The Chronic Psychosocial Stress Paradigm in Male Tree Shrews: Evaluation of a Novel Animal Model for Depressive Disorders

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To improve our knowledge of the causal mechanisms of stress-related disorders such as depression, we need animal models that mirror the situation in patients. One promising model is the chronic psychosocial stress paradigm in male tree shrews, which is based on the territorial behaviour of these animals that can be used to establish naturally occurring challenging situations under experimental control in the laboratory. Co-existence of two males in visual and olfactory contact leads to a stable dominant–subordinate relationship, with subordinates showing distinct stress-induced behavioural and neuroendocrine alterations that are comparable to the symptoms observed during episodes of depression in patients such as constantly elevated circulating glucocorticoid hormones due to a chronic hyperactivity of the hypothalamic-pituitary-adrenal axis. To elucidate whether the chronic psychosocial stress model in tree shrews besides its "face validity" for depression also has "predictive validity", we treated subordinate tree shrews with the tricyclic antidepressant clomipramine and found a time-dependent restoration of both endocrine and behavioural parameters. In contrast, the anxiolytic diazepam was ineffective. Although the chronic psychosocial stress model in tree shrews requires further validation, it has sufficient face, predictive, and construct validity to become an interesting non-rodent model for research on the etiology and pathophysiology of depression.

Keywords: HPA axis; Clomipramine; Diazepam; Cortisol; Behaviour; Drug metabolism

INTRODUCTION

Depressive disorders are among the most common human diseases in that approximately 11% of all adult human beings experience a time period of depression at least once in their lives (Judd, 1995). Among the most potent factors that trigger or induce depressive episodes are stressful life events (Paykel, 1978; Anisman and Zacharko, 1982; Kessler, 1997). Stress responses are usually depicted as adaptive physiological and psychological processes activated in animals and man. They are regarded as alarm systems, which are initiated whenever there is a discrepancy between what an organism is expecting and what really exists (Levine and Ursin, 1991). Loss of control, lack of information or uncertainty about what will happen in future induce alarm reactions, whereas the presence of reliable information, control and social support reduce these alarm reactions. Therefore, the stress response *per se* cannot be regarded as harmful or pathological in itself. Only when demanding, prolonged and sustained, body and brain homeostasis can be

threatened, and health of the individual may be endangered. The stress hypothesis of mood disorders has led to the development of a number of putative animal models of depression (Willner, 1991; Yadid *et al.*, 2000).

Depressive disorders are a collection of symptoms which occur together with a sufficient frequency and chronicity to constitute a recognizable clinical condition. Patients suffering from major depression frequently show a phase shift in circadian activity patterns, early morning wakenings, psychomotor retardation or agitation, and appetite disturbances (Benoit *et al.*, 1985; DSM-IV, 1994; Raoux *et al.*, 1994). These symptoms closely match results from animal experiments designed to examine the effects of different stressors on behavioural patterns. In rats, brief social stress leads to dramatic changes in nocturnal behaviour (Blanchard and Blanchard, 1990), and a long-lasting decrease in the amplitude of the circadian patterns for heart rate and body-core temperature (Tornatzky and Miczek, 1993). In mice, psychosocial stress induces restricted activity and disturbances in urinary marking patterns with similar effects also reported for rats

TABLE I   Signs and symptoms of major depression (DSM-IV criteria, 1994) in comparison to effects of centrally administered CRH in laboratory animals, and to effects of chronic psychosocial stress in tree shrews (with modifications from (Owens and Nemeroff, 1991)

DSM-IV major depression	Effects of centrally administered CRH in laboratory animals	Effects of chronic psychosocial stress in tree shrews
Significant weight loss or weight gain when not dieting or decrease in appetite	Decreases food consumption in rats	Significant weight loss, reduced food and water intake
Insomnia or hypersomnia, early morning wakening	Disrupts normal sleep patterns with concomitant EEG changes	Disturbances in sleep patterns, early morning wakening
Marked diminished interest or pleasure in all or almost all activities most of the day, nearly every day	Diminishes sexual behaviour in male and female rats	Reduced activity of the gonads
Depressed mood most of the day, as indicated either by subjective account or observation by others	Mimics the behavioural despair syndrome observed after maternal separation in rhesus monkey infants	Reduced locomotor activity and grooming behaviour

(Desjardins *et al.*, 1973; Luciano and Lore, 1975). Rats, subjected to chronic unpredictable mild stress, show a reduced intake of and preference for saccharin or sucrose solutions. These findings seem to imply a defective reward system in the stressed animals, and is taken as a model of anhedonia, which is also a characteristic of depressed patients (Willner *et al.*, 1987; 1992). Although the above-mentioned approaches have been extremely useful in elaborating and detecting the effects of antidepressant drugs, one has to consider that animal models for psychiatric disorders such as depression should fulfil three major criteria: The first criterion is "face validity" and assesses how well the symptoms observed in the animals resemble those in human patients. The second criterion, "predictive validity", addresses the question how well animals in the model respond favourably to the same drugs as humans do under the same treatment conditions. The third criterion, "construct validity", assesses to what extent the model is consistent with the theoretical rationale (Willner, 1991).

However, only very few of the existing animal models satisfactorily meet the criteria for predictive, face and construct validity (Yadid *et al.*, 2000). Furthermore, some of the stressors used in animal studies bear little or no relationship to the biology of the species investigated (Koolhaas *et al.*, 1997).

THE CHRONIC PSYCHOSOCIAL STRESS PARADIGM IN TREE SHREWS—FACE VALIDITY FOR DEPRESSION

Chronic Stress Symptoms in Tree Shrews Resemble Symptoms of Depression

Rodents are frequently used to model human psychiatric disorders. In the recent years, however, evidence has accumulated that chronic psychosocial stress in a non-rodent species, the male tree shrew (*Tupaia belangeri*) may represent a suitable and naturalistic paradigm to study the causal mechanisms of stress-related disorders such as depression. From the phylogenetic point of view, the day active animals are regarded as an intermediate between insectivores and primates (Martin, 1990). DNA-sequences of genes for the glucocorticoid and the mineralocorticoid receptor (Meyer *et al.*, 1998), for corticotrophin-releasing factor receptors (Palchaudhuri *et al.*, 1998; 1999), and for the α_{2A}-adrenoceptor (Meyer *et al.*, 2000) revealed high homologies to the respective human genes which are in the range of 90–98% identity with the human sequences versus an average of 80% homology with the corresponding sequences in rats.

Tree shrews are widely distributed in South-East Asian forests and plantation areas where they live singly or in pairs in territories which they defend vigorously against intruding conspecifics. This pronounced territoriality, especially of the males, can be used to establish a naturally occurring challenging situation under experimental control in the laboratory. When living in visual and olfactory contact with a male conspecific by which it has been defeated, the subordinate tree shrew shows dramatic behavioural, physiological, and neuroendocrine changes. As revealed by detailed quantitative behavioural analysis, subordinates tend to withdraw from the field of vision of the dominant, reduce their locomotor activity, and cease auto-grooming behaviour (Aue, 1988). Their sleeping pattern is characterized by an increasing number of early morning wakening episodes (Aue, 1988) and their circadian rhythm is profoundly disturbed (Stöhr, 1986; Fuchs and Schumacher, 1990). In subordinates, the reduction of body weight is due to a diminished food and water intake and to a significantly elevated metabolic rate (Fuchs and Kleinknecht, 1986; Aue, 1988; Jöhren *et al.*, 1991; Kramer *et al.*, 1999). Analysis of endocrine parameters in subordinates revealed constantly increased concentrations of the adrenocortical hormone cortisol (Fuchs *et al.*, 1993), increased adrenal weights (Raab and Storz, 1976; von Holst *et al.*, 1983; Fuchs *et al.*, 1993), and reduced functions of the gonads (von Holst *et al.*, 1983; Fischer *et al.*, 1985). In the brains of subordinate tree shrews, 5-HT$_{1A}$-receptors are decreased in a time and region specific manner (Flügge, 1995) and α_2-adrenoceptors are

TABLE II Serum concentrations of clomipramine and its pharmacologically active metabolites in tree shrews after a treatment period of 5 days (50 mg/kg/day) and in depressed patients who had received daily oral doses of 150 mg for at least one week. Data (ng/ml) are given as means $\pm$ SD of three tree shrews and 10 patients

	Tree shrews	Patients
Clomipramine	176 $\pm$ 83	151 $\pm$ 36
Desmethylclomipramine	62 $\pm$ 10	163 $\pm$ 77
8-Hydroxyclomipramine	32 $\pm$ 14	42 $\pm$ 11
8-Hydroxydesmethylclomipramine	38 $\pm$ 6	86 $\pm$ 46

down-regulated in areas mainly involved in the regulation of autonomic functions (Flügge *et al.*, 1992). In the hippocampus, a brain structure which plays a crucial role in negative feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Herman *et al.*, 1993), and which is involved in spatial information processing as well as in aspects of learning and memory (Eichenbaum and Otto, 1992; Shen *et al.*, 1994), we recently demonstrated downregulation of glucocorticoid, mineralocorticoid and corticotrophin-releasing factor receptors (Jöhren *et al.*, 1994; Fuchs and Flügge, 1995; Meyer *et al.*, 2001), specific structural changes in pyramidal neurons (Fuchs and Flügge, 1998), and reduced neurogenesis (Gould *et al.*, 1997). These distinct stress-induced behavioural, physiological, and central nervous alterations in subordinates are based exclusively on the cognitive interpretation of the continuous visual presence of the dominant conspecific (Raab, 1971; Raab and Storz, 1976). Importantly, in dominant tree shrews, on the contrary, no noticeable behavioural and physiological alterations that might represent stress reactions can be observed (Aue, 1988; Fuchs *et al.*, 1995).

The behavioural and neuroendocrine reactions observed in subordinate tree shrews are similar to those produced by centrally administered CRH in rodents and primates to mimic depressive-like symptoms. But importantly, they are also comparable to the symptoms observed in depressed patients (DSM-IV, 1994; see Table I). Though it must be admitted that key symptoms of affective disorders such as depressed mood, loss of interest, loss of energy or recurrent thoughts of death, all raised by subjective account, cannot be modelled in animals, the bio-behavioural responses observed in subordinate tree shrews are comparable to symptoms in depressed patients. Thus, the chronic psychosocial stress model in tree shrews has an obvious "face validity" for depression (Willner, 1991).

THE CHRONIC PSYCHOSOCIAL STRESS PARADIGM IN TREE SHREWS—PREDICTIVE VALIDITY FOR DEPRESSION

The Tricyclic Antidepressant Clomipramine

To elucidate whether the tree shrew model, besides its obvious "face validity" for depression also has a "predictive validity" (Willner, 1991; Willner *et al.*, 1992), we treated subordinate animals with the tricyclic

antidepressant clomipramine (Fuchs *et al.*, 1996). We used clomipramine since it is a potent inhibitor of serotonin re-uptake (McTavish and Benfield, 1990) and aberrations in the regulation of the serotonergic system are implicated in the etiology of depressive illness (Maes and Meltzer, 1995). Moreover, desmethylclomipramine, the pharmacologically active metabolite of clomipramine, is a potent noradrenaline re-uptake inhibitor (McTavish and Benfield, 1990), which is also a primary target of clinically validated antidepressants. To minimize uncontrollable stress effects due to injections, and to mimic the most common route of administration in humans, clomipramine was administered orally to the tree shrews.

In humans, therapeutically effective doses of clomipramine result in serum concentrations of clomipramine and its metabolite desmethylclomipramine in the range of 230–450 ng/ml (Härtter *et al.*, 1995). In a pilot study, we determined the clomipramine dose necessary to reach similar serum concentrations in tree shrews. Clomipramine and its major metabolites were determined with a high-performance liquid chromatography (HPLC) method (for details see Fuchs *et al.*, 1996). Daily oral application of 50 mg/kg during five consecutive days resulted in clomipramine and desmethylclomipramine serum concentrations of about 240 ng/ml, which has recently been considered as the lower threshold concentration of these two compounds (Gex-Fabry *et al.*, 1999). We therefore decided to use this dose for the treatment of the subordinate tree shrews. Other investigators used an antidepressant dose between 10 and 20 mg/kg/day injected i.p. in rats (Heydorn *et al.*, 1982; Peiffer *et al.*, 1991). The pattern of metabolites observed in tree shrews after the 5-day treatment indicated a metabolic pathway of clomipramine similar to that in humans. In tree shrews, major metabolites of clomipramine were desmethylclomipramine, 8-hydroxyclomipramine, 10-hydroxydesmethylclomipramine and 8-hydroxydesmethylclomipramine (Table II). The concentrations measured were comparable to steady state blood serum concentrations of depressed patients, who had received daily oral doses of 150 mg clomipramine (Table II). Moreover, didesmethylclomipramine and 10-hydroxylated metabolites could be identified in blood of the tree shrews (data not shown) and man (Nielsen and Brösen, 1993). In serum of rats who had received an acute dose of clomipramine (20 mg/kg) desmethylclomipramine but not 8- or 10-hydroxylated metabolites were found (Weigmann *et al.*, 1998) which is in contrast to tree shrews or man. To our knowledge, this was the first preclinical study to determine the dose of an antidepressant necessary to reach—in analogy to human patients—therapeutically relevant serum concentrations in animals. It clearly demonstrates the need for monitoring the concentrations of circulating antidepressants and their pharmacologically active metabolites in animal studies. Otherwise it cannot be excluded that sub- or supra-effective doses are used.

To obtain a realistic situation of an antidepressant intervention, the treatment started when the stress-induced

behavioural and endocrine alterations had been manifested. Although the therapeutic effects of antidepressant drugs appear not earlier than 2 or 3 weeks after the onset of the treatment, only a few animal studies have employed chronic administration of antidepressants during a clinically relevant time (Willner *et al.*, 1992; Reul *et al.*, 1993; 1994). In our study in tree shrews, the drug was provided daily, while the psychosocial stress continued, and the therapeutic action of clomipramine was followed across a clinically relevant time period of 4 weeks. We investigated the effect of the antidepressant on locomotor activity, marking behaviour, and urinary cortisol in the subordinate animals.

Experimental Procedure

Experimentally naive adult male tree shrews (*Tupaia belangeri*) were from the breeding colony at the German Primate Center in Göttingen, Germany. The animals were singly housed on a regular day/night cycle (lights on from 08:00 to 20:00 h) with free access to food and water and were used to handling (for details see Fuchs, 1999). All animal experiments were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/EEC), and had been approved by the Government of Lower Saxony, Germany.

During a *No stress* period of 10 days, from five singly housed male tree shrews body weight was recorded daily and the activity of the pituitary–adrenocortical axis was determined by measuring cortisol in morning urine which was collected daily between 07:45 and 08:00 h after a gentle massage of the hypogastrium. Samples were stored frozen and later urinary free cortisol was determined with a radioimmunoassay. To correct for physiological dilution of urine, the resulting concentrations were related to creatinine concentrations (for details see Fuchs *et al.*, 1996). By collecting morning urine samples, we aimed to obtain integrated estimates of endocrine activity over a defined period of time (Seeman *et al.*, 1997). Tree shrews spent the night in their nesting boxes while sleeping most of the time. Therefore, the use of an overnight collection protocol provides an estimate of basal, non-stimulated cortisol levels by minimizing the potential influences of confounding factors such as differences in physical activity (Seeman *et al.*, 1997). In addition, urine analysis is advantageous in tree shrews because blood sampling is stressful for the animals (Stöhr, 1986).

After the *No stress* period (days 1–10), psychosocial stress (*Stress*; days 11–20) was induced according to our standard procedure (for details, see Fuchs *et al.*, 1996). Briefly, one naive male was introduced into the cage of a socially experienced male. This resulted in active competition for control over the territory, and after establishment of a clear dominant/subordinate relationship, the two animals were separated by a wire mesh barrier. As in earlier studies (Flügge *et al.*, 1992; Fuchs *et al.*, 1993; 1995; Jöhren *et al.*, 1994; Flügge 1995; Fuchs and Flügge, 1995) all of the naive animals turned out to become subordinate. The barrier was removed every day

for approximately 1 h between 08:30 and 10:00 h allowing physical contact between the two males only during this time. By this procedure, the subordinate animal was protected from repeated attacks, but it was constantly exposed to olfactory, visual and acoustic cues from the dominant. Under these conditions, subordinate animals displayed characteristic subordination behaviour. They reduced their level and sphere of activity in their cages, showed tail ruffling, and elicited alarm cries. After 10 days of daily psychosocial conflict, subordinate animals received clomipramine dissolved in 0.9% saline (50 mg/kg) orally in the morning. For the next 30 days, the subordinate animals remained in the psychosocial conflict situation as described above and were treated daily with clomipramine (*Stress+clomipramine*; days 21–49). Before and during the stress experiments each animal was video taped daily between 18:45 and 19:15 h and behavioural parameters (locomotor activity and marking behaviour) were analysed later.

Locomotor activity was measured by marking the cages with adhesive tape, which allowed a visual subdivision of the cage into six areas of equal size (width: 25 cm × height: 40 cm; head–tail length of the animals: approximately 30 cm). Similar to quantification of motor activity in an open field, movements were counted as one event whenever an animal changed its position within the cage from one of these areas to an adjacent area. The visual subdivision of the cages allowed measurement of the time an animal spent in the defined areas. Marking behaviour was quantified by measuring the total time the animal spent with different forms of marking such as marking with the abdominal gland, marking with the sternal gland, and urinary marking (Martin, 1968; von Holst and Buergel-Goodwin, 1975; Aue, 1988). All behaviour analyses were performed using the Hindsight 1.3 Behavioural Observer software (Weiss, 1995).

Statistical analysis of the data was performed using the GB-Stat 5.3 software (Dynamic Microsystems, Silver Spring, MD, USA). To avoid the influence of inter-individual pre-test differences, all values were transformed into percent values by relating them to the mean value of the No stress phase. Furthermore, values were divided into five data blocks, representing the arithmetic mean of 10 days for the first four data blocks, and 9 days for the fifth data block. This procedure allowed detection of significant within-group differences between the *No stress*, *Stress*, and *Stress+clomipramine* period. To test for significant differences of means we used the one-way ANOVA procedure, followed by Fisher's LSD test.

Effects of Chronic Stress on Behaviour

In animal models of depression, the behaviour most frequently studied is locomotor activity (Willner *et al.*, 1992). Decreased motor activity as a consequence of intensive stress has been widely reported mostly on the basis of open field studies (Anisman and Zacharko, 1982). In addition, a symptom often observed in depressed patients

Urinary free cortisol | **Locomotor activity** | **Marking behavior**
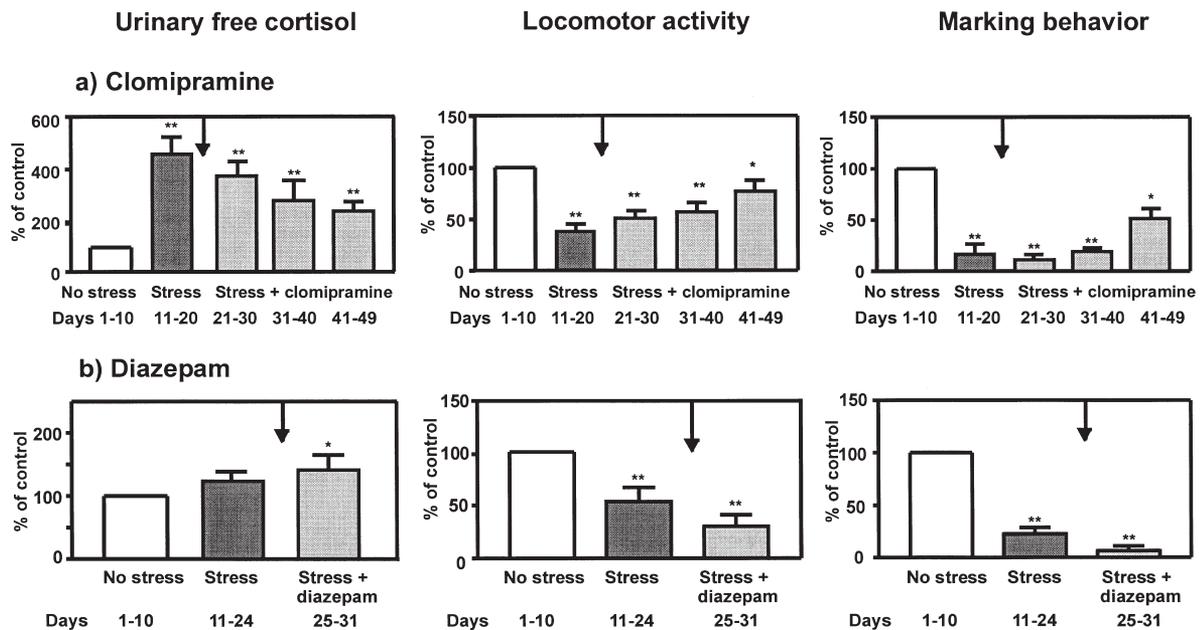
a) Clomipramine

FIGURE 1 Psychosocial stress in male tree shrews. The effect of treatment with
(a) the tricyclic antidepressant clomipramine. Male tree shrews ($n = 5$) received clomipramine orally (50 mg/kg) starting on day 20 of the experiment (indicated by the arrow) and the daily treatment was continued until day 49. Data were transformed into percent values by relating them to the individual's mean value in the *No stress* period and are given as means $\pm$ SD. Significant differences: Urinary free cortisol: *No stress* (days 1−10) vs. *Stress* (days 11−20) $p < 0.01$; *No stress* (days 1−10) vs. *Stress+clomipramine* (days 21−49) in all cases $p < 0.01$; Locomotor activity: *No stress* (days 1−10) vs. *Stress* (days 11−20) $p < 0.01$; *No stress* (days 1−10) vs. *Stress+clomipramine* (days 21−40) in all cases $p < 0.01$; *No stress* (days 1−10) vs. *Stress+clomipramine* (days 41−49) $p < 0.05$; Marking behaviour: *No stress* (days 1−10) vs. *Stress* (days 11−20) $p < 0.01$; *No stress* (days 1−10) vs. *Stress+clomipramine* (days 21−40) in all cases $p < 0.01$; *No stress* (days 1−10) vs. *Stress+clomipramine* (days 41−49) $p < 0.05$. With modifications from Fuchs *et al.* (1996);
(b) the anxiolytic diazepam on urinary free cortisol, locomotor activity, and marking behaviour. Male tree shrews ($n = 5$) received diazepam orally (5 mg/kg) starting on day 25 of the experiment (indicated by the arrow) and the daily treatment was continued until day 31. Data were transformed into percent values by relating them to the individual's mean value in the *No stress* period and are given as means $\pm$ SD. Significant differences: Urinary free cortisol: *No stress* (days 1−10) vs. *Stress+diazepam* (days 25−31) $p < 0.05$; Locomotor activity: *No stress* (days 1−10) vs. *Stress* (days 11−24) $p < 0.01$; *No stress* (days 1−10) vs. *Stress+diazepam* (days 25−31) $p < 0.01$; Marking behaviour: *No stress* (days 1−10) vs. *Stress* (days 11−24) $p < 0.01$; *No stress* (days 1−10) vs. *Stress+diazepam* (days 11−31) $p < 0.01$. With modifications from van Kampen *et al.* (2000).

is retardation of psychomotor activity (Kapur and Mann, 1992). Like in other animal models, chronic psychosocial conflict induced a significant decrease in motor activity in subordinate tree shrews. The significance of this change as indicator for "depressive-like" behaviour is demonstrated by the fact that clomipramine but not the anxiolytic compound diazepam reversed the adverse effects on the locomotor activity in subordinate tree shrews (Fig. 1a,b; see below). Interestingly, we recently found in subordinate tree shrews a positive correlation between the locomotor activity and the number of dopamine transporter (DAT) binding sites in the caudate nucleus and the putamen (Isovich *et al.*, 2000). This finding indicates that the reduction in motor activity is related to downregulation of DAT binding sites in these motor related brain areas.

Chemical signals play an important role in territorial behaviour of male tree shrews (Martin, 1968; von Holst and Buergel-Goodwin, 1975; von Holst and Lesk, 1975). Scent substances are found in glandular secretes, urine, faeces and saliva, and contain information about the identity and physiological state of the individual (von Holst and Lesk, 1975). The production of the scent substances is controlled by androgens (von Holst and Buergel-Goodwin, 1975). Since gonadal activity and in consequence, circulating

testosterone is reduced in subordinate tree shrews (Fischer *et al.*, 1985; Flügge *et al.*, 1998), marking behaviour nearly disappears under chronic stress. Exogenous testosterone antagonizes the stress-induced decrease in marking behaviour in subordinate tree shrews indicating that this type of behaviour is under the control of androgens (von Holst and Buergel-Goodwin, 1975; Flügge *et al.*, 1998). Long-term antidepressant treatment in subordinate tree shrews induced a stepwise reactivation of the testosterone-dependent marking behaviour (Fig. 1a). To our knowledge, few data exist on the relationship between antidepressant medications and androgen levels in experimental animals (Hendrick *et al.*, 2000). In adult male rats, testosterone levels are not affected by fluoxetine or trimipramine treatment (Taylor *et al.*, 1996). Thus, future research should delineate potential mechanisms of interaction between antidepressants and the male reproductive axis.

Effects of Chronic Stress on Body Weight and HPA Axis

Body weight of the animals was monitored every day to evaluate the animals' health state and to classify the tree shrews as being subordinate (von Holst *et al.*, 1983; Fuchs

et al., 1993). In line with several studies from our group (Fuchs *et al.*, 1993; Flügge *et al.*, 1998; Isovich *et al.*, 2000) and similar to studies in rats (Rybkin *et al.*, 1997) we found that chronic psychosocial stress reduced body weight in subordinate tree shrews from the onset of the conflict situation onwards (-5%; $p < 0.05$). In tree shrews, this weight loss is predominantly due to a stress-induced enhancement of metabolic activity (Fuchs and Kleinknecht 1986; Jöhren *et al.*, 1991) and to a certain extent due to reduced food intake (Kramer *et al.*, 1999). When treating subordinate tree shrews with clomipramine body weight returned to pre-stress levels within 20 days. The restoration in body weight can be explained by a clomipramine-induced normalization of consummatory behaviour in the stressed animals (Kramer *et al.*, 1999).

The intensity of psychosocial stress in subordinate tree shrews was also demonstrated by a sustained activation of the HPA axis as indicated by a pronounced and non-adapting elevation of urinary cortisol excretion. However, daily treatment of subordinate animals with clomipramine evoked a time-dependent and significant re-normalization of urinary cortisol excretion (Fig. 1a).

Among the more consistent observations in patients with major depression is dysfunction of the HPA axis (Sachar *et al.*, 1973; Holsboer *et al.*, 1983; Rubin *et al.*, 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). Treatment of subordinate tree shrews with the tricyclic antidepressant clomipramine normalized the activity of HPA axis. This may be due to direct interactions of the drug with serotonergic and/or noradrenergic circuits in various brain areas, which in turn modulate the activity of the corticotrophin-releasing factor (CRF) system. The latter system is suggested to be a modulator of synthesis and release of ACTH and other pro-opiomelanocortin products from the pituitary and to regulate autonomic activity (Owens and Nemeroff, 1991; Holsboer *et al.*, 1992).

These findings are indicative for the predictive validity of the tree shrew model by demonstrating that stress-induced behavioural and neuroendocrine alterations can be reversed by an antidepressant treatment. It is important to mention that we found a slow onset of clomipramine action across several weeks of chronic treatment as is the case in patients.

Effects of the Anxiolytic Diazepam

As demonstrated by studies in humans (Lemoine *et al.*, 1991; Suetsugi *et al.*, 1998) and animals (Molewijk *et al.*, 1995), clomipramine has besides its antidepressant effects, also anxiolytic properties. To test whether the beneficial effects of clomipramine in stressed tree shrews might be due to its anxiolytic properties and to further evaluate the tree shrew paradigm as a model for depression we investigated the action of the prototypic benzodiazepine receptor agonist diazepam on locomotor activity, marking behaviour, and urinary cortisol in subordinate animals (van Kampen *et al.*, 2000).

To mimic a realistic situation of anxiolytic intervention in tree shrews, the treatment started—similar to the previous experiment—after the stress-induced behavioural and endocrine alterations had been established. Furthermore, the drug was administered orally, while the psychosocial stress continued. Oral administration was used again, since it provides some advantages: (i) it mimics the clinical situation that uses oral application for most patients; (ii) due to the first path metabolism, resulting drug and metabolite concentrations differ from that obtained after i.p. or i.v. administration.

We determined in a pilot study the diazepam dosage necessary to reach a serum concentration in tree shrews similar to that known to be therapeutically effective in humans. A recently established HPLC method enabled us to detect the major metabolites of diazepam (see van Kampen *et al.*, 2000). Based on the results of the pilot study, the animals were given 5 mg/kg diazepam dissolved in 6% ethanol and distilled water in the morning. In view of clinical patterns it was of importance to examine the effects of treatment following subchronic rather than acute administration. Thus the potential therapeutic action of diazepam was followed across 7 days.

Experimental Procedure

The experimental procedure was comparable to the one used in the clomipramine study. During a *No stress* period (days 1–10) individually housed male tree shrews ($n = 5$) were weighed daily and the basal activities of the pituitary–adrenocortical axis was determined by measuring cortisol in morning urine. The induction of psychosocial conflict was carried out as described before (*Stress*; days 11–24). After 14 days of psychosocial conflict, subordinate animals received diazepam (5 mg/kg) orally every day in the morning. For the next 7 days, the subordinate animals remained in the psychosocial conflict situation and were treated daily with diazepam (*Stress+ diazepam*; days 25–31). All animals were weighed daily, morning urine samples were collected and the behaviour was video taped between 18:45 and 19:15 h. In a 15 min interval locomotor and marking behaviour was quantified from the tapes. In this experiment locomotor activity was measured using the Insight Software program from OCTEC (Bracknell, UK) but was similar to the quantification of motor activity in the clomipramine experiment. All analysis and quantifications of marking behaviour were performed with the Observer 3.1 software (Noldus Technology, Wageningen, The Netherlands).

The statistical analysis of the data was performed using the GB-Stat 5.3 software (Dynamic Microsystems). To avoid interferences between interindividual pre-test values, all results were transformed into percent values by relating them to the individual's mean value of the

control period. The data of every experimental group were divided in three blocks: *No stress* (days 1–10), *Stress* (days 11–24) and *Stress+diazepam* (days 25–31). For significance testing the means of the three treatment blocks were compared. To test for significant differences of means in one experimental group we used the Friedman test with the Wilcoxon signed-rank test as *post hoc* test.

After the 7-day treatment with diazepam (5 mg/kg/day) and 24 h after the last drug application, blood was collected from all animals by puncturing the tail's venous plexi. Blood serum concentrations of diazepam and its pharmacologically active metabolites were analysed by solid phase extraction and subsequent HPLC analysis (see van Kampen *et al.*, 2000). Mean concentrations (means ± SEM) in the serum of the five experimental animals were 7 ± 5 ng/ml for diazepam, 106 ± 58 ng/ml for nordiazepam, 22 ± 12 ng/ml for temazepam, and 30 ± 8 ng/ml for oxazepam. These results indicate that in tree shrews the *N*-demethylation and hydroxylation pathways are similar to those known for humans and rats (Greenblatt and Shader, 1987). However, our results pointed towards a situation slightly different from rats where temazepam is a major metabolite (Jing *et al.*, 1995), giving evidence that in tree shrews the degradation route is more similar to humans with the limitation that not all metabolites could be detected with our system.

Effects of Diazepam on Body Weight, Behaviour and HPA Axis

In line with earlier findings, the chronic psychosocial stress resulted in subordinate animals in (i) a significant reduction of body weight, (ii) an activation of the HPA axis indicated by increased urinary cortisol, and (iii) distinct changes in behavioural variables such as marking behaviour and locomotor activity (Fig. 1b). Despite use of a similar stress paradigm in the diazepam experiment to that used in the experiments with clomipramine, we observed a less pronounced activation of the HPA axis. One explanation of this finding is that there are differences among individuals in their stress responses, with more reactive animals in the clomipramine experiment. In other recent studies psychosocially stressed tree shrews showed a comparable stress-induced cortisol increase of up to 200% compared to control values (Flügge *et al.*, 1998; Isovich *et al.*, 2000).

The decrease in body weight is an indicator by which tree shrews my be classified as being subordinate (von Holst *et al.*, 1983; Fuchs *et al.*, 1993). During the *Stress* period (days 11–24) subordinate animals displayed a significant reduction in body weight (-5%; $p < 0.05$) and the decline continued during the *treatment* period (days 25–31; for details, see van Kampen *et al.*, 2000).

The important finding of this study is that a subchronic treatment of stressed tree shrews with diazepam—in a dose that did not have sedative effects in control animals and which has been shown to be pharmacologically effective in rats (Schmitt and Hiemke, 1998)—did not significantly

affect the stress-related endocrine and behavioural alterations. This clearly contrasts with the findings of the previous study showing that decreases in both locomotor activity and marking behaviour, and the hyperactivity of the HPA axis produced by the psychosocial stress paradigm are sensitive to chronic treatment with clomipramine.

Stimulation of the HPA-axis as indicated by raised plasma glucocorticoid hormone concentrations is one hallmark of the neuroendocrine responses to emotional stressors in mammals (Henry and Stephens, 1977). During stress conditions brain benzodiazepine receptors appear to participate in the physiological regulation of adrenocortical activity (De Boer *et al.*, 1990). Several reports have demonstrated that benzodiazepine receptor ligands with anxiolytic actions like diazepam can prevent or oppose the stress-induced activation of the HPA system (Lahti and Barsuhn, 1974; File, 1982; Pericic *et al.*, 1984; McElroy *et al.*, 1987; De Boer *et al.*, 1991). Consequently, it would be expected that diazepam reduces the activity of the stress-activated neuroendocrine system, which was not observed in the present study. One explanation may arise from earlier findings demonstrating that stress exposure may alter the responses to benzodiazepines over prolonged periods of time. In rats, exposure to a brief stressful event up to at least 1 month earlier prevented completely the effect of diazepam on plasma corticosterone (Antelman *et al.*, 1987). Furthermore, several laboratories have reported that the effects of benzodiazepines on HPA axis activity in intact animals are not simple. Small doses do not alter basal activity of the axis (Marc and Morselli, 1969; Lahti and Barsuhn, 1975) whereas larger doses markedly increase the activity resulting in elevated corticosterone levels (Marc and Morselli, 1969; Lahti and Barsuhn, 1974; 1975; Keim and Sigg, 1977).

In psychosocially stressed tree shrews, therapeutic efficacy has been shown by treatment with the tricyclic antidepressant clomipramine which is known to inhibit serotonin uptake. 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 psychopathologies (Rickels *et al.*, 1993; Hoehn-Saric and McLeod, 1994). To test the hypothesis of a functional relationship between the endogenous anxiolytic system and the endogenous stress system via serotonergic mechanisms, the effect of psychotropic drugs such as buspirone should be evaluated in the tree shrew paradigm of psychosocial stress in order to further validate this non-rodent model as a tool in preclinical research of psychopathologies.

THE CHRONIC PSYCHOSOCIAL STRESS PARADIGM IN TREE SHREWS—CONSTRUCT VALIDITY FOR DEPRESSION

The term construct validity is borrowed from psychological testing and means that a procedure is based on a sound

theoretical rationale. As pointed out by Willner (1991) it is doubtful if any animal model could meet fully the requirements of construct validity. Despite this constraint, the construct validity of subordinate tree shrews in the chronic psychosocial stress model for depression is based on the following observations. Subordinate or submissive behaviour in people is often associated with low self-esteem, which is regarded as vulnerability factor for depression (Brown *et al.*, 1990). Social encounters resulting in defeat of one participant can induce an unfavourable self-esteem insofar as the submitted individual has to acknowledge defeat. Thus, subordinate behaviour in psychosocially stressed tree shrews may be a model for some aspects of low self-esteem in humans. In humans, loss of favourable social position is increasingly recognized as a stressful life event associated with increased risk of depression (Brown, 1993).

CONCLUSIONS

Animal models for chronic stress represent an indispensable preclinical approach to human psychopathology since clinical data point to a major role of psychological stress experiences, either acute and/or chronic, to the development of behavioural and physiological disturbances. Valid animal models that are used to study the pathophysiology of depression and the specific bio-behavioural responses to antidepressant drug treatments are of central interest. When transferring results from the treatment of depression-like symptoms in experimental animals to the clinical situation, little attention has so far been paid to potential species-specific differences in the metabolism of the applied drugs. Our studies revealed species differences in the metabolism of the tricyclic antidepressant clomipramine in rats, tree shrews and humans showing that the degradation route of this antidepressant in tree shrews is similar to that in humans. Thus, the chronic psychosocial stress model in tree shrews might have advantages over the widely used rat models.

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