonhomme and Esposito 1998). The mechanism of this effect is not well understood and may be related to a variety of regulatory factors, including changes in DA receptor sensitivity or sensitisation of the excitability of DA neurons in the VTA (Stewart and Rajabi 1996; Ainsworth, Smith et al. 1998; Bonhomme and Esposito 1998). The mechanism for this is apparently distinct from that involved in elevating DA neurotransmission in the mPFC, as baseline levels of extracellular DA are not increased in the nucleus accumbens by antidepressant treatment (Tanda, Carboni et al. 1994; Stewart and Rajabi 1996; Reith, Li et al. 1997). Nevertheless these effects may be important in the behavioural response to antidepressants. The enhancement of DA activity in cortical and limbic areas by SNRI such as RBX may contribute to improvement of certain deficits, increasing motivation and the ability to experience pleasure and reward.

It is worth to notice that similar to previous studies with CIT, the animals treated with antidepressant RBX in their drinking water consumed less fluid than rats receiving only water. This effect is likely evoked by aversive taste of the drug however since similar way of HAL administration did not change fluid consumption, it is possible that diminished fluid intake was due to minor gastrointestinal disturbances induced by RBX. Such gastrointestinal disturbances might lead to reduced food intake and, because rats are prandial drinkers, also to lower water intake. RBX-treated rats gained significantly less weight than controls, an effect that was also observed in a previous human studies (Poyurovsky, Isaacs et al. 2003; Lu, Kupa et al. 2005)

In humans, at therapeutically effective doses, plasma concentrations of RBX are in the range of 10–100 ng/ml (Baumann et al. 2004). In the present study the RBX dose necessary to reach similar plasma concentrations in rats was determined in a pilot study. Because daily oral application of the target dose 40 mg/kg for five consecutive days resulted in the average RBX serum concentration of about 12 ng/ml, this dose was chosen to treat the experimental animals. Interestingly, drug monitoring performed at the end of the four weeks of treatment revealed average plasma levels of RBX to be more than double that found in the pilot experiment after five days of drug treatment, suggesting time-dependent accumulation during long-term

treatment. In spite of these shortcomings, the present data show that the animals were treated with sufficiently high doses of the drug to attain antidepressant effects.

4.5 Effects of haloperidol

Haloperidol due to its strong central antidopaminergic action is classified as a highly potent neuroleptic. It is approximately 50 times more potent than chlorpromazine on a weight basis. Haloperidol possesses a strong activity against delusions and hallucinations, most likely due to an effective dopaminergic receptor blockage in the mesocortex and the limbic system of the brain. Haloperidol blocks the dopaminergic action in the nigrostriatal pathways and has also antihistaminic and anticholinergic properties. Mixture of these effects is the probable reason for the high frequency of extrapyramidal motoric side-effects (dystonias, akathisia, pseudoparkinsonism). Haloperidol also has sedative properties and displays a strong action against psychomotor agitation, due to a specific action in the limbic system and is therefore an effective treatment for mania and states of agitation.

In present study, four weeks of oral treatment with HAL resulted in average plasma drug concentrations of about 5 ng/ml. This is in the recommended therapeutic range in human (5- 17 ng/ml) for treatment of psychosis (Baumann, Hiemke et al. 2004).

Haloperidol had robust, negative influence on sucrose preference in both groups of treated animals. This effect is explainable by blockade of central dopaminergic receptors. Indeed since HAL decreases locomotor activity by blockade of D2 receptors (Janssen 1967; van den Buuse and de Jong 1989) the very same effects are likely to occur in mesolimbic dopaminergic system being responsible for reward. Similar effects were observed in other reward related behavioural paradigms such as intracranial self stimulation (ICSS) (Borowski and Kokkinidis 1992). In these studies HAL administration elicited pronounced reduction in ICSS already after 3 days of treatment and these changes translated into increased thresholds after 9 and 12 and 21days. Moreover, a pronounced HAL induced reduction of ICSS was evident even after 50 days after cessation of treatment. From other hand, HAL has been shown to have a week protective effects against the stress induced anhedonic state (Orsetti, Colella et al. 2005). In contrast, (Papp, Moryl et al. 1996) reported

that HAL is devoid of antidepressant properties in CMS model of depression. The apparent discrepancy between present study and those described above can be explained, at least in part, taking into account that different doses have been employed (0.2 mg/kg/day and 1.0 mg/kg/day vs. 2 mg/kg/day in present study). Interestingly in other studies by (Papp and Wieronska 2000) the stress induced decrease in sucrose consumption was rapidly reversed by administration of 5 or 10 mg/kg of the antipsychotic amisulpride, whereas higher doses of the drug were ineffective. As indicated by other authors (Lecrubier, Boyer et al. 1997) the antidepressant effects of amisulpride appeared in the dose range lower than that used to treat schizophrenia and probably reflect the selective blockade of presynaptic dopamine receptors. Thus the weak antidepressant effects of low dose HAL observed by (Orsetti, Colella et al. 2005), lack of effects observed by (Papp, Moryl et al. 1996) and "anhedonic" effects observed in present study may be explained in light of these considerations.

Immobility in the swim test may be reversed not only by antidepressants, but also by D2/D3 receptor agonists, applied systemically or to the nucleus accumbens (Willner 1997). Conversely, a number of studies have reported that antidepressant effects in the swim test were reversed by DA antagonists (Willner 1997); these include studies in which antidepressants were administered chronically. Dopaminergic drugs, especially all dopamine mimetic substances tested, reduced immobility time (Porsolt, Anton et al. 1978; Porsolt 1979; Zebrowska-Lupina, Kozyrska et al. 1980; Borsini, Bendotti et al. 1981; Araki, Kawashima et al. 1985; Duncan, Paul et al. 1985; Kitada, Miyauchi et al. 1986). However since they also increase motor activity, it is difficult to ascertain whether or not their effectiveness was ascribable to this property. Dopamine blocking agents were either inactive or increased immobility time (Porsolt, Anton et al. 1978; Porsolt 1979; Kitada, Miyauchi et al. 1983; Vaccheri, Dall'Olio et al. 1984). Neuroleptics did not reduce immobility time over 20% even after a chronic treatment (Gorka and Janus 1985; Kawashima, Araki et al. 1986). In the present study the neuroleptic drug HAL not only failed to shorten immobility time in FST but showed itself depressive like properties prolonging significantly immobility behaviour. This is in agreement with previous studies. (Weiner, Schiller et al. 2003) showed that HAL increases immobility in FST, similar results were obtained by (Steru,

Chermat et al. 1987) in tail suspension test. In the study by (Kawashima, Araki et al. 1986) chronic administration of chlorpromazine, HAL and DZP enhanced the duration of immobility, whereas their acute administration had no effect on it. Like stimulant drugs, neuroleptics (in low doses) are sometimes used in the management of delusional depression, but their efficacy as antidepressants is questionable. Indeed depression as a side effect of neuroleptic therapy (Randrup and Munkvad 1975; Siris, Bermanzohn et al. 1991) and antidepressant effects on withdrawal of neuroleptics (Randrup and Munkvad 1975; Del Zompo, Bocchetta et al. 1986) are both well documented.

HAL exerted its effects on rat behaviour rapidly and intensively, characteristics that have been reported earlier by (Rupniak, Jenner et al. 1986; Lynch 1990; Paulus and Geyer 1991). The drug treatment strongly affected locomotor activity and measured elements of exploratory behaviour in open field. It is known that treatment with HAL decreases both rat locomotor activity (Patacchioli, Di Grezia et al. 1996) and amphetamine or apomorphine (Magnus-Ellenbroek and Havemann-Reinecke 1993) induced hyperactivity behaviour. The blockade of locomotor activity may be due at least partly to the inhibition of dopaminergic transmission in the limbic forebrain (Jorgensen 2004). Indeed, also other studies suggested that decreased locomotor activity was associated with the excess of dopamine in the synapses due to the blockade of the D2 dopamine autoreceptor (Garris, Budygin et al. 2003). Reid et al. (Reid and Turner 1998) also found that typical neuroleptics have a high correlation between catalepsy and D2 receptor binding in the striatum, which has been identified as the key brain structure for causing neuroleptics-related locomotor deficiencies. Vasconcelos et al. (Vasconcelos, Nascimento et al. 2003) reported that high doses of HAL decreased locomotor activity, which could be due to inhibition of dopaminergic action in the limbic forebrain. Although HAL is able to stimulate the release of dopamine in the striatum (Westerink, Kawahara et al. 2001) and in nucleus accumbens (Liegeois, Ichikawa et al. 2002) via acting on presynaptic D2 receptor, antipsychotic properties have been best correlated with blockade of post synaptic D2 receptors. Summarizing, in the present model HAL was not only devoid of antidepressant treatment but even worsened the stress induced adverse changes.

4.6 Effects of diazepam

The therapeutic effects of diazepam are a result of its effect on CNS GABA activity. Diazepam and the other benzodiazepines appear to either enhance or facilitate GABA activity by binding to the benzodiazepine receptor, which is part of a complex including an aminobutyric acid receptor, benzodiazepine receptor, and barbiturate receptor. Binding to the complex results in increased CNS inhibition by GABA. The anticonvulsant and other effects of diazepam are believed to be produced by a similar mechanism, possibly involving various subtypes of the receptor.

Several reports from clinical studies indicated that antidepressants can be effective in treating anxiety and that a number of anxiolytic drugs have antidepressant actions, leading to the suggestion that there may be a common underlying mechanism between these two conditions. To evaluate the fear related compounds in the chronic social stress paradigm, the effects of DZP on sucrose preference, forced swimming, locomotor activity/exploration and performance in elevated plus maze have been tested. In order to mimic clinical conditions and to avoid its addictive effects, DZP was administered to experimental animals only acutely at the end of 5 weeks period of social stress. Three injections of DZP (1 mg/kg i.p.) during 24hr sucrose preference test performed after 5 weeks of social stress had no effects on sucrose preference neither in stressed nor in control animals. This result is in line with other studies in rats (Muscat, Papp et al. 1992) indicating lack of beneficial effects of DZP on sucrose preference. The results obtained in elevated plus maze indicate that the dose used in present study was effective, exerting anxiolytic effects on naïve rats. The lack of effects of DZP on sucrose preference shows that the stress induced reduction in sucrose preference is not anxiety related.

Diazepam (1 mg/kg i.p.) had no significant influence on behaviour of rats in FST. This is in agreement with studies of (Marti and Armario 1993) where acute DZP administration did not modify the behaviour of rats in the test. Also sub-chronic administration (6 days) of two different doses of DZP did not alter the behaviour of rats in the FST, but significantly decreased the defecation rate, suggesting that the drug was effective as an anxiolytic (Marti and Armario 1993). In other studies chlordiazepoxide and DZP were ineffective (Porsolt 1979; Ogawa, Mizuno et al. 1984; Duncan, Paul et al. 1985) DZP was

ineffective even after chronic treatment (Kawashima, Araki et al. 1986). In studies of (Nishimura, Ida et al. 1989) similar like in studies by (Nagatani, Sugihara et al. 1984) DZP caused prolongation of immobility time. However DZP was reported to potentate antiimmobility effects of desipramine (Flugy, Gagliano et al. 1992). From other hand the anti-immobility effect of the tricyclic antidepressants, desipramine and imipramine (16-32 mg/kg) were antagonized by the acute co-administration of a benzodiazepines, DZP (0.25-2 mg/kg) and lorazepam (0.125 mg/kg) (Van der Meersch-Mougeot, da Rocha et al. 1993). This range of effects may be related to the dose used (high doses induce non-specific sedative effects) and also to subtle differences in species or in procedures. It was also suggested that the genetic background of the animals maybe responsible for the observed variations (Shephard, Nielsen et al. 1982; Tamborska, Insel et al. 1986).

During stress conditions brain benzodiazepine receptors appear to participate in the physiological regulation of adrenocortical and neurosympathical activity (De Boer, Van der Gugten et al. 1990)(deboer 90). Numerous reports indicated that benzodiazepine receptor ligands with anxiolytic actions like DZP can prevent and oppose the stress induced activation of HPA and sympathetic adrenomodullatory system (Lahti and Barsuhn 1975; File 1982; Pericic, Lakic et al. 1984; De Boer, Van der Gugten et al. 1990). In present study DZP had no significant effects on locomotor activity of control animals however evoked slight effects on locomotor activity and rearing in stressed animals. (Fernandes, Arnot et al. 1999) reported similar effect in naïve animals at the dose of 2 mg/kg. These results may suggest existence of fear related compound in social stress induced behaviour. However this hypothesis is opposed by the results from elevated plus maze test where DZP had no anxiolytic effects on stressed animals. It is worth to notice that in order to minimize masking of the anxiolytic effects of DZP by its sedative effects, injections were administered 30 min prior to testing (Dailly, Hascoet et al. 2002). Further detailed studies should determine to which extend fear related behaviour constitutes to the effects of chronic social stress.

The elevated plus maze is one of the most widely employed behavioural screening assays for anxiolytic agents in rodents. The elevated plus maze is based on the creation of a conflict between the exploratory drive of rat and its innate fear of open exposed areas. It is one of the most widely used animal

models of anxiety and has been extensively validated pharmacologically and ethologically (Pellow 1986; Pellow and File 1986; Dawson and Tricklebank 1995). In the present study DZP reduced anxiety of control animals as measured by increased number of entries and increase in time spent in open arms of the experimental apparatus. Interestingly, the drug had no effects on stressed animals. Since stress itself had also no effects on anxiety this is interesting observation suggesting some silent effect of stress preventing DZP induced anxiolysis. That observation is in agreement with studies showing that exposure even to a brief stressful event prevents the behavioural effects of DZP (Antelman, Kocan et al. 1987).

4.7 Corticosterone and testosterone

Among the more consistent observations in patients with major depression is dysfunction of the HPA axis (Holsboer 1983; Rubin 1987). This correlation between the hypersecretion of cortisol and depression is one of the oldest observations in biological psychiatry – at least in a sub-population of depressed patients - and normalizes upon successful therapy (Holsboer and Barden 1996). Elevated levels of corticosteroids have been observed in about 50% of depressed patients (Checkley 1996) and a normalization of corticosteroid levels is seen in patients treated with antidepressants (Kasper, Vieira et al. 1990). In line with these findings in the present study it has been assumed that increased corticosterone levels may support the hypothesis that observed behavioural alterations are correlates of depressive symptoms in humans. Following this hypothesis the decrease of social stress elevated levels of corticosterone by antidepressant treatment would be likely the additional proof for predictive validity of the present model. Although all presented here studies consequently failed to show any differences in basal corticosterone plasma levels between control and socially stressed rats, the results from dexamethasone suppression test show a diminished response to peripheral DEX challenge in stressed animals. Furthermore the stressed animals consequently showed hypertrophy of adrenal glands and decreased testosterone levels suggesting hyperactivity of HPA axis. The possible explanation of these contradictory results includes habituation to social stress on a basal level but increased sensitivity to subsequent challenges in socially stressed rats. From other side, social isolation of control animals might cause uncontrollable stress- in fact the levels of corticosterone in control animals

were in some of the present studies relatively high. A few studies showed that isolation results in anxiety followed by elevated corticosterone levels (Bartolomucci, Palanza et al. 2003; Weiss, Pryce et al. 2004). It is possible that daily exposure of rats to a social stressor replaces the daily social interaction of which the isolated control animals were deprived. Furthermore several studies showed that some mild stressors may be even anxiolytic (Roth and Katz 1979; D'Aquila, Brain et al. 1994; Morato and Brandao 1997). One can postulate that short term defeats were not sufficiently strong to induce persistent corticosterone elevation while social isolation in control animals elevated corticosterone levels to the amounts comparable with stressed animals. This hypothesis however does not explain hypertrophy of adrenal glands observed in stressed animals. Another plausible explanation would include a stress induced alteration in circadian rhythm followed by change in hormone profile. Plasma levels of corticosterone display a circadian rhythm, with the higher values occurring during the dark phase in nocturnally feeding animals. Stressful situations induce a rise of corticosterone levels and this endocrine response to stress also presents circadian variations. The higher increase of corticosterone in response to stress occurs when the hormone is in its lower circadian level, and the minimum responses occurring at the peak The available literature data on circadian rhythms in animals subjected to stress are limited although changes in daily rhythms of activity, body temperature and heart rate under conditions of chronic stress have been found in rats (Kant, Bauman et al. 1991; Meerlo, De Boer et al. 1996) and tree shrews (Fuchs and Flugge 2002). It has been shown that social defeat causes profound decreases in amplitude of the rhythm of body temperature and heart rate lasting for 10 days (Tornatzky and Miczek 1993). Furthermore social defeat causes disturbances in the pattern of vigilance states, i.e. sleep and wakefulness (Meerlo, Pragt et al. 1997). It is known that sleep and waking as well as correlated with them body temperature and activity exert a direct effect on corticosterone release (Franken, Tobler et al. 1992). Therefore although the blood sampling period for corticosterone analysis was equal for all animals, the stressed individuals could have been in a shifted phase of their circadian corticosterone rhythm. In other words the stress induced disturbance of sleep-wakefulness pattern followed by shift in circadian rhythm of corticosterone could have made the direct comparison between control and

stressed groups impossible. Further studies are necessary to elucidate this issue.

Activation of HPA axis by stress exerts inhibitory effects on hypothalamic pituitary gonadal (HPG) axis (Collu, Gibb et al. 1984; Collu, Gibb et al. 1984; Calogero, Burrello et al. 1999). Indeed also in present studies chronic social stress caused decrease in circulating testosterone levels. Increased glucocorticoid levels disrupt and suppress endocrine signalling in the male reproductive axis (Lamperti and Baldwin 1979; Suter and Schwartz 1985). Glucocorticoid levels in circulation rise sharply in response to stress, resulting in testicular involution and a significant drop in testosterone secretion. These changes are accompanied by diminished libido and fertility (Phillips, Lakshmi et al. 1989). A similar pattern is observed in rats subjected to immobilization stress (Monder, Sakai et al. 1994). All these changes in HPA-HPG related parameters suggest hyperactivation of HPA axis by chronic social stress. However the lack of effects of chronic social stress on circulating corticosterone level itself, demands further, detailed investigation.

4.8 Conclusion

In rats, adverse effects of chronic social stress can be reversed by chronic treatment with different classes of antidepressant drugs. Results of this study may help to fulfil all three (construct, face and predictive) criteria of validity for the rat chronic social stress paradigm as a model of depressive symptoms (Willner and Mitchell 2002). As discussed, the model may have construct validity based upon the fact that socio- or psychogenic factors play an important role in many cases of human depressive disorders. Furthermore, in rats, chronic social stress triggers the changes that could be considered behavioural correlates of depressive symptoms in humans (face validity). Finally, the effectiveness of antidepressant treatment in ameliorating socially induced behavioural disturbances as well as its selectivity (no beneficial effects of other classes of drugs) may be taken as an argument for the predictive validity of the present model as a model of depression.

5 Summary

In order to develop a model of chronic social stress in rats, in the first study the male *Wistar* rats were subjected to five weeks daily exposure to social defeat. This protocol resulted in a variety of behavioural changes in the rodents that might be regarded as behavioural correlates of depressive-like symptoms in humans. They are: 1) the prolonged immobility time in the forced swimming test symptomatic of decreased motivation and behavioural despair (Porsolt 1979); 2) the reduced preference for sweet sucrose solution, a putative indicator of anhedonia in rodents (Willner, Muscat et al. 1992; Moreau 1997); and 3) the reduced locomotor and exploratory activity suggesting changes in incentive motivation and emotionality (Roth and Katz 1979; Katz, Roth et al. 1981). Furthermore, these effects were associated with decreased body weight gain and increased weight of adrenal glands, both organic correlates of stress effects (Sapolsky, Romero et al. 2000).

Based on the obvious 'face validity' and to investigate whether this model also has 'predictive validity' the question has been addressed how well stressed rats respond favourably to the different classes of antidepressant drugs such as SSRI (CIT 30 mg/kg/day and FLX 10 mg/kg/day) or SNRI (RBX 40 mg/kg/day). To mimic a realistic situation of antidepressant intervention, the animals started to be treated after stress-induced alterations had been established such as reduction in locomotor and exploratory activity. Four weeks of daily treatment with all the tested antidepressants reversed the majority of adverse effects of chronic social stress at least on behavioural level. The drugs showed different profiles of action what is in agreement with a clinical data. Importantly, antidepressant drugs did not influence the behaviour of control animals. This is in agreement with the effects of antidepressant drugs in healthy human and animal subjects. The drug monitoring performed at the end of the experiment allowed to determine plasma levels of each drug and their metabolites at which the particular behavioural and physiological effects were observed. After four weeks of oral treatment the plasma concentrations of the drug were similar to those reported in human patients treated with therapeutically effective doses (Baumann, Hiemke et al. 2004; Baumann, Hiemke et al. 2004).

In the last part of the study that was designed to evaluate the specificity of antidepressant treatment, the animals were treated with neuroleptic drug haloperidol and anxiolytic diazepam. In order to mimic clinical situation, HAL (2 mg/kg/day) was administered chronically while DZP (1 mg/kg i.p.) was applied acutely. The treatment with HAL resulted in reduced locomotor and exploratory activity, decreased preference to sucrose solution and prolonged immobility time in forced swim test. The treatment worsened the adverse effects of chronic social stress having effects similar to stress on reward and motivation related behaviours. Treatment with DZP caused reduction of anxiety related behaviours as measured in elevated plus maze in control animals. However had no beneficial effects on socially stressed individuals. Neither sucrose preference nor performance in forced swim test was affected by DZP treatment. These results provided the proof for specificity of antidepressant treatment in reversing socially induced correlates of depressive symptoms in rats.

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