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Psychosocial Stress in Tree Shrews: Clomipramine Counteracts Behavioral and Endocrine Changes

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FUCHS, E., M. KRAMER, B. HERMES, P. NETTER AND C. HIEMKE. *Psychosocial stress in tree shrews: Clomipramine counteracts behavioral and endocrine changes.* PHARMACOL BIOCHEM BEHAV **54**(1) 219-228, 1996. – Male tree shrews (*Tupaia belangeri*) provide an animal model to study the neurobehavioral consequences of chronic psychosocial stress. When living in visual and olfactory contact with a male conspecific by which it has been defeated, the subordinate tree shrew shows dramatic behavioral, physiological, and neuroendocrine changes. Because the over all pattern of these changes resemble a depression-like symptomatology, we investigated to what extent the behavioral and endocrine changes in subordiante animals can be reversed by treatment with the tricyclic antidepressant clomipramine. In the present study, animals were subjected to a 10-day period of psychosocial conflict to elicit stress-induced behavioral and endocrine alterations before the onset of drug treatment, and psychosocial stress continued throughout the treatment period of 30 days. Clomipramine was administered orally once daily at a dose of 50 mg/kg. The drug had a time-dependent restorative influence on marking and grooming behavior, locomotor activity, risk assessment, as well as on urinary cortisol and norepinephrine excretion. It, thus, appears that the clomipramine treatment counteracts the behavioral and endocrine effects of chronic psychosocial stress in tree shrews, and the time course of recovery corresponds closely to that observed when treating depressed patients in the clinic.

Antidepressant Cortisol Depression Grooming HPA axis Locomotor activity Marking behavior Norepinephrine Risk assessment

A VARIETY of evidence is indicative of an association between acute and/or chronic life stressors and the pathogenesis of mental illnesses such as anxiety disorders and depression (3,25,37,49). As simulations within which aspects of depression are investigated in animal models, stressors mostly involve noxious stimuli or perturbations of the physical environment such as electric foot shock, tail pinch, forced swimming, physical restraint, water and food deprivation, cold exposure, or soiled cages. Most common stressors in humans, however, are of a more psychological type. To bridge the gap between these experimental models and the situation in humans, there is a need to use more psychological types of stressors in animal studies. Evidence has accumulated in recent years that male tree shrews (*Tupaia belangeri*) may represent a suitable model to study the biobehavioral consequences of psychosocial stress. From the phylogenetic point of view, the day-active animals are regarded as an intermediate between insectivores and primates (32). They are widely distributed in South-East Asian forestral and plantation areas where they live singly or in pairs in territories, which they defend vigorously against intruding conspecifics. This pronounced territoriality, especially in 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 behavioral, physiological, and neuroendocrine changes. As revealed by detailed quantitative behavioral analysis, subordinates tend to withdraw from the field of vision of the dominant, reduce their locomotor activity, and cease auto-grooming behavior (1). Their sleeping pattern is characterized by an increasing number of early waking episodes in the second half of the night (1), and their circadian

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rhythm is profoundly disturbed (13,48). In subordinates, the reduction of body weight is due to a diminshed food and water intake and to a significantly elevated metabolic rate (1,12,27). Analysis of endocrine parameters in subordinates revealed constantly increased concentrations of both adrenocortical and adrenomedullary hormones (11,15), increased adrenal weights (15,40,56), and reduced activities of the gonads (7,56). In the brains of subordinate tree shrews, 5-HT_{1A}-receptors are decreased in a time- and region-specific manner (8) and alpha$_2$-adrenoceptors are downregulated in areas mainly involved in the regulation of autonomic functions (9). In the hippocampus, a brain structure that plays a crucial role in negative feedback regulation of hypothalamic-pituitary-adrenal axis stress response (22,26), and which is involved in spatial information processing as well as in aspects of learning and memory (6,45), we recently demonstrated a downregulation of hippocampal glucocorticoid and corticotropin-releasing hormone receptors (16,28) and specific structural changes in pyramidal neurons (17,30). These distinct stress-induced behavioral and physiological alterations in subordinates are based exclusively on the cognitive interpretation of the continuous visual presence of the dominant conspecific (39,40,56). In dominant tree shrews, on the contrary, no noticable behavioral and physiological alterations can be observed (1).

Phenomenologically, many of these behavioral and neuroendocrine reactions are similar to those produced by centrally administered CRH in laboratory animals and are comparable to the symptoms observed in depressed psychiatric patients (5,36). To elucidate whether the tree shrew model, besides its face validity for depression also has a predictive validity (56), we investigated the therapeutical action of the antidepressant clomipramine on locomotor activity, marking behavior, self-grooming, risk assessment, and urinary cortisol and norepinephrine in subordinate tree shrews. To mimic a realistic situation of antidepressant intervention, the treatment started after the stress-induced behavioral and endocrine alterations had been established. The drug was administered orally, while the psychosocial stress continued during the whole treatment period, and the therapeutic action of clomipramine was followed across a clinically relevant time period of 4 weeks.

METHOD

Animals and Housing

The experiments were conducted with adult male tree shrews (*Tupaia belangeri*) from the breeding colony at the German Primate Center (Göttingen, Germany). The animals were housed individually on a regular day/night cycle with illumination from 0800 to 2000 h in air-conditioned rooms [for details, see (13)].

In our study, tree shrews received the tricyclic antidepressant clomipramine orally. This route of administration was chosen to minimize uncontrollable stress effects elicited by IP injections of the drug. In addition, oral application of antidepressant drugs is the most common route of administration also in humans. In a pilot study, we determined the clomipramine dosage necessary to reach a plasma concentrations in tree shrews similar to that known to be therapeutically effective in humans. For 5 consecutive days animals received clomipramine dissolved in 0.9% saline in the morning. Four hours after the last drug application, blood was collected by puncturing the tail's venous plexus.

Determination of Drug Concentrations in Serum Samples

Clomipramine and ist metabolites desmethylclomipramine, 8-hydroxyclomipramine, 10-hydroxyclomipramine, and 8-hydroxydesmethylclomipramine (Ciba-Geigy, Basel, Switzerland), were determined in serum with minor modifications by a high-performance liquid chromatography (HPLC) method with column switching that was originally established for the determination of amitriptyline and its major metabolites in human plasma and serum (19). The fully automated analysis uses direct injection of 100 μl serum or plasma without prior sample pretreatment. Clomipramine and its metabolites were retained on a clean-up column (20 × 4.6 mm i.d.) filled with reversed C8 material (Hypersil CPS) of 10 μm particle size. Proteins, lipids, and other interfering compounds were washed to waste using 5% methanol in deionized water (v : v) at a flow rate of 1.5 ml/min. After changing the flow (1.5 ml/min) onto the analytical column (Spherisorb CN, 5 μm by MZ Analysentechnik, Mainz, Germany), clomipramine and its metabolites were separated within 20 min by acetonitril : methanol : 0.01 M phosphate buffer, pH 6.2 (58 : 19 : 23; v : v) as eluent and quantified by ultraviolet detection at 214 nm. The recovery rates were always better than 80%. Using serum specimens spiked with 25-350 ng/ml of pure drug substance the interassay coefficients of variation ranged between 3 and 11%. The limit of detection was about 15 ng/ml. Pilot studies with serum blank samples of untreated tree shrews did not reveal compounds interfering with the analysis of the drugs in the treated animals.

Experimental Procedure

During a 10-day control period, with individually housed animals (n = 20), the basal activities of the pituitary-adreno-

TABLE 1

SERUM CONCENTRATIONS OF CLOMIPRAMINE AND ITS METABOLITES AFTER A TREATMENT PERIOD OF 5 AND 28 DAYS, RESPECTIVELY

	Treatment Period	
	5 Days	28 Days
Clomipramine	176.4 ± 82.5 (n = 3)	2148.3 ± 307.9 (n = 9)
Desmethylclomipramine	62.3 ± 10.4 (n = 3)	252.0 ± 22.6 (n = 9)
8-Hydroxyclomipramine	32.0 ± 13.6 (n = 3)	ND
10-Hydroxyclomipramine	13.8 ± 2.8 (n = 3)	1026.8 ± 250.2 (n = 3)
8-Hydroxydesmethylclomipramine	38.4 ± 6.2 (n = 3)	ND

Data (ng/ml) are given as means ± SD; n = number of animals; ND = not detectable.

cortical axis and of the sympathetic system were determined by measuring cortisol and norepinephrine in the morning urine, which was collected daily between 0745 and 0800 h after a slight massage of the hypogastrium.

In the first experimental group, the induction of psychosocial conflict was carried out according to our standard procedure. After the control period, the opaque partition between the neighboring cages of two males unknown to one another was removed. This resulted in an active competition for control over the enlarged territory. After establishment of a stable dominant/subordinate relationship, the two males were separated by a transparent wire mesh. Daily morning urine samples were collected as described above, and the wire mesh was removed every day for 1.5 h between 0830 and 1000 h, allowing physical contact between the two males. After 10 days of psychosocial conflict, subordinate animals received clomipramine (50 mg/kg) orally dissolved in 0.9% saline in the morning (stress + clomipramine group; $n = 5$). For the next 30 days, the subordinate animals remained in the psychosocial conflict situation as described above. Daily morning urine samples were collected and the animals were treated daily with clomipramine. In the second group treated according to the same schedule as the first one, the subordinate animals received 0.9% saline instead of clomipramine (stress + placebo group; $n = 5$). Animals of the third group remained singly housed in their home cages after the control period. For the next 10 days, morning urine samples were collected daily. After this period, the animals received clomipramine (50 mg/kg) orally dissolved in 0.9% saline in the morning (control + clomipramine group; $n = 5$). The treatment and handling of the animals was continued for the next 30 days. In the fourth group, the animals remained singly housed in their home cages after the control period. For the next 10 days, morning urine samples were collected daily. After this period, the animals received 0.9% saline in the morning (control + placebo group; $n = 5$). The treatment and handling of the animals was continued for the next 30 days.

Monitoring and Analysis of Behavior

The behavior from each experimental animal was video taped twice daily between 0945 and 1015 h and between 1845 and 1915 h. The behavior recorded by video between 1000 and 1005 h and between 1900 and 1905 h was analyzed from coded tapes, meaning that the investigator was blinded as to the origin of the tapes. In the two 5-min intervals, four different types of behavior were quantified.

Locomotor activity was measured by marking the cages with adhesive tape that allowed a visual subdivision of every cage into six areas of equal size. 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 measuring the time every animal spent in defined areas of the cage. According to Aue (1), subordinate tree shrews tend to avoid the dominant conspecific by maintaining the longest possible distance between the two animals. To quantify this risk assessment behavior, we measured the time every animal spent in the upper third, the lower third, and the central third of the cage. Self-grooming was quantified by measuring the total time the animal spent with scratching, fur licking, fur cleaning, and wiping the muzzle after food intake (46). Marking behavior was quantified by measuring the total time the animal spent with different forms of scent marking such as marking with the abdominal gland, marking with the sternal gland, and urinary marking (31).

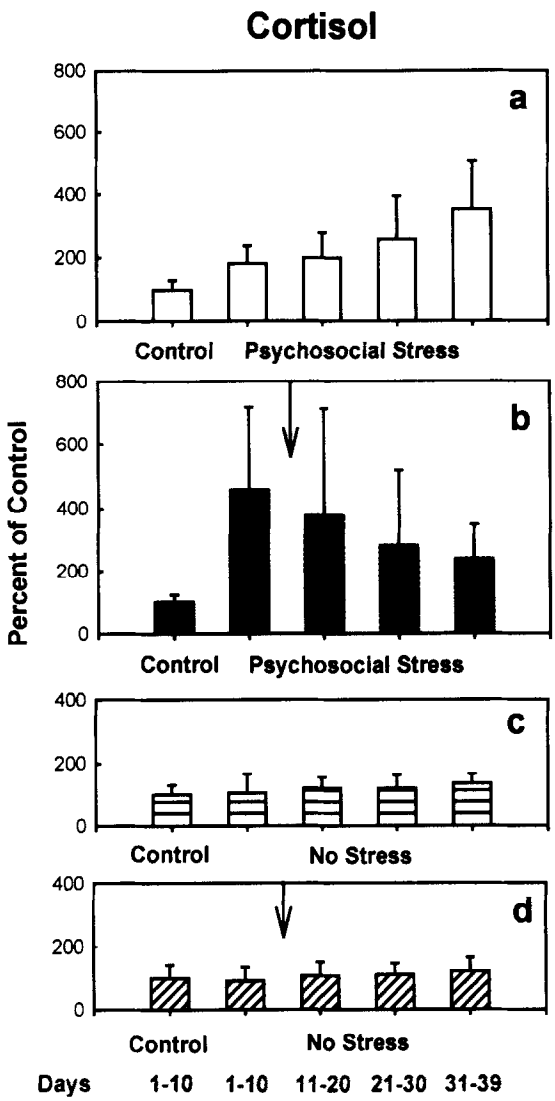

FIG. 1. Effect of chronic psychosocial stress and long-term clomipramine treatment on urinary cortisol excretion in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. The antidepressant was applied orally (50 mg/kg) starting on day 10 of the psychosocial stress period (arrow) and the daily clomipramine treatment was continued until day 39 of psychosocial stress. (c) Control + placebo group. (d) Control + clomipramine group. The arrow indicates the beginning of the daily antidepressant treatment (orally, 50 mg/kg), which was continued until the end of the experiment. Data are given as means ± SD. Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress days 31-39 $p < 0.01$.

All behavior analyses were performed using the Hindsight 1.3 Behavioral Observer software (Scott Weiss, Leeds, UK). This program allows using a standard computer keyboard, for

TABLE 2

INFLUENCE OF PSYCHOSOCIAL STRESS AND CLOMIPRAMINE TREATMENT ON URINARY CORTISOL AND NOREPINEPHRINE

	Cortisol	Norepinephrine
Stress + placebo	353 ± 155	329 ± 157
Stress + clomipramine	239 ± 110	303 ± 134
Control + placebo	135 ± 31	115 ± 39
Control + clomipramine	122 ± 44	220 ± 49

Data from experimental days 31–39 from each group are expressed as percent of pretreatment values (means ± SD; $n = 4$). Note that in the control group, long-term clomipramine treatment induced an increase in urinary norepinephrine excretion. Statistical differences for cortisol: stress + placebo vs. stress + clomipramine $p < 0.01$. Statistical differences for norepinephrine: stress + placebo vs. stress + clomipramine $p < 0.01$; control + placebo vs. control + clomipramine $p < 0.01$.

monitoring and measuring frequency, as well as duration of behavioral events. The Hindsight 1.3 software converts the computer keyboard into an event counter, by assigning certain behaviors to certain keys. The program measures the frequency as well as the duration of key activations during every scoring session.

Analysis of Urine Samples

Urinary free cortisol was measured by a scintillation proximity radioimmunoassay (51) using antirabbit cortisol antiserum (Paesel-Lorei, Frankfurt, Germany), antirabbit IGG-coated fluomicrospheres (scintillation proximity assay antirabbit reagent type I, Amersham, Braunschweig, Germany), [^{3}H]-cortisol (Amersham) as tracer. Urinary norepinephrine was quantified by HPLC with coulometric detection after extraction on BioRex 70 cation-excange columns (BioRad, Munich, Germany) as described recently (14). To correct for physiological alteration in urine dilutions, the resulting concentrations were related to creatinine concentrations, which were determined with a Beckman Creatinine Analyzer 2.

Statistical Analysis

The statistical analysis of the data was performed using the GB-Stat 5.3 software (Dynamic Microsystems, Silver Spring, MD). To avoid the influence of interindividual pretest differences, all values were transformed into percent values by relating them to the mean value of the control phase. Furthermore, values of every experimental group (stress + clomipramine; stress + placebo; control + clomipramine; control + placebo) were divided into five data blocks, of which everyone represented the means of 10 days. This procedure allowed detection of significant within-group differences between control period, stress period, and treatment period. To test for significant differences of means we used the one-way ANOVA procedure, followed by Fisher's LSD test.

To detect possible interaction effects of our experimental groups (stress + clomipramine; stress + placebo; control + clomipramine; control + placebo), we used each group's last data block (day 31–39), to perform a two-factorial ANOVA procedure, followed by Fisher's LSD test, determining significant mean differences as well as significant interactions of treatment factors.

RESULTS

Drug Concentrations in Serum

After a 5-day period of treatment with clomipramine, blood samples were taken from three animals for monitoring drug concentrations in serum. In these samples, clomipramine, desmethylclomipramine, 8-hydroxylated metabolites, and 10-hydroxyclomipramine were detected with marked interindividual variabilities (Table 1). The highest levels were found for clomipramine with a mean concentration of 176 ng/ml. The mean concentration for desmethylclomipramine was 62 ng/ml, while those of the hydroxylated metabolites ranged between 13 and 38 ng/ml. When analyzing the serum samples obtained at the end of the treatment period, clomipramine concentrations were about 10-fold higher (mean 2418 ng/ml), and also desmetyhlclomipramine was increased (mean 252 ng/ml). At this time the concentrations of 8-hydroxylated metabolites were below the limit of detection (3 to 5 ng/ml). Relatively high levels of 10-hydroxyclomipramine were found in three animals, while this metabolite was not detectable in the other five animals controlled for their serum drug levels. No differences in serum concentrations of clomipramine and its metabolites were found between stressed and unstressed animals treated with the antidepressant compound for 4 weeks.

Urinary Parameters

The intensity of psychosocial stress in subordinate tree shrews is demonstrated by a sustained and significant activation of the HPA axis as indicated by the pronounced and highly significant elevation of urinary cortisol excretion in the stress-placebo group (Fig. 1a; $p < 0.01$). However, daily treatment of subordinate animals with clomipramine (50 mg/kg) evoked a time-dependent and significant decrease of urinary cortisol excretion (Fig. 1b). No effects on urinary cortisol excretion were obserevd in animals from both the control + clomipramine and the control + placebo group (Fig. 1c and d; Table 2).

Besides the activation of the HPA axis, chronic psychosocial stress also resulted in an activation of the neurosympathetic system, as demonstrated by the significant increase of urinary norepinephrine in the stress + placebo group compared to the control + placebo group (Fig. 2a and b; p

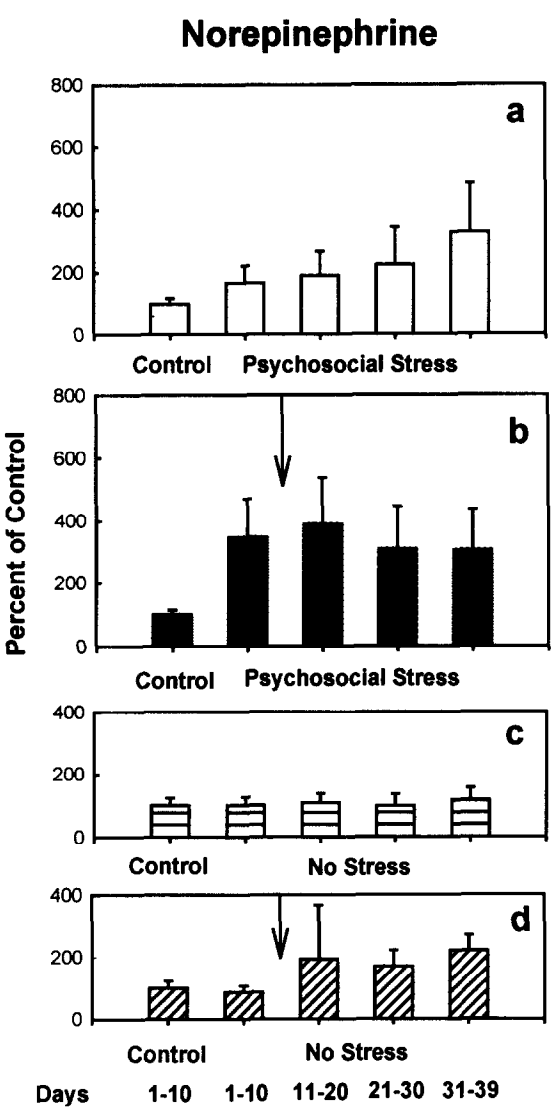

FIG. 2. Effect of chronic psychosocial stress and long-term clomipramine treatment on urinary norepinephrine excretion in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. (c) Control + placebo group. (d) Control + clomipramine group (for details, see Fig. 1). Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (d) Control vs. days 11-20 $p < 0.01$; control vs. days 21-30 $p < 0.01$; control vs. days 31-39 $p < 0.01$.

< 0.01). Daily antidepressant treatment produced a marked decline in the neurosympathetic tone of the chronically stressed animals; that is, the urinary excretion of the catecholamine was decreased in the stress + clomipramine group as compared to the stress + placebo group. Treatment of control animals with clomipramine, however, resulted in significant increment of urinary norepinephrine concentration as compared to the control + placebo group (Fig. 2c and d; Table 2).

Behavioral Parameters

Figure 3 summarizes the effects of psychosocial conflict and of oral clomipramine treatment on marking behavior in tree shrews. In subordinate animals that received no antidepressant treatment (stress + placebo group), marking behavior significantly declined in a time dependent manner (Fig. 3a). However, clomipramine treatment (50 mg/kg/day) starting after the first 10 days of psychosocial conflict induced a step-wise and significant restoration of the marking behavior

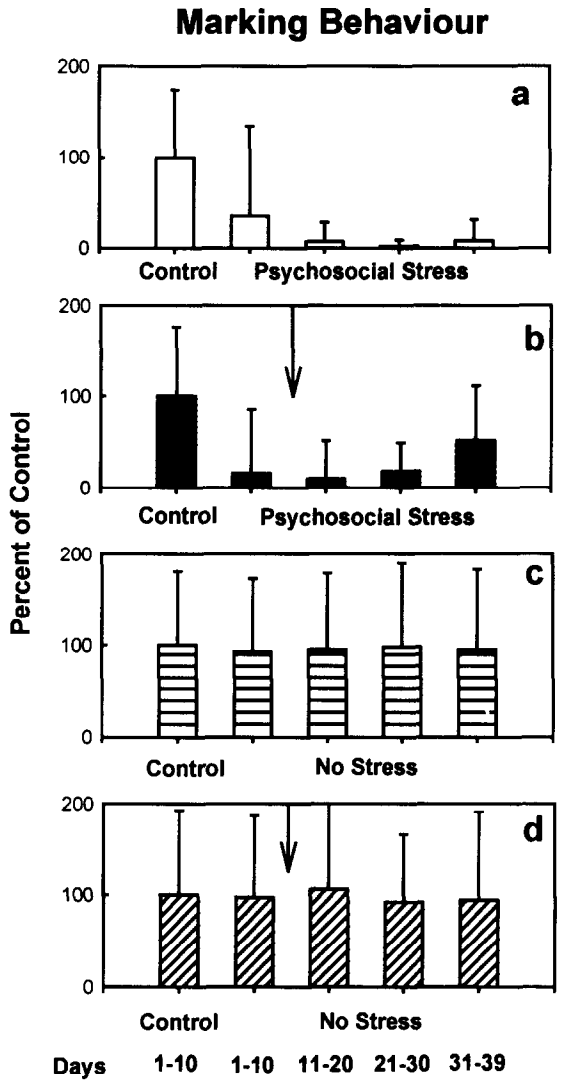

FIG. 3. Effect of chronic psychosocial stress and long-term clomipramine treatment on marking behavior in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. (c) Control + placebo group. (d) Control + clomipramine group (for details, see Fig. 1). Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress days 31-39 $p < 0.05$.

within 4 weeks (Fig. 3b). No effects on marking behavior, either with clomipramine or with placebo, were observed in the control groups (Fig. 3c and d).

A similar pattern of drug effect was observed with self-grooming behavior. In subordinate animals receiving placebo, autogrooming was significantly reduced within the first 10 days of psychosocial conflict and remained on this low level for the next 4 weeks (Fig. 4a; $p < 0.01$). When treating subordinate tree shrews with clomipramine, self-grooming returned to control levels within 20 days (Fig. 4b).

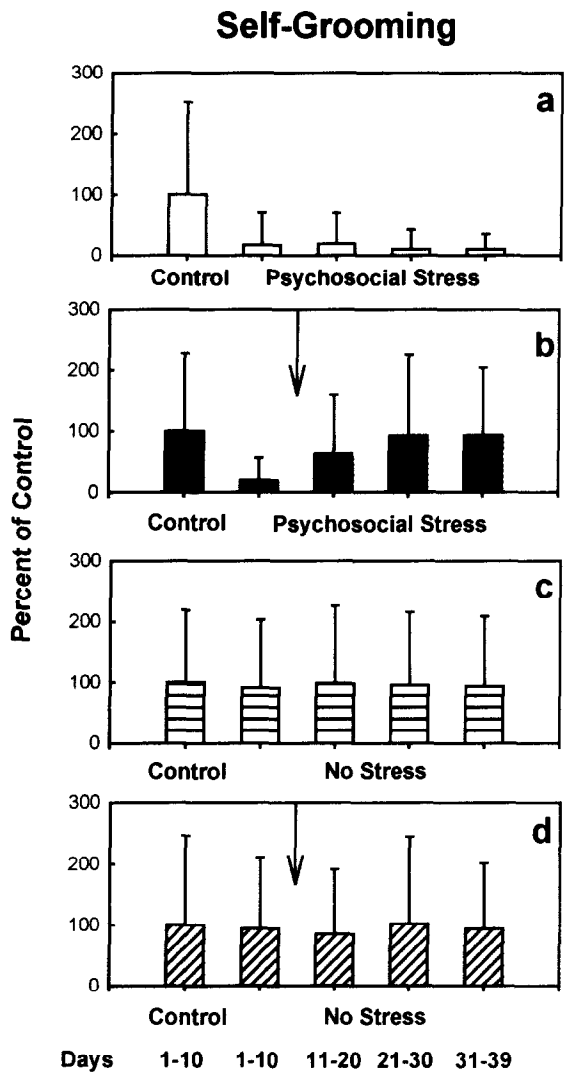

FIG. 4. Effect of chronic psychosocial stress and long-term clomipramine treatment on self-grooming in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. (c) Control + placebo group. (d) Control + clomipramine group (for details, see Fig. 1). Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.05$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress days 21-30 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress days 31-39 $p < 0.01$.

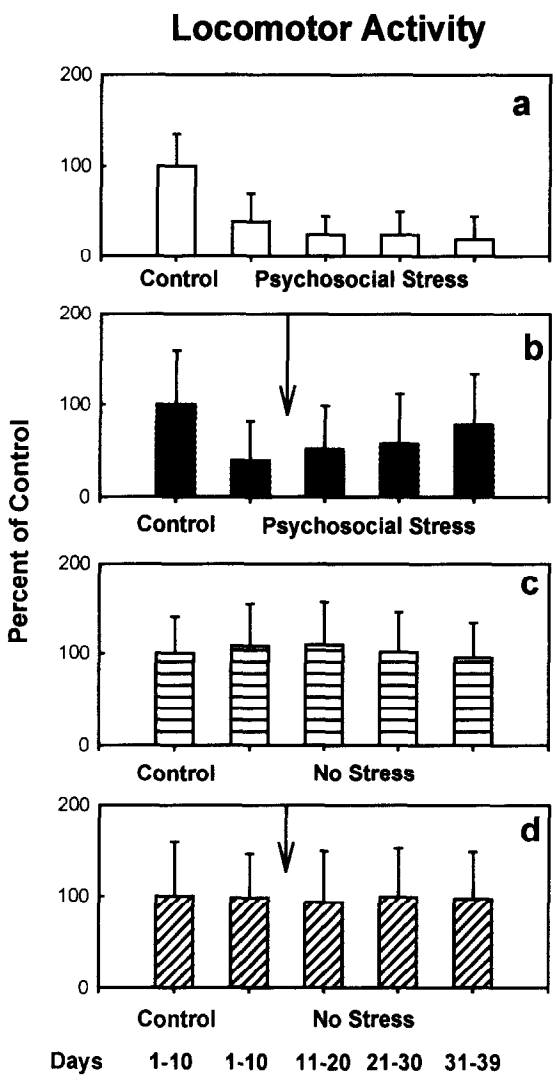

FIG. 5. Effect of chronic psychosocial stress and long-term clomipramine treatment on locomotor activity in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. (c) Control + placebo group. (d) Control + clomipramine group (for details, see Fig. 1). Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress days 31-39 $p < 0.01$.

In line with the data of stress-induced reduction of marking behavior and self-grooming, a pronounced decrease of locomotor activity was observed in subordinate animals from the beginning of the psychosocial conflict onwards. As shown in Fig. 5a, motor activity was reduced for about 70% within the first 10 days of psychosocial conflict and remained on this low level during the whole experiment. Daily administration of clomipramine moderately restored motor activity in subordinate tree shrews resulting in levels close to controls within the next 4 weeks (Fig. 5b).

Subordinate tree shrews tend to avoid the dominant conspecific by maintaining the longest possible distance to the latter one. This risk assessment and the effect of antidepressant treatment on this behavior is summarized in Fig. 6. In the stress + placebo group, risk assessment significantly increased (Fig. 6a). However, clomipramine treatment starting after the first 10 days of psychosocial conflict resulted in a step-wise and significant normalisation of risk assessment within the next 4 weeks (Fig. 6b).

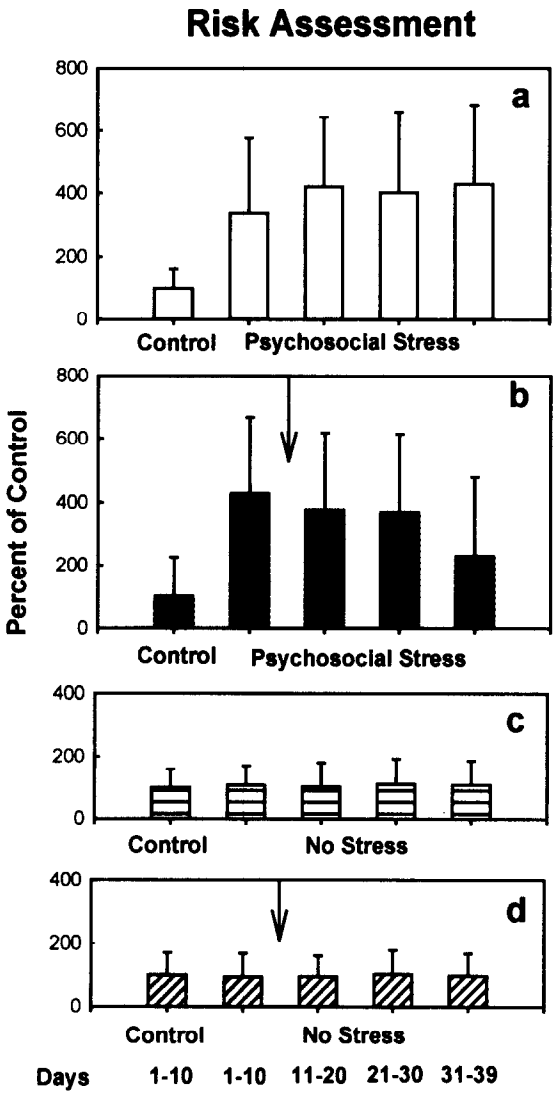

FIG. 6. Effect of chronic psychosocial stress and long-term clomipramine treatment on risk assessment in male tree shrews. (a) Stress + placebo group. (b) Stress + clomipramine group. (c) Control + placebo group. (d) Control + clomipramine group (for details, see Fig. 1). Significant differences: (a) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$. (b) Control vs. psychosocial stress days 1-10 $p < 0.01$; control vs. psychosocial stress days 11-20 $p < 0.01$; control vs. psychosocial stress days 21-30 $p < 0.01$; control vs. psychosocial stress days 31-39 $p < 0.01$; psychosocial stress days 1-10 vs. psychosocial stress day 31-39 $p < 0.01$.

DISCUSSION

Although the therapeutic effects of antidepressant drugs appear after 2 or 3 weeks of treatment, only a few animal studies have employed chronic administration of antidepressants during a clinically relevant time (41,42,58). In the present experiment we intended to mimic a realistic situation of an antidepressant intervention in animal studies. We, therefore, followed the therapeutic action of the drug across 4 weeks, which is a clinically relevant time period. The antidepressant was applied orally. This is the most common route in humans and minimizes uncontrollable stress effects elicited by injection of the drug the route mostly used in animal studies. The stress paradigm had a strong psychological component. The antidepressant treatment started after the stress-induced biobehavioral alterations had been established, and the stressful influences were continuously present during the whole treatment period. Using this approach, the results of the present study show that long-term oral treatment of subordinate male tree shrews with clomipramine counteracts the behavioral and endocrine effects of chronic psychosocial stress. We used clomipramine because it is a potent and preferential inhibitior of serotonin reuptake (33), and aberrations in the regulation of the serotonergic system are thought to be implicated in the etiology of depressive illness (52). Moreover, desmethylclomipramine, the pharmacologically active metabolite of clomipramine, is a potent norepinephrine reuptake inhibitor (33).

In humans, at therapeutically effective doses, plasma concentrations of clomipramine and desmetylclomipramine are in the range of 350 ng/ml (20). In a pilot study, we determined the clomipramine dose necessary to reach similar plasma concentrations in tree shrews. Because a daily oral application of 50 mg/kg for 5 consecutive days resulted in a clomipramine and desmethylclomipramine serum concentration of about 240 ng/ml, we decided to use this dose for the treatment of the subordinate animals. As reported from other groups, the dosage of antidepressants in rats were in the range between 10 and 20 mg/kg/day after IP injection (23,38). The pattern of metabolites observed in tree shrews after the 5-day treatment indicated similar metabolic pathways of clomipramine as in humans. However, this aspect was not confirmed when serum samples were analyzed after a 30-day drug treatment. Now, lower *N*-methylation, and especially lower hydroxylation rates, were found in tree shrews. This difference is probably due to species-specific properties of liver enzymes being involved in the degradation of clomipramine. As opposed to other investigations, in the present study it was for the first time to determine the dose of an antidepressant necessary to reach—in analogy to patients—therapeutically relevant plasma concentrations in animals. In addition, plasma concentrations of the drug at the end of the experiment were also determined for the first time. The unexpected increase in plasma clomipramine is an important finding because 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 supraeffective doses had been used.

Chronic psychosocial conflict induces an activation of the neurosympathetic system and of the HPA axis. The resulting elevation of circulating norepinephrine and glucocorticoids may have detrimental effects on behavior and various bodily

functions by affecting the endocrine, the immune, the cardiovascular, and the nervous system (2,10,17,21,29,30,57). In subordinate tree shrews, the urinary excretion of cortisol and norepinephrine was increased, which indicates hyperactivity of the HPA axis and of the neurosymapthetic system. These data are in accordance with earlier findings of our group (14–17,28). Increased adrenal cortisol responses and baseline hypersecretion of cortisol in depressed patients are some of the best-replicated findings in psychiatry (24,35,43,44). In depressed patients, dysregulation of the HPA axis and of the sympathetic system occur together, and increased cortisol levels are combined with a hypersecretion of norepinephrine (35). Like in depressed patients (18,24), treatment of subordinate tree shrews with the tricyclic antidepressant clomipramine normalizes both the activity of HPA axis and of the sympathetic system. 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 corticotropin-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 (36,50).

Concentration of urinary norepinephrine is the endproduct 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 time dependent responses to challenging situations (4,34). In addition, urine analysis is advantageous in tree shrews because blood sampling is stressful for the animals (48). Interestingly, urinary norepinephrine excretion was significantly elevated in control animals that received clomipramine treatment vs. controls with placebo treatment. This result may be due to the fact that desmethylclomipramine, the pharmacologically active metabolite of clomipramine that was also found in tree shrew serum, is a potent norepinephrine reuptake inhibitor (33).

The behavior most frequently studied in animal models of depression is locomotor activity (58). As in other experimental paradigms and species, chronic psychosocial conflict in subordinate tree shrews induces a significant decrease in motor activity. This motor retardation can be returned to normal levels when treating the animals with clomipramine. Subordinate animals not only reduce their motor activity but also their sphere of action. According to Aue (1), they tend to avoid the dominant animal and try to have the largest distance possible from the dominant, a behavior termed risk assessment in the present study. As expected, risk assessment increased stepwise during psychosocial stress in subordinates. Because clomipramine not only acts as a pure antidepressant but also proved to be effective in treating anxiety disorders (33), treatment of subordinate tree shrews for 4 weeks normalized risk assessment in a time-dependent manner.

Self-grooming is a behavioral feature often related to HPA axis activity (47). Recent studies in rats demonstrated that grooming cannot be simply understood as an immediate response necessary to reduce arousal following stress exposure (53). Self-grooming rather seems to be suppressed in defeated rats (53). Similarly, we found in the present and in an earlier study (1) a clear reduction of self-grooming activity in subordinate tree shrews, resulting in rough and dirty looking fur in these animals. Though the central nervous circuits for grooming are still a matter of dicussion, it is interesting that long-term clomipramine treatment induced a step-wise normalization of grooming behavior in subordinate tree shrews. Presumably, grooming is an essential behavior for mammals, because it is thought to have many functions, ranging from cleaning the fur and spreading of olfactory active chemicals to temperature regulation (47).

Chemical signals play an important role in territorial behavior of male tree shrews (31,46). Scent substances are found in glandular secretes, urine, feces, and saliva, and contain information as to the identity and physiological state of the individual (55). Laboratory experiments have shown that both the production of the scent substances and the marking behavior are controlled by androgens (54). Because gonadal activity, and in consequence, circulating testosterone, is reduced in subordinate tree shrews (7,56), marking behavior nearly disappears under stressful conditions. Probably by acting on the hypothalamus–pitutary–gonadal axis, long-term antidepressant treatment induced a step-wise reactivation of the testosterone-dependent marking behavior in subordinate tree shrews.

As noted in the introduction, our previous studies have implicated face validity for depression in subordinate tree shrews. In the present investigation, we further validated the tree shrew model by demonstrating that stress-induced behavioral and neuroendocrine alterations can be reversed by antidepressant treatment. The experiments aimed of mimicing aspects of clinical conditions in terms of drug administration and duration of treatment. In this context it is important to mention that we found a slow onset of clomipramine action across several weeks of chronic treatment. This encouraging result is an essential requirement for animal models designed to elucidate the mechanisms underlying the therapeutic action of antidepressants (58). To understand the underlying central nervous processes, ongoing studies investigate the biochemical consequences of chronic antidepressant adiministration that alleviate depression-like symptoms and stimulate the normalization of behavior.

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