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Social stress in tree shrews: Effects on physiology, brain function, and behavior of subordinate individuals

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Abstract

Social stress is known to be involved in the etiology of central nervous disorders such as depression. In recent years, animal models have been developed that use chronic stress to induce neuroendocrine and central nervous changes that might be similar to those occurring in the course of the development of depressive disorders. The present review gives a summary of observations made in the tree shrew chronic social stress model. During periods of daily social stress, male tree shrews develop symptoms that are known from many depressed patients such as persistent hyperactivities of both the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system, disturbances in sleeping patterns, and reduced motor activity. Moreover, various physiological parameters indicate an acceleration of the over all metabolic rate in socially stressed tree shrews. Some of these parameters can be renormalized by antidepressants thus supporting the view of the tree shrew social stress paradigm as model for major depression. In the brains of socially stressed animals, monoamine receptors show dynamic changes that reflect adaptation to the persistent monoaminergic hyperactivity during periods of chronic stress. In addition to the changes in neurotransmitter systems, there are structural changes in neurons, e.g., retraction of the dendrites of hippocampal pyramidal neurons. Together, these processes are suggested as a cause of behavioral alterations that can be counteracted by antidepressants in this naturalistic social stress model.

Keywords: Antidepressants; Behavior; Hippocampus; HPA axis; Monoamine receptors; Sleep; Sympathetic nervous system; Urinalysis

1. Introduction

Although depressive disorders are among the most common human diseases and despite concerted clinical and preclinical research, the neurobiological processes that lead to major depression are currently not completely understood (Judd, 1995; Murray and Lopez, 1997; Manji et al., 2001). In view of the fact that stressful life events often contribute to the etiology of depressive episodes (Kessler, 1997; Paykel, 1978), animal models have been developed to study central nervous mechanisms that lead to depressive symptoms. However, such models have to be assessed on the basis of how well they fulfill three major criteria, namely *face validity*, *predictive validity*, and *construct validity* (Willner, 1984). At present, only a few of the existing preclinical models for depressive disorders satisfactorily meet these criteria (Yadid et al., 2000).

In the recent years, evidence has accumulated that chronic social stress in a nonrodent species, the male tree shrew (*Tupaia belangeri*) may represent a suitable and naturalistic experimental paradigm to study the causal mechanisms of major depression (Fuchs et al., 1996). Tree shrews are day-active so that their biological rhythms might be more similar to those of humans than the diurnal rhythms of night-active rodents such as rats. From the phylogenetic point of view, tree shrews are regarded as an intermediate between insectivores and primates (Martin, 1990). Genes for the glucocorticoid and the mineralocorticoid receptors (MRs) (Meyer et al., 1998), for receptors of the corticotropin-releasing factor (Palchaudhuri et al., 1998, 1999), for the α_{2A}-adrenoceptor (Meyer et al., 2000), and for the amyloid-β protein (Pawlik et al., 1999) revealed a high grade of homology with the respective human proteins in the range of 90–98% amino acid sequence identity, whereas the homology with the

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corresponding rat proteins was only about 80%. These molecular biology findings are in accord with a report based on morphological characteristics (Novacek, 1992). The validity of a recent phylogenetic reconstruction based on DNA sequencing claiming that lagomorphs (e.g., hares) are the closest relatives of tree shrews (Schmitz et al., 2000) is at present difficult to evaluate.

The natural habitats of tree shrews are forestial and plantation areas in Southeast Asia where they are widely distributed. They are solitary and males defend their territories against intruding conspecifics of the same sex (Kawamichi and Kawamichi, 1979). This pronounced territoriality of the males could be used to establish naturally occurring challenging situations under experimental control in the laboratory. When two adult males are housed together in one cage, there are territorial fights and as a consequence, a social hierarchy is established with a dominant and a subordinate male. Separating the animals by a wire mesh

Table 1
Changes detected in male tree shrews during chronic social stress

	Effect of chronic stress	References
Physiological parameters		
Body weight	5–10% decrease	Fuchs et al. (1993)
Sympathetic nervous system	urinary noradrenaline: two- to threefold increase	Fuchs et al. (1993) and Kramer et al. (1999)
Adrenomedullary system	urinary adrenaline: transient increase	Fuchs et al. (1993)
Systolic blood pressure	slight, transient increase	Fuchs et al. (1993)
Heart rate	persistently elevated	Stöhr (1986)
Sleep	reduced slow-wave sleep, more/longer awake phases	Aue (1988)
Pteridines	increased urinary biopterin and isoxanthopterin	Fuchs et al. (1992)
RNA metabolites	increased 7-methyl-guanine in urine	Jöhren et al. (1991)
Oxygen consumption	increased during the night	Fuchs and Kleinknecht (1986)
Endocrine parameters		
Cortisol	urinary cortisol: two- to fivefold increase (HPA axis hyperactivity)	e.g., Jöhren et al. (1994)
Testosterone	decrease	Fischer et al. (1985)
Melatonin	increased excretion of the metabolite aMT6s	Fuchs and Schumacher (1990)
Receptors and transporters in the brain		
5-HT_{1A} receptors	gradual down-regulation of heteroreceptors in hippocampus and cortical regions; fast renormalization after stress or hormonal replacement	Flügge (1995) and Flügge et al. (1998)
α_2-Adrenoceptors	down-regulation in brain regions involved in autonomic functions	Flügge (1996), Flügge et al. (1992), and Meyer et al. (2000)
β_1-Adrenoceptors	after 4 weeks down-regulation in hippocampus and parietal cortex; transient effects in prefrontal cortex, olfactory area, and pulvinar nucleus	Flügge et al. (1997)
β_2-Adrenoceptors	after 4 weeks up-regulation in pulvinar nucleus; transient effects in prefrontal cortex	Flügge et al. (1997)
DAT	reduced DAT binding sites in caudate nucleus and putamen	Isovich et al. (2000)
Hippocampal GRs	down-regulation of GR; regional up- and down-regulation of MR	Meyer et al. (2001)
11β-HSD	attenuation of activity	Jamieson et al. (1997)
CRH receptors	down-regulation of binding sites for ^{125}I-ovine CRH in anterior pituitary, dentate gyrus, CA1 and CA3 of the hippocampus, area 17, superior colliculus; up-regulation of binding sites for ^{125}I-ovine CRH in cortical regions, amygdala, choroid plexus	Fuchs and Flügge (1995)
Morphological changes in the brain		
Neurogenesis in the dentate gyrus	inhibition of the proliferation of granule cell precursors	Gould et al. (1998) and Czeh et al. (2001)
Nuclear ultrastructure of hippocampal pyramidal neurons	more heterochromatin	Fuchs et al. (1995) and Vollman-Honsdorf et al. (1999)
Retraction of dendrites	retraction of apical dendrites of pyramidal neurons in the hippocampus	Magariños et al. (1996)
Volume of the hippocampal formation	volume reduced by approximately 10%	Ohl et al. (2000) and Czeh et al. (2001)
Behavior		
General motor activity	reduced	Fuchs et al. (1996) and Kramer et al. (1999)
Self-grooming	reduced	Aue (1988) and Kramer et al. (1999)
Scent marking activity	reduced	Kramer et al. (1999)
Feeding and water intake	reduced	Kramer et al. (1999)

barrier does not interfere with the hierarchy. As long as the subordinate lives in visual and olfactory contact with the dominant by which it has been defeated, it experiences chronic stress characterized by clear physiological, neuroendocrine, and central nervous changes and behavioral alterations that resemble symptoms in depressive patients. Previous investigations have shown that these stress reactions in subordinate tree shrews are due to the cognitive interpretation of the continuous visual presence of the dominant conspecific (Raab, 1971; von Holst et al., 1983). Physiological and neuroendocrine parameters indicate no adaptation to the stress situation, meaning that changes persist as long as the subordinate animal is aware of the presence of its dominant counterpart.

The present paper summarizes work from our group during recent years demonstrating a variety of changes in peripheral, central nervous, and behavioral parameters occurring in subordinate tree shrews during long-term periods of social stress (Table 1). Our experiments are aimed at investigating how social conflict influences physiology and behavior, as well as molecular and structural parameters in the brains of subordinate individuals. Recent experiments were designed to assess whether the social stress paradigm in male tree shrews fulfills the criteria of a suitable experimental model for depressive disorders.

2. Animal housing and experimental procedures

For more than 10 years, we have bred tree shrews (*Tupaia belangeri*) at the German Primate Center, Göttingen, Germany. After weaning, males are housed singly under controlled conditions (lights on from 8:00 a.m. to 8:00 p.m., relative humidity ±60%; temperature ±27 °C) in steel cages (size $50 \times 80 \times 130$ cm, $w \times d \times h$). Each cage contains a system of tree branches and a wooden nest box is located at the bottom of the cage. Tree shrew diet (Altromin, Lage, Germany) and tap water are available ad libitum (for details of the housing conditions, see Fuchs, 1999). The health status of the animals is constantly controlled by the veterinary staff. All animal experiments described 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.

As stated above, adult male tree shrews are territorial and therefore have to be kept single housed from puberty onwards (for details, see Fuchs, 1999). To induce stress, a socially naive adult male is introduced into the cage of another male that had already become dominant in a previous confrontation with a subordinate. The encounter results in active competition for control over the territory, and after establishment of a clear dominant/subordinate relationship, the two animals are separated by a wire mesh barrier. Thus, direct physical contact is only allowed for approximately 1 h everyday while the rest of the time, the animals are separated by the wire mesh barrier. Using this procedure, the subordinate male is protected from repeated attacks but is constantly exposed to olfactory, visual, and acoustic cues from the dominant. These conflict conditions can last several days or even weeks (*chronic stress*) during which the subordinate animal is unable to control the situation. In humans, lack of both control and predictability of a situation are known to be extremely stressful factors (Checkley, 1992).

3. Social stress and physiology

Chronic psychosocial stress in subordinate male tree shrews leads to a variety of changes in endocrine, physiological, central nervous, and behavioral parameters that are listed in Table 1.

3.1. Reaction of the sympathetic nervous system and systolic blood pressure

An important parameter that is routinely used to document that the subordinate experiences stress is the activation of its sympathetic nervous system reflected by elevated plasma noradrenaline levels (Fuchs, 1984). To detect whether the sympathetic hyperactivity persists throughout the period of daily social stress, long-term recordings have to be performed. Usually, physiological parameters in laboratory animals are determined by analyzing blood samples, e.g., drawn from chronically implanted catheters. There are however many species including tree shrews, in which, for various reasons, catheters cannot be chronically implanted. Furthermore, it is possible to measure plasma hormones in small volumes of blood obtained by puncturing the venous plexus of the tree shrew tail with a small scalpel (Fuchs, 1999) but this procedure is stressful and can induce changes in basal heart rate that might persist during several days (Stöhr, 1986). To circumvent the problems related to these invasive techniques, noninvasive methods such as urine analysis have certain advantages. Firstly, urine samples can easily be collected over any period and sampling does not require special equipment. Secondly, several compounds measurable in blood can also be detected in urine. As a routine, we collect the urine of tree shrews every morning shortly before lights are turned on, performing a slight massage of the hypogastrium thus inducing urination (Fuchs, 1999). This procedure is not stressful when the animals are used to the daily handling procedure (Fuchs et al., 1993). Since tree shrews spent the nighttime in their nesting boxes and most of it sleeping urinary norepinephrine is the end product of whole body release and reuptake processes, metabolic degradation, and redistribution into multiple physiological compartments. Despite this complicated process, daily analysis of urinary norepinephrine provides a reliable insight into the organism's responses to challenging situations (Moleman et al., 1992).

Socially stressed subordinate tree shrews show a two- to threefold increase in urinary noradrenaline, and these elevated levels persist during the whole chronic stress period (Fuchs et al., 1993; Kramer et al., 1999).

Social defeat in wild rats has been shown to produce a shift of the autonomic balance towards sympathetic dominance characterized by cardiac tachyarrhythmias (Sgoifo et al., 1999). In view of the activation of the sympathetic nervous and the adrenomedullary system in chronically stressed male tree shrews, it was tempting to assume that that there were cardiovascular changes also in these animals. Therefore, we measured systolic blood pressure in tree shrews using the tail cuff method (Lorenz, 1968). It turned out that subordinates show a slight but not significant increase in blood pressure (from 110 to approximately 120 mmHg). The rise in blood pressure was also only transient suggesting adaptive mechanisms, either centrally or peripherally. In contrast, heart rate has been shown to be significantly increased over several days in male tree shrews that have been once defeated by a dominant (Stöhr, 1986).

3.2. Reaction of the hypothalamic–pituitary–adrenal (HPA) axis

Among the more consistent observations in patients with major depression is the dysfunction of the HPA axis (Holsboer et al., 1983; Rubin et al., 1987; Sachar et al., 1973). The correlation between hypersecretion of cortisol and depression is one of the oldest observations in biological psychiatry—at least in a subpopulation of depressed patients—and is regarded as one cause of depressive symptoms since HPA axis activity normalizes upon successful therapy (Holsboer and Barden, 1996). By determining free cortisol from morning urine samples, we aimed to obtain in tree shrews integrated estimates of HPA axis activity over a defined period (Seeman et al., 1997). As mentioned above, the animals spend the night in their nesting boxes, and the use of an overnight urine collection protocol provides an estimate of basal, nonstimulated cortisol levels by minimizing the potential influences of confounding factors such as differences in physical activity (Seeman et al., 1997).

In subordinate tree shrews, urinary cortisol increases two- to fivefold from the first day of the stress period onwards and the high level of the steroid hormone persists during the entire stress period showing no adaptation of the HPA axis to the recurrent stress stimuli (daily confrontations with the dominant male; Jöhren et al., 1994). This chronic hyperactivity of the HPA axis may be responsible for many of the physiological changes addressed above, but also for changes in the brain (see below).

It has been proposed that the effects of psychosocial stress in tree shrews can be differentiated into two distinct endocrine stress reactions (von Holst et al., 1987). According to this two-axis model of the stress response, so-called submissive tree shrews are characterized by an activation of the HPA axis and behaviorally, by resignation and helplessness, whereas *subordinate* tree shrews are characterized by an activation of the sympatho-adrenomedullary system and active coping strategies in stressful situations. Our results provide no evidence for two types of defeated animals. On the contrary, cortisol and norepinephrine concentrations in the morning urine of socially stressed tree shrews show a positive correlation indicating a parallel activation of both the HPA axis and the sympatho-adrenomedullary system (Kramer et al., 1999).

3.3. Body weight

It could be assumed that the hyperactivity of the sympathetic nervous system and of the HPA axis induces a general metabolic activation that would result in body weight reduction. Indeed, a decrease in body weight is detectable in subordinate tree shrews from the morning after the first encounter with the dominant onwards. Body weight reduction thus represents an easy measure that indicates the inferior social status of the subordinate individual and the beginning of the stress reaction, respectively (Fuchs et al., 1993). In male tree shrews, social stress results in a reduction of body weight in the range from 5% to 10%, an effect that persists as long as the animal is daily confronted with the dominant. However, as outlined below, the decrease in body weight is not only related to the metabolic activation in the animals but is in part also related to reduced food intake (Table 1).

3.4. Urinary pteridines and RNA degradation products

In recent years, there has been increasing interest in quantifying pteridines that are synthesized from guanosine triphosphate in body fluid from mammals including man (Sander et al., 1986). The main attention has been focused on neopterin, which serves as a marker for the activation of the T-lymphocyte macrophage system. Although neopterin itself does not appear to play a role in immune processes, its measurement in urine and blood has been proven useful for monitoring diseases affecting the immune system (Müller et al., 1991). Another member of the pteridine family is biopterin whose fully reduced form, terahydrobiopterin, is a cofactor for the pteridine-dependent monooxygenases, enzymes that play important roles in the biosynthesis of biogenic amines such as catecholamines and indolamines.

We determined pteridines in the urine of socially stressed and unstressed tree shrews using HPLC methods (Fuchs et al., 1992). It turned out that social stress increases urinary excretion of biopterin and of the catabolite isoxanthopterin, probably reflecting an increased turnover rate of biogenic amines in the stressed animals.

Modified RNA catabolites (nucleosides and nucleobases) have been found in the urine of mammals including man. These modified nucleosides are generated from cellular RNA (mRNA, rRNA, and tRNA) through posttranscriptional modification, primarily methylation. They cannot be

used for de novo synthesis of RNA but are catabolized or excreted in the urine. We analyzed concentrations of the RNA metabolite 7-methyl-guanine and found that it was significantly increased in morning urine of subordinate tree shrews during the period of daily social confrontations reflecting an acceleration of nucleic acid turnover in the stressed animals (Jöhren et al., 1991). Plotting the changes of 7-methyl-guanine excretion versus the corresponding changes of body weight resulted in a highly significant negative correlation. This close correlation supports the idea of a relationship between metabolic status and the amount of some excreted modified RNA catabolites as indicators of whole-body RNA degradation.

3.5. Oxygen consumption

The abovementioned data indicate that the overall metabolic rate of stressed animals is elevated, which would imply that also oxygen consumption is increased. We therefore measured oxygen consumption of stressed male tree shrews and controls during the night (12 h) using an open-flow oxygen analysis system (Fuchs and Kleinknecht, 1986). It turned out that oxygen consumption in subordinates was significantly increased from 2 h until 7 h after lights off, indicating that chronic social stress is accompanied by a general acceleration of the metabolism during the resting period.

3.6. Sleep

To investigate whether there might also be sleep disturbances in stressed male tree shrews, we performed EEG (electroencephalogram) measurements (Aue, 1988). An EEG electrode was fixed to the skull of anesthetized tree shrews above the right hemisphere as described for the rat (Knight et al., 1985). In addition to the EEG electrode, an EMG (electromyogram) electrode was fixed to the edge of the orbita to record activity of the eyelid. Four weeks after implantation of the electrodes, EEGs were recorded. Measurements were performed in two dominant and two subordinate animals during five entire nights (8 p.m. to 8 a.m.) of a stress period with daily social confrontations. EEGs from unstressed control animals were also recorded during five nights.

The following four phases of brain activity could be recorded in tree shrews (Berger and Walker, 1972), (i) *awake, motorically active:* the amplitude of the cortical EEG was 170–330 μV with high frequency (6–10 Hz); (ii) *awake, no motor activity:* 30–65 μV, 8–13 Hz; (iii) *light slow-wave sleep:* the animal is motorically inactive and the

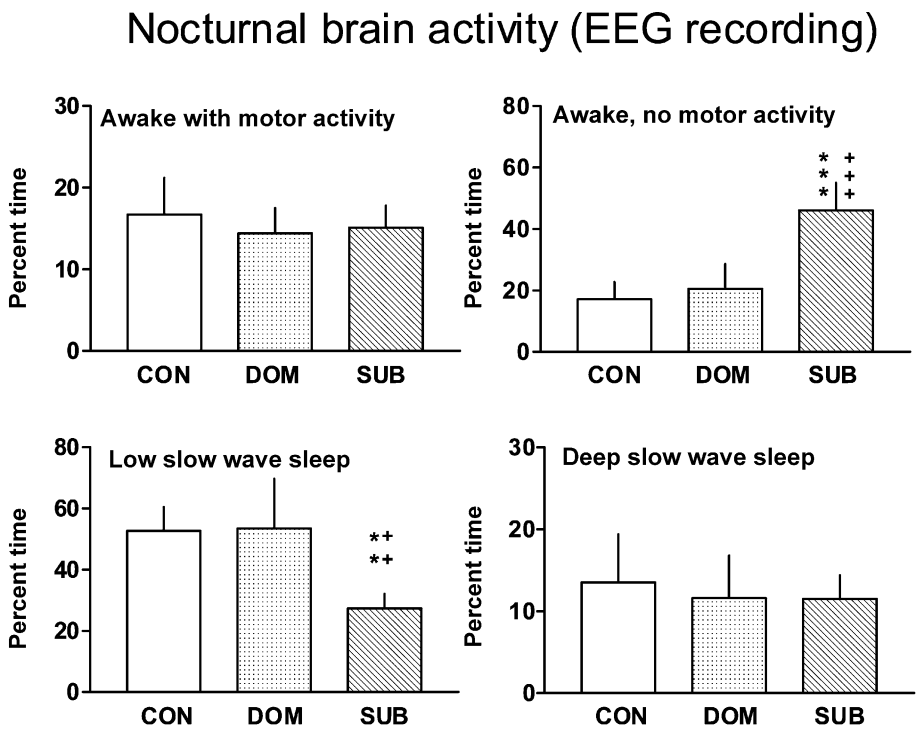

Fig. 1. Nocturnal brain activity and sleep phases of tree shrews. EEGs were recorded during five consecutive nights: The four states of activity/sleep correspond to the following EEG patterns: *awake, motorically active:* amplitude 170–330 μV, frequency 6–10 Hz; *awake, no motor activity:* 30–65 μV, 8–13 Hz; *light slow-wave sleep:* the animal is motorically inactive, 70–90 μV, 0.5–4 Hz; *deep slow-wave sleep:* 100–160 μV, 0.5–5 Hz. CON = unstressed controls; DOM = dominant animals during the period of daily social confrontations with the subordinate; SUB = subordinate animals during the period of daily social confrontations with the dominant. Data are expressed as percentage of time spent in the respective phase. They were analyzed by one-way ANOVA. Asterisks: significant difference to controls: ** $P<.01$, *** $P<.001$. Crosses: significant difference to dominants; $^{++}P<.01$, $^{+++}P<.001$ (with modifications from Aue, 1988).

amplitude is higher than in the awake phase (70–90 μV), frequency is low (0.5–4 Hz); (iv) *deep slow-wave sleep:* amplitudes 100–160 μV, frequency maximum 0.5–5 Hz.

The EEG measurements showed profound disturbances in the nocturnal sleeping patterns of subordinate tree shrews (Fig. 1). Awake phases without motor activity were significantly increased in these stressed animals, whereas low slow-wave sleep was reduced. The sleeping pattern of chronically stressed male tree shrews thus shows similarities with that of depressed human patients, which is characterized by frequent "early-morning waking" (Rüther, 1989). In contrast, the sleeping/activity patterns of dominant male tree shrews did not differ from controls.

3.7. Melatonin

The mammalian pineal gland can be influenced by a variety of exogenous stimuli among which light and darkness play important roles. Moreover, it became clear that nonphotic stressful stimuli modulate the secretion of melatonin, which has extremely widespread effects on possibly every organ in the body (Reiter et al., 2000). Chronic stress in subordinate male tree shrews caused an increase in urinary concentrations of the principal melatonin metabolite 6-sulfatoxymelatonin that persisted during the entire stress period and decreased only after cessation of the daily stress exposures (Fuchs and Schumacher, 1990). These findings substantiated the function of the pineal gland in transforming stimuli from the social environment to endocrine information.

4. Social stress and the brain

4.1. Monoamine receptors

Activation of monoamine systems in the brain is a major component of the stress response. The hyperactivity of the central nervous monoamine systems is of special interest because it may lead to psychopathologies such as anxiety disorders and depression, and is regarded as a basis for changes in behavior (Stanford, 1993; Holsboer, 1995; Meerlo et al., 1997). As demonstrated for the first time about 30 years ago, release and turnover of monoamines in the brain are increased during stressful experiences thus leading to high concentrations of neurotransmitters such as noradrenaline and serotonin (5-HT) or their metabolites, respectively (Thierry et al., 1968; Raab, 1971; Raab and Storz, 1976).

Fluctuations in concentrations of monoamines can have an impact on receptors that are stimulated by these endogenous agonists. High agonist concentrations lead to down-regulation of G-protein coupled receptors (decrease in receptor numbers), whereas low monoamine concentrations can induce up-regulation (for a review, see Flügge, 2000). The resulting changes in the responsiveness of the respective receptor system and, consequently, the imbalance between different systems probably constitute an important component of the pathophysiological processes that occur during chronic stress in the brain. To analyze which neurotransmitter systems in the brain are affected by chronic stress and to visualize the time course of presumptive changes, we studied different monoamine receptors.

The impact of chronic stress on the serotonergic system was demonstrated by analyzing 5-HT_{1A} receptors. In the dorsal raphe nucleus, somatodendritic 5-HT_{1A} autoreceptors trigger the release of 5-HT, whereas 5-HT_{1A} heteroreceptors in other brain regions modulate the activity of other neurons. 5-HT_{1A} receptors have been proposed to be involved in states of anxiety and stress (Coplan et al., 1995; Graeff et al., 1996). Experiments in rats indicated that stimulation of the 5-HT_{1A} autoreceptors induces anxiolysis, whereas stimulation of the heteroreceptors induced anxiety (File et al., 1996).

In the brains of socially stressed male tree shrews, 5-HT_{1A} heteroreceptors in the hippocampus and the occipital cortex were gradually down-regulated during a 4-week period of chronic stress (Flügge, 1995). In contrast, the number of the somatodendritic autoreceptors in the dorsal raphe nucleus did not change during this period indicating that stress affects only the postsynaptic receptors whereas the autoreceptor system remains unaffected. The changes in the hippocampal 5-HT_{1A} receptors are probably due to a regulatory influence of glucocorticoids that are known to decrease expression of these receptors (McKittrick et al., 1995). However, gonadal hormones also appear to play a role and a testosterone substitution in the subordinate male tree shrews leads to renomalization of brain 5-HT_{1A} receptor numbers (Flügge et al., 1998).

Whereas in the serotonergic system, a number of somatodendritic autoreceptors are stable during a 4-week period of chronic stress, α_2-adrenergic autoreceptors that trigger the release of noradrenaline from locus coeruleus neurons were already decreased after 2 days of social stress (Flügge, 1996). After 3 weeks of stress, receptors were down-regulated in brain regions that are involved in the regulation of autonomic functions, namely nuclei in the medulla oblongata, the periaqueductal gray, hypothalamic, and limbic regions (Flügge et al., 1992). These data show that the noradrenergic systems or at least its presynaptic autoreceptors react more quickly to stress exposure than the 5-HT_{1A} receptors. Our recent experiments indicate that the changes in the α_2-adrenoceptor system might have an impact on glutamatergic neurotransmission as α_2-adrenoceptor are expressed in glutamatergic neurons, at least in the lateral reticular nucleus in the brain stem (Meyer et al., 2000).

Not only the α_2-adrenoceptor but also the β-adrenoceptor system in the brain shows plastic changes during chronic stress. β_1-Adrenoceptors were transiently down-regulated after 2 days of stress in the prefrontal cortex and in the olfactory area, and were decreased after 4 weeks in the parietal cortex and the hippocampus (Flügge et al., 1997). A transient up-regulation of β_1-adrenoceptors occurred in the pulvinar nucleus after 10 stress days. β_2-Adrenoceptors were

transiently down-regulated after 2 days of social in the prefrontal cortex and up-regulated in the pulvinar nucleus after 4 weeks. These data demonstrate that chronic social stress leads to time-dependent changes in the central nervous β-adrenoceptor system.

4.2. Dopamine transporter (DAT)

Dopamine responses to stress received particular attention because of the involvement of the dopaminergic system in human psychopathologies (Fibiger, 1995). In rodents, the effect of different types of physical stress on brain dopaminergic functioning has been well established (Puglisi-Allegra et al., 1991; Imperato et al., 1992). However, the role of the dopaminergic system in more naturalistic stress situations is poorly understood. Therefore, we investigated in tree shrews the effect of chronic social stress on the DAT, an important component in the regulation of dopaminergic neurotransmission. We found a positive correlation between locomotor activity (which is reduced in stressed animals; see below) and the total number of DAT binding sites in motor-related brain areas (Isovich et al., 2000). Our findings suggest a functional connection between the stress-induced reduction in locomotor activity and the down-regulation of DAT binding sites.

In accordance with the above findings in chronically stressed male tree shrews, we also showed that social defeat in male rats induces a decrease in striatal DAT binding sites. However, this was only the case in rats that were singly housed after the defeat. In contrast, a familiar environment after the social defeat can prevent changes in the dopaminergic system, at least in rats (Isovich et al., 2001).

4.3. Glucocorticoid (GR) and mineralocorticoid (MR) receptors in the hippocampal formation

As pointed out above, stress is characterized by a hyperactivity of the HPA axis, and the adrenal glucocorticoid hormones cortisol and corticosterone are principal effectors in the stress response. They have profound effects on mood and behavior and modulate neurotransmission and neuroendocrine control (De Kloet et al., 1998). The broad range of physiological effects that are influenced by glucocorticoids are mediated via cellular GRs and MRs, which themselves are subject to autoregulation. GRs and MRs are not only present in peripheral organs but also in the brain where they are highly expressed in the hippocampal formation, a brain region important for the regulation of memory processes and for mediation of the stress response. The hippocampus exerts an inhibitory control over the hypothalamus and the activity of its pyramidal neurons is regulated via the GRs and MRs, which themselves are subjected to negative feed back inhibition by the glucocorticoids (De Kloet et al., 1998; Dijkstra et al., 1998).

We studied GR and MR expression at the single cell level using semiquantitative in situ hybridization to evaluate effects of chronic stress. As expected, stress exposure induced a down-regulation of GR mRNA in the hippocampal formation that reflects an impairment of the glucocorticoid-mediated negative-feedback control mechanism in the chronically stressed animals (Jöhren et al., 1994). Interestingly, mRNA for the MR in anterior subfields of the hippocampus was also clearly reduced. On the contrary, in a more posterior location on the longitudinal axis of the tree shrew hippocampus, the MR message was increased in subfields CA1, CA3, and in the dentate gyrus (Meyer et al., 2001).

4.4. 11-β-Hydroxysteroid-dehydrogenase (11β-HSD)

The influence of steroids on the brain is not only related to the presence of appropriate receptors but also to enzymes that degrade these hormones. Thus, the bioavailability of glucocorticoids is greatly determined by the enzyme 11β-HSD type 1 (11β-HSD-1), which catalyzes the reversible conversion of physiological glucocorticoids (cortisol, corticosterone) to their inert 11-keto metabolites (cortisone, 11-dehydrocorticosterone), thus acting as a tissue-specific regulator of glucocorticoid access to intracellular corticosteroid receptors (Monder and White, 1993; Seckl, 1993). We studied 11β-HSD-1 activity in different organs of male tree shrews following 28 days of chronic social stress. In the hippocampus, the stress attenuated 11β-HSD-1 activity by approximately 30%, and also in the liver, enzyme activity was reduced by stress to approximately 50% (Jamieson et al., 1997). It remains to be determined whether the reduction in 11β-HSD-1 activity leads to high concentrations of physiologically active glucocorticoids in the respective tissues, or whether it reflects a homeostatic mechanism designed to minimize the adverse affects of prolonged stress and/or glucocorticoid excess.

4.5. Receptors for the corticotropin-releasing hormone (CRH)

As suggested by diverse preclinical and clinical studies, the peptide CRH is one of the major substances eliciting an organism's autonomic, behavioral, endocrine, and immune responses to stressors (De Souza and Nemeroff, 1990). To elucidate whether chronic social stress has an impact on the CRH system, we measured binding sites for ^{125}I-labeled ovine CRH in the brain via in vitro receptor autoradiography. It turned out that 24 days of social conflict reduced the number of binding sites in the anterior lobe of the pituitary, in the dentate gyrus, in regions CA1 to CA3 of the hippocampus, in area 17, and in the superior colliculus (Fuchs and Flügge, 1995). These changes may indicate that increased concentration of the "stress peptide" CRH leads to the down-regulation of CRH receptors. In contrast, significant enhancements of ^{125}I-CRH binding were observed in the frontal cortex, cingulate cortex, claustrocortex, amygdala, and choroid plexus. These regional response patterns of the CRH system reflect distinct neuroendocrine processes

that are presumed to coordinate autonomic, endocrine, and behavioral reactions during long-lasting stress exposure. The finding that besides the CRH receptor changes in the amygdala there were very pronounced stress effects in several neocortical regions indicates that chronic stress does not only affect the limbic system but also cortical brain regions.

4.6. Structural changes in the brain

Some years ago, Sapolsky et al.'s (1986) findings that stress induces neuronal death in the hippocampus draw public attention to the fact that our brain is plastic and reacts flexibly to diverse environmental stimuli. However, exact quantification of numbers of neurons in defined brain regions such as the hippocampal formation requires special techniques such as the optical fractionator method (Keuker et al., 2001). Using this unbiased approach, we could not confirm that chronic stress induces neuronal death in the hippocampus (Vollmann-Honsdorf et al., 1997). Very recent experiments using in situ end labeling technique to identify apoptotic cells coincide with our earlier findings. When all hippocampal subareas were analyzed together, a significant decrease in the number of apoptotic cells was found in the chronic stress group (Lucassen et al., 2001). Moreover, the nuclear ultrastructure of the hippocampal pyramidal neurons changes during stress yielding increased staining intensities of nuclei of these neurons (Fuchs et al., 1995). Our recent electron microscopic analysis indicates that this effect is due to increased heterochromatin formation (Vollmann-Honsdorf et al., 1999). In addition, chronic social stress has an influence on neuronal morphology in that it induces a retraction of the dendrites of CA3 pyramidal neurons (Magariños et al., 1996).

As pointed out above, many of the stress-induced changes, either in the periphery or in the brain, are attributed to HPA axis hyperactivity due to the high circulating levels of glucocorticoids. To investigate whether exogenous cortisol changes the overall morphology of the hippocampal formation, the region with the greatest number of GRs in the brain, we measured the volume of the hippocampal formation in cortisol-treated tree shrews. Treatment with cortisol for 28 days reduced the volume of the tree shrew hippocampus by 10–15%. As expected, chronic stress also reduced the hippocampal volume by up to 10% (Ohl et al., 2000; Czeh et al., 2001).

4.7. Stress affects neurogenesis

Although the adult brain has classically been thought to be a structure with very limited regenerative capacity, neural stem cells have recently been shown to exist in the adult central nervous system, and it is now evident that the adult brain is efficiently generating specific neuronal populations (Fuchs and Gould, 2000). There are two regions of active proliferation in the mammalian brain that generate neurons continuously throughout life, the subependymal zone of the lateral ventricles and the dentate gyrus of the hippocampal formation, the latter being a structure intimately involved in the processing and storage of new information. Cell proliferation in the dentate gyrus can be modulated by environmental signals and experience. In tree shrews, both acute and chronic social stresses have been shown to inhibit granule cell production in the dentate gyrus (Gould et al., 1997, Fuchs and Gould, 2000). It is likely that the changes in granule cell generation are triggered by the stress-induced activation of the HPA axis and ultimately by the elevated circulating glucocorticoid hormones.

5. Social stress and behavior

Dominant and subordinate males can easily be distinguished according to their behavior. Like in other species, the dominant tree shrew chases the subordinate, attacks, and tries to bite, while the latter displays flight and freezing behavior (Blanchard et al., 2001). During acute confrontation, both counterparts perform characteristic vocalizations ("squeak"; Kirchhof et al., 2001). Squeaks of subordinates are longer in duration and higher in frequency than those of dominants. However, the qualitative and quantitative differences in behavior are most apparent after the chronic confrontation.

5.1. Motor activity

Tree shrews display a high locomotor activity (Kurre and Fuchs, 1988a,b) and motor activity is the type of behavior most frequently studied in animal models of depression (Willner et al., 1992). As in other experimental paradigms and species, chronic social conflict in subordinate tree shrews induces a significant decrease in motor activity. Subordinate animals not only reduce their motor activity but also their sphere of action. According to Aue (1988) and demonstrated by Fuchs et al. (1996), they tend to avoid the dominant animal and try to keep the largest distance possible from the dominant. Reduced motor activity appears to be a parameter showing the motivational state of the animal, as treatments with antidepressants such as clomipramine and fluvoxamine, which probably have beneficial effects on the emotional state, also restore normal motor activity (Fuchs et al., 1996). In contrast, a testosterone substitution in the subordinate males, which is able to restore scent marking and self-grooming behavior, cannot renormalize motor activity (Flügge et al., 1998).

5.2. Self-grooming

Self-grooming is a behavioral feature often related to HPA axis activity and is presumably an essential behavior for mammals, since it is thought to have many functions ranging from cleaning the fur and thus spreading olfactory active chemicals to temperature regulation (Spruijt

et al., 1992). Studies in rats demonstrated that grooming cannot be simply understood as an immediate response necessary to reduce arousal following stress exposure. Self-grooming rather seems to be suppressed in defeated rats (van Erp et al., 1994). Similarly, we found a clear reduction in self-grooming activity of subordinate tree shrews resulting in rough and dirty looking fur of these animals (Aue, 1988). Though the central nervous circuits for grooming are still a matter of discussion (Spruijt et al., 1992), it is interesting that long-term treatment with the antidepressant clomipramine induced a stepwise normalization of grooming behavior in subordinate tree shrews (Fuchs et al., 1996).

5.3. Marking activity

Chemical signals play an important role in regulating the behavior of tree shrews. Scent substances are found in glandular secretions, urine, feces, and saliva and contain information concerning the identity and physiological state of the individual. Both the production of the scent substances and the marking behavior are controlled by androgens (von Holst and Buergel-Goodwin, 1975). During chronic stress, marking behavior of subordinates is reduced, most probably due to the low testosterone levels (Kramer et al., 1999). As known from males of other species, e.g., gerbils (Probst, 1985), marking activity in tree shrews can be restored by testosterone substitution (Flügge et al., 1998).

5.4. Consumatory behavior

An organism's functioning is partially guaranteed by normal consumatory behavior. Thus, reduction of food and water intake could result in disturbances of the bodily homeostasis and loss of body weight. Similar to a study in rats, where the effect of restraint stress on consumatory behavior was found to be daytime-dependent (Rybkin et al., 1997), we detected a clear difference in food and water intake dependent on the observation time (Kramer et al., 1999). It should be noted, however, that the reduction in body weight in subordinate tree shrews is also due to the general acceleration of the metabolism, e.g., reflected by high oxygen consumption (see above).

6. Effects of antidepressants in chronically stressed tree shrews

Behavioral and neuroendocrine reactions observed in socially stressed tree shrews are similar to those produced by centrally administered CRH in laboratory animals to mimic depressive-like symptoms. But importantly, they are also comparable to the symptoms observed in depressed patients (DSM-IV, 1994; Table 2). Thus, the chronic social stress model in tree shrews has an obvious *face validity* for depression. However, 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, which are raised by subjective account, cannot be modeled simultaneously in animals.

To elucidate whether the chronic social 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 endocrine and behavioral parameters that corresponded closely to that observed when treating depressed patients (Fuchs et al., 1996; Kramer et al., 1999). Interestingly, treating subordinate animals with the anxiolytic diazepam was ineffective supporting the view that in tree shrews, the emotional state induced by social stress might be more depression than anxiety related (van Kampen et al., 2000). Our recent experiments also show that the selective 5-HT reuptake inhibitor fluvoxamine counteracted effects of chronic social stress with respect to behavioral and endocrine parameters (van Kampen et al., unpublished data). Interestingly, fluvoxamine induced a pronounced down-regulation of the somatodendritic 5-HT_{1A} autoreceptors on the serotonergic neurons in the dorsal raphe nucleus that coincides with clinical studies showing that antidepressant treatments lead to reduced sensitivity of these serotonergic autoreceptors (Lesch et al., 1991). Furthermore, the atypical antidepressant tianeptine restored normal rate of neurogenesis in the

Table 2
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 social 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 social 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 everyday	Diminishes sexual behavior 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 behavioral despair syndrome observed after maternal separation in rhesus monkey infants	Reduced locomotor activity and grooming behavior

dentate gyrus of chronically stressed male tree shrews (Czeh et al., 2001).

7. Conclusions

Preclinical projects on stress represent an important branch of biomedical research since clinical data point to a crucial role of psychological and social stress, either acute or chronic, in the etiology of affective disorders (Brown, 1993). In this field, valid animal models that can be used to study the pathophysiology of major depression and the specific bio-behavioral responses of animals to antidepressant drug treatments are of central interest. Based on the data summarized in the present report, the chronic social stress paradigm in male tree shrews can be regarded as a suitable nonrodent model for research on the etiology and pathophysiology of depressive disorders because (1) behavioral and endocrine symptoms of subordinate tree shrews resemble those of depressed patients and (2) antidepressant treatments lead to an improvement of these symptoms. Using the tree shrew model, we demonstrated that chronic stress leads to various central nervous changes including alterations in the morphology of neurons and time-dependent changes at the level of neurotransmitter receptors and transporters indicating imbalances in these systems. Recently, we also demonstrated that stress-induced alterations in brain metabolism, hippocampal volume, and neurogenesis are prevented by concomitant treatment with the modified tricyclic antidepressant tianeptine (Czeh et al., 2001). Together, these findings provide experimental evidence for new theories stating that impairments of brain structural plasticity are important features of depressive disorders (Duman et al., 1999).

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