Chapter 2

Effect of the Substance P (NK₁ receptor) antagonist L-760735 and clomipramine on endocrine and behavioral parameters

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Psychopharmacology (2005), 181(2): 207-216
Abstract

Substance P and its preferred receptor, the neurokinin 1 receptor (NK₁), have been proposed as possible targets for new antidepressant therapies, although results of a recently completed phase III trial failed to demonstrate that the NK₁ antagonist MK-869 is more effective than placebo in the treatment of depression. In the present study we compared the effects of the NK₁ antagonist L-760735 with the tricyclic antidepressant clomipramine on endocrine and behavioral parameters in chronically stressed tree shrews. Animals were subjected to a 7-day period of psychosocial stress before receiving daily oral administration of L-760735 (10 mg/kg/day) or clomipramine (50 mg/kg/day). The psychosocial stress continued throughout the treatment period of 21 days. Daily morning urine was collected to measure cortisol and norepinephrine levels. All animals were videotaped daily and three types of behavior were analyzed. Chronic psychosocial stress resulted in a significant increase of urinary cortisol and norepinephrine concentrations. Moreover, stressed animals displayed decreased marking behavior and locomotor activity, while grooming remained unaffected. Neither treatment with clomipramine nor with L-760735 was able to normalize the stress-induced elevation of cortisol or norepinephrine. On the behavioral parameters, L-760735 had a time-dependent restorative influence on marking behavior close to normal levels, without affecting locomotor activity. Grooming behavior was significantly increased by the three weeks of drug treatment. These results suggest that L-760735 was able to counteract certain stress-induced behavioral alterations in an animal model of depression.
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2.1 Introduction

Recently substance P (SP) and its preferred neurokinin 1 receptor (NK₁) have been in focus for their possible beneficial role in affective disorders. The neuropeptide SP is distributed throughout the mammalian brain and is highly abundant in the limbic system, which is known for its involvement in stress, emotion and arousal (Harbuz & Jessop, 2001; Pioro et al., 1990). Several animal studies have shown that the SP system is also involved in the regulation of stress and aversive behaviors. After a microinjection of SP into the dorsal part of the periaqueductal grey, rats spent less time in the open arms of an elevated plus maze (Aguiar & Brandao, 1996). It was also shown that neonatal separation of guinea pig pups increased the release of substance P in the amygdala (Kramer et al., 1998). During maternal separation guinea pig pups emitted distress vocalizations, which were inhibited by intra-amygdala infusion of L-760735, a NK₁ antagonist (Boyce et al., 2001). Moreover, null mutation of the NK₁ receptor in NK₁⁻/⁻ mice caused a marked decrease in separation induced ultrasound vocalizations compared to wild type pups (Rupniak et al., 2000; Santarelli et al., 2001).

L-760735 and other NK₁ antagonists are effective in a wide range of anxiolytic screens, including the gerbil and rat social interaction test (Cheeta et al., 2001; File, 2000), elevated plus maze (Varty et al., 2002) and fear conditioning (Ballard et al., 2001). Anxiolytic drugs are also able to reduce SP levels in certain brain areas (Brodin et al., 1994). Chronic treatment with different types of antidepressants reduced the levels of substance P in several brain structures rats ((Shirayama et al., 1996). In a chronic mild stress paradigm performed on rats, the NK₁ antagonist NKP608 had an antidepressant-like effect (Papp et al., 2000).

These results indicate that the substance P system in the brain plays a role in the pathophysiology of affective disorders like anxiety and depression. Medication that affects the serotonin system is widely used in the clinic to treat depression and anxiety (Den Boer et al., 2000). An interaction between the serotonin and SP system has been reported. A post-mortem study in humans demonstrated that in the raphe nuclei, approximately of 50% of the neurons co-express serotonin and substance P (Sergeyev et al., 1999). It appears that changes within the SP system can have a major impact on the serotonin system in the brain. Several electrophysiological studies have shown that NK₁ blockade or deletion can enhance neuronal firing in the dorsal raphe (Santarelli et al., 2001; Conley et al., 2002). NK₁⁻/⁻ mice also show 5-HT1A somatodendritic autoreceptor desensitization that can be seen after chronic treatment with selective serotonin reuptake inhibitors (Froger et al., 2001). It also appears that NK₁ antagonists increase the firing rate of the norepinephrine neurons in the locus coeruleus (Maubach et al., 2002).
In humans, the first clinical study testing the NK₁ antagonist MK-869 demonstrated that it was as effective as paroxetine in treating depression (Kramer et al., 1998). The antidepressant efficacy has since been replicated in clinical trials with two further NK₁ antagonists: L-759274 (Kramer et al., 2004), and CP 122721 (Chappell et al. presented at Association for European Psychiatry Congress, Stockholm, May 2002). However, results of a recently completed phase III trial showed that MK-869 was not more effective than placebo in the treatment of depression (see footnote in Kramer et al. (2004)).

A major complication in substance P research is that of species variants in NK₁ receptor pharmacology (Beresford et al., 1991). This makes the conventional rat and mouse models of depression and anxiety unsuitable for preclinical evaluation of the clinically interesting NK₁ antagonists. NK₁ antagonists, such as L-760735, are close analogues of L-759274, active in a wide range of preclinical assays for antidepressant and anxiolytic drugs. These preclinical assays were adapted for the use in species, which have more homology with the human NK₁, such as guinea pigs and gerbils (Rupniak et al., 2001). Tree shrews (Tupaia belangeri) are animals which are phylogenetically placed between insectivores and primates (Martin, 1990). Tree shrews are solitary animals and defend their territory from intruding conspecifics. This behavior, which is especially seen between males, is used as a psychosocial stress paradigm in our laboratory. The psychosocial stress paradigm in tree shrews has a high face and construct validity for depression (Fuchs & Flugge, 2002; van Kampen et al., 2002b).

Recently, we have demonstrated that L-760735 penetrates into the brain of the tree shrew and is able to block the NK₁ effectively, which indicates that the NK₁ of the tree shrew shows homology with the human NK₁ in this aspect (van der Hart et al., 2002). In this study the NK₁ antagonist L-760735 had a comparable effect to the tricyclic antidepressant, clomipramine, on counteracting chronic psychosocial stress-induced alterations in the central nervous system (CNS), such as changes within in vivo brain metabolite levels and hippocampal cytogenesis (van der Hart et al., 2002). In this study we evaluated whether the beneficial effects of treatment with L-760735 seen in the CNS are manifested in the neuroendocrine and behavioral parameters of the stressed animals. Furthermore we investigated whether these effects are comparable with clomipramine treated animals.
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Animals, Materials and Methods

2.1.1 Animals

Experimentally naïve adult male tree shrews (Tupaia belangeri) were obtained from the breeding colony at the German Primate Center in Göttingen, Germany. These day active animals were housed singly on a regular day/night cycle (lights on from 08:00 h to 20:00 h) at 26 °C, 55% relative humidity, with free access to food and water (Fuchs, 1999). Animal experiments were conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/EEC) and were approved by the Government of Lower Saxony, Germany.

2.1.2 Drug administration

Animals received the highly brain-penetrating NK1 receptor antagonist L-760735 (10 mg/kg/day, Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, UK) or the tricyclic antidepressant clomipramine (50 mg/kg/day, Sigma) daily. Both L-760735 and clomipramine were dissolved in water and were administered orally in the morning between 7:30 – 8:00 a.m. The solutions were stored at 4 °C and protected from light. Since the majority of the available NK1 antagonists are designed to have a high affinity for the human NK1 receptor (Rupniak, 2002), we conducted a pilot study. In this pilot study we established the dose of L-760735 that was adequate in blocking NK1 receptors in the tree shrew brain (for details see; van der Hart et al. (2002)).

2.1.3 Experimental Design

The experimental design was carried out according to our standard protocol ((Czech et al., 2001; van der Hart et al., 2002). The four experimental groups (Control; Stress; Stress + L-760735; Stress + Clomipramine) are illustrated in Figure 1. The first experimental phase (‘Control’) lasted for 7 days, during which all animals remained undisturbed. The second phase of the experiment consists of a 7-day period, during which animals of the Stress (n=5), Stress + L-760735 (n=5) and Stress + Clomipramine (n=6) were submitted to daily psychosocial conflict.
For the induction of psychosocial conflict one naïve male was introduced into the cage of a socially experienced male. After establishment of a clear dominant/subordinate relationship, the two animals were separated by a wire mesh barrier. As in earlier studies (Czeh et al., 2001; Fuchs & Flugge, 2002) all of the naïve animals became subordinate. The barrier was removed every day for approximately 1 h allowing physical contact between the two males only during this time. By this procedure, the subordinate animal was protected from repeated attacks, but was constantly exposed to visual, auditory and olfactory cues from the dominant animal for the rest of the day. Under these conditions, subordinate animals displayed characteristic subordination behavior such as reduced marking behavior as well as eliciting alarm cries. The third experimental phase consisted of the treatment of the test compounds lasting 21 days. During this time, the subordinate animals remained in the psychosocial conflict situation and were treated daily with the drug or vehicle, respectively. Animals of the Stress group were treated according to the same experimental schedule, but received vehicle only. The animals of the control group (n=6) were run in a separate experiment. They were individually housed and undisturbed in separate quarters elsewhere in the facility and received only vehicle during the last phase of the experiment.

Figure 1: Experimental design and animal groups: Control, Stress, Stress + Clomipramine and Stress + L-760735. The experiment consisted of 3 experimental phases. During the 7 days of the first phase all experimental animal groups received No Stress. In the second phase of the experiment, which lasted again for 7 days, animals from the Stress, Stress + Clomipramine and the Stress + L-760735 groups were submitted daily to psychosocial conflict. The unstressed control group (Control) remained undisturbed. The third experimental phase lasted for 21 days in which the unstressed control group stayed undisturbed, the stress group (Stress) remained under the stressful situation, and received daily administration of vehicle (water). Besides stressing the animals the treatment groups received clomipramine 50 mg/kg/day (Stress + Clomipramine) or the NK1 receptor antagonist L-760735 10 mg/kg/day (Stress + L-760735).
During the entire experiment the daily routine was to collect urine samples from the animals, by a slight massage over the hypogastrium every morning between 7.30 and 8.00, before the lights went on. At this time point there is still relatively large amount of urine in the bladders. Morning urine was used to monitor the activity of the hypothalamus-pituitary-adrenocortical (HPA) axis. This was immediately followed by oral administration of the drug. Later between 9.00 and 14.00, at an unpredictable time point the psychosocial confrontations took place for approximately 1 h. Finally, all animals were videotaped for behavioral assessment between 19:00 to 19:15 during all experimental phases. This time point was chosen to avoid any unwanted confounding factors (e.g. human activity around the animals).

2.1.4 **Analysis of urinary cortisol and norepinephrine**

Urinary free cortisol was measured with a scintillation proximity radioimmunoassay, using anti-rabbit IGG-coated fluomicrospheres (scintillation, proximity assay anti-rabbit reagent type I, Amersham, Braunschweig, Germany), anti-rabbit cortisol antiserum (Paesel-Lorei, Frankfurt, Germany) \(^{3}H\)-cortisol and as radioactive tracer (Amersham, Braunschweig, Germany) (Udenfriend et al., 1985). Urinary norepinephrine was quantified by RP-HPLC with coulometric detection after extraction on BioRex 70 cation-exchange columns (BioRad, Munich, Germany). To correct for physiological alterations in urine dilutions, the resulting concentrations were related to creatinine concentrations, which were determined using a Beckman Creatinine Analyzer 2.

2.1.5 **Organ weights**

Post-mortem, the adrenals, testes and epididymi were removed and the weights were determined on an analytical balance. Relative organ weights were obtained by dividing the organ weights by the body weights and then expressed as percentage.
2.1.6 Behavioral analysis

During the entire experiment all animals were videotaped from 19:00 until 19:15 daily. The videotapes were coded to ensure that the observer was blind to the experimental treatment of the animal. Fifteen minutes of each day were analyzed using the Observer 3.1 software (Noldus Information Technology, Wageningen, the Netherlands). Throughout the observation time the duration of scent marking (marking with abdominal gland, the sternal gland and urinary marking) was scored. Self-grooming (licking, cleaning, scratching and washing) was also scored. Locomotor activity was scored during the same trial using Ethovision 2.1 (Noldus Information Technology, Wageningen, the Netherlands). The home cage of the animal was divided into six zones, and each crossing of the border between the different zones was counted during the 15-minute trial.

2.1.7 Data analysis

Data were statistically analyzed using Statistica 5.0 (Statsoft, Inc., Tulsa, OK, USA). The mean of the neuroendocrine and behavioral data of each animal was determined for each week and used to perform two-way ANOVA for repeated measures (between factor: groups; within factor: experimental weeks). Subsequently, Duncan’s post hoc t-test was performed to detect significant differences between the weeks and groups. The level of significance was set at p<0.05. Data are expressed as mean ± SEM.
2.2 Results

2.2.1 Neuroendocrine parameters

Figure 2 Effect of chronic psychosocial stress and drug treatment with clomipramine or L-760735 on urinary free cortisol. (A) In the unstressed Control group, the concentration of urinary cortisol remained constant throughout the entire experiment. (B) In the Stress group, stress induced a significant and sustained elevation of urinary cortisol, with a slight habituation in the last two weeks of the experimental period. (C) In the Stress + Clomipramine group, and the Stress + L-760735 group (D), urinary free cortisol was significantly elevated throughout the whole stress period. * p<0.05, compared with the pre-stress phase (week 1).
The activity of the HPA axis and the sympathetic system were monitored for the duration of the experiment. Daily, morning urine was collected and prepared for analysis for urinary free cortisol and norepinephrine. In the Control (unstressed) group, the concentration of urinary cortisol remained constant throughout the experiment. All groups that were subjected to psychosocial stress showed a significant increase in urinary free cortisol. Psychosocial stress induced up to 50% elevation of urinary cortisol compared to pre-stress baseline levels. The increase in free cortisol in urine during stress (Fig. 2) revealed a significant difference between the experimental groups (F(3.18)=4.50, p<0.05) and between the experimental weeks (F(4,72)=6.73, p<0.001). In the Stress group, the elevation in urinary cortisol was maintained for the first 2 weeks of psychosocial stress, thereafter returning towards normal levels. In contrast, urinary cortisol remained elevated throughout the study in the animals treated with either clomipramine or L-760735.
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Figure 3 Effect of chronic stress and antidepressant treatment on urinary norepinephrine. (A) In the unstressed Control group, urinary norepinephrine remained unaltered throughout the whole experiment. (B) In the Stress group, stress induced an immediate and sustained elevation of urinary norepinephrine. (C) In the Stress + Clomipramine group, stress resulted in an instant elevation of norepinephrine, which further increased during the progress of the experiment. (D) In the Stress + L-760735 group, the sustained elevation of urinary norepinephrine demonstrates that these animals were subjected to a stressful situation throughout the experiment. * p < 0.05, compared with the pre-stress phase (week 1) and # p < 0.05 compared to the Stress group in the same week.

In unstressed control animals, urinary norepinephrine concentrations were stable throughout the experiment. Urinary norepinephrine concentrations were significantly increased during psychosocial stress (Fig. 3). Statistical analysis
revealed a significant effect between groups (F[3,18]=10.620, p<0.001), and within weeks (F[4,72]=19.236, p<0.001) and an interaction between these two factors (F[12,72]=4.495, p<0.001). Psychosocial stress resulted in up to a 70% elevation of urinary norepinephrine concentrations compared to pre-stress baseline levels in all three stress groups. In vehicle or L-760735-treated shrews, urinary norepinephrine levels remained elevated to a similar extent throughout the study. In the clomipramine-treated group, there was a continuous rise of urinary norepinephrine compared to baseline levels during the treatment.

2.2.2 Organ weights

Chronic psychosocial stress induced a significant increase in relative adrenal gland weights (Fig. 4.A, p<0.05). Relative adrenal gland weights of the Stress group showed a non-significant increase of 46%, while significantly larger adrenals were measured in animals which were stressed and treated with clomipramine (p<0.001) or L-760735 (p<0.01). Adrenal gland weights of the animals treated with clomipramine showed a larger increase compared to the Stress group or to the Stress+L-760735 group (Fig. 4.A).

![Figure 4](image-url)

**Figure 4** Effect of chronic stress and treatment with clomipramine or L-760735 on relative adrenal, testis and epididymis weights. (A) Relative adrenal weights. Stress tends to increase the relative adrenal weight, whereas animals of the drug treated groups had significantly larger adrenals compared to unstressed controls. (B) Relative testis weights: Stress significantly reduced testis weight, this reduction could be counteracted by antidepressant treatment. (C) Relative epididymis weights: Stress had a non-significant decreasing effect on the relative epididymis...
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weight whereas drug treatments had an opposite effect. * p<0.05, compared with the Control group; # p<0.05, compared with the Stress group.

Psychosocial stress resulted in a significantly smaller (-49%) relative testis weight in the Stress group compared to the unstressed Control group (Fig 4.B, p<0.001). Treatment with either clomipramine or L-760735 induced a normalization of the relative testis weight and no difference compared with the unstressed Control group was observed. Both compounds were able to counteract the stress-induced testis weight reduction (Stress vs. Stress + Clomipramine: p<0.005, Stress vs. Stress + L-760735: p<0.05). Relative epididymis weight tended to be reduced by chronic psychosocial stress (Fig. 4.C). Treatment with clomipramine or L-760735 counteracted this tendency (Stress vs. Stress + Clomipramine: p<0.05, Stress vs. Stress + L-760735: p<0.05). Treatment with L-760735 resulted in a significant increase in relative epididymis weight compared to the unstressed Control group (p<0.05).
2.2.3 Behavioral parameters

**Figure 5** Effect of chronic stress and antidepressant treatment on locomotor behavior. (A) In the Control group locomotor activity was unaltered. (B) Stress significantly suppressed duration of locomotor activity in the Stress group. Neither treatment with clomipramine (C) nor with the NK₁ antagonist L-760735 could counteract the reduction in locomotor activity (D). * p<0.05, compared with the pre-stress phase (week 1) and # p<0.05 compared to the Stress group in the same week.
Chronic psychosocial stress in tree shrews had a significant effect on all behavioral parameters, except for grooming. Locomotor activity of the unstressed Control group remained constant throughout the experiment, whereas stress significantly suppressed this activity (Fig. 5). Statistical analysis revealed a significant difference between groups and within the different weeks ($F[12,72]=2.468, p<0.01$). Neither clomipramine nor L-760735 treatment altered the stress-induced decrease in locomotor activity.

Figure 6 Effect of chronic stress and treatment with clomipramine or L-760735 on marking behavior. (A) Duration in marking behavior remained relatively stable in the Control group. (B) Stress had a dramatic suppressive effect on marking behavior, which was unaltered by clomipramine treatment (C), whereas treatment with L-760735 could eventually, counteracted the effect of chronic psychosocial stress (D). * $p<0.05$, compared with the pre-stress phase (week 1) and # $p<0.05$ compared to the Stress group in the same week.
Scent marking behavior was markedly reduced by chronic psychosocial stress (Fig. 6). During the first 7 days of psychosocial stress (Week 2) animals in all three stressed groups spent approximately 50% less time on scent marking behavior compared to the pre-stress baseline period (Week 1). A significant effect of chronic psychosocial stress was seen between the groups (F[3,17]=4.944, p<0.05) and within the weeks (F[4,68]=11.316, p<0.001), and a significant interaction was detected between these factors (F[12,68]=2.086, p<0.05). Marking behavior was continuously suppressed in the Stress group throughout the entire stress period (Fig. 6.B). Three weeks of treatment with clomipramine did not counteract this suppression, and only a tendency to normalization of marking behavior was observed (Fig. 6.C). In contrast, two weeks of treatment with L-760735 was able to normalize the stress-induced decrease in marking behavior (Fig. 6.D).
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Figure 7  Effect of chronic stress and treatment with clomipramine or L-760735 on self-grooming behavior. The time spent on self-grooming was constant in the unstressed Control group throughout the experiment (A). In the Stress group, stress resulted initially in a decrease of self-grooming behavior to 25% of the pre-stress baseline level. The observed reduction recovered spontaneously (B). In the Stress + Clomipramine group, stress induced an initial increase in self-grooming behavior to 178% of the control week, which was then normalized. In the last week of the experiment, a significant increase in self-grooming behavior was observed (C). In the Stress + L-760735 group, stress had no apparent effect on self-grooming behavior, but a substantial increase in this behavior was observed in the last 2 weeks of the stress period (D). * p < 0.05, compared with the pre-stress phase (week 1) and # p<0.05 compared to the Stress group in the same week.
Stress did not consistently alter grooming behavior, although there were marked changes in grooming in the first week of the psychosocial confrontation (Week2, Fig. 7). Interestingly, treatment with either clomipramine or L-760735 increased self-grooming behavior above baseline levels at the end of the experiment (Fig. 7. C-D). Significant differences were seen in time spent on self-grooming behavior on the factor weeks (F[4,68]=3.484, p<0.05), and on the interaction between groups and weeks (F[12,68]=1.995, p<0.05).
2.3 Discussion

In the present study we used the chronic psychosocial stress paradigm in male tree shrews, an established model for research on the pathophysiology of major depression (Fuchs & Flugge, 2002; van Kampen, 2002). In this model we investigated the effects of chronic treatment with a NK₁ antagonist L-760735 on stress-induced alterations of behavioral and endocrine parameters. Furthermore, we treated a group of animals with clomipramine, so that we could compare the effect of the NK₁ antagonist to the effect of a well-known tricyclic antidepressant drug. The main findings of the present study are that treatment with both the NK₁ antagonist and clomipramine attenuated certain stress-induced alterations on organ weight, whereas neither drug treatment counteracted the stress-induced elevation of cortisol and norepinephrine. Furthermore, the NK₁ antagonist counteracted the stress-induced reduction on marking behavior.

2.3.1 The effect of clomipramine and L-760735 treatment on the stress induced activation of the HPA axis.

Chronic psychosocial stress activates the HPA axis and the sympathetic nervous system. Increased concentrations of cortisol and norepinephrine signal noxious stimulation to the CNS, cardiovascular and immune systems (McEwen, 2000). The increased concentration of urinary cortisol and norepinephrine demonstrates that the animals in the Stress, Stress + Clomipramine and Stress + L-760735 groups, were severely stressed throughout the entire experimental stress period. Pharmacological intervention did not attenuate the stress-induced elevation of urinary cortisol concentrations. Habituation to the psychosocial stress was observed in the Stress group as the cortisol levels decreased slightly by the end of the experiment. This phenomenon has also been observed in previous chronic stress studies based on social conflict (Dal Zotto et al., 2002). However, in the present study, this habituation of the HPA axis to chronic stress was not seen in the drug treated animals, suggesting that the habituation mechanism may be compromised in this study by pharmacological intervention.

Besides cortisol, urinary norepinephrine concentrations were also significantly elevated throughout the four weeks of stress, in all stressed groups. The increase of urinary norepinephrine concentration of the Stress + Clomipramine group was the most pronounced among the groups. The main pharmacological property of clomipramine and its active metabolite des-clomipramine is that they inhibit the re-uptake of norepinephrine. This may account for the higher norepinephrine concentration in the urine of these animals (McTavish & Benfield, 1990).
The increased relative adrenal weight confirms the observation seen in the urinary cortisol levels. The adrenals of the three stressed groups weighed more than the adrenals of the unstressed Control group. Furthermore, the ratio of the relative adrenal weights appears to be similar to the ratio of the cortisol concentrations seen in the last week of the experiment. Apparently, the increased excretion of cortisol led to an increased adrenal weight in the stressed animals.

2.3.2 The effect of clomipramine and L-760735 treatment on the behavior of chronically stressed tree shrews.

Locomotor activity gradually decreased after the onset of stress. Neither clomipramine nor L-760735 could counteract the effect of stress. In the Stress + Clomipramine group, stress resulted in a prompt decrease of locomotor activity (Fig 5.C), probably because these animals experienced the most severe stress right from the start of the stress procedure. A previous study by our group showed a restoration of locomotor activity after 30 days of treatment with clomipramine (Fuchs et al., 1996). The duration of antidepressant treatment therefore appears to be important in order to see a clear drug effect on locomotor behavior.

Marking behavior was significantly reduced by chronic psychosocial stress. The duration of marking behavior during the observation period decreased drastically until 10-15% of the pre-stress baseline levels. In a previous study, our group demonstrated that marking behavior is related to the gonad function of the animals. Marking behavior in male tree shrews is controlled by androgens and the stress-induced reduction in marking behavior can therefore be counteracted by testosterone treatment (Flugge et al., 1998). Chronically stressed tree shrews have been shown to have a decreased testosterone plasma concentration and a decreased testis and epididymis weight (Fischer et al., 1985). The present study showed that animals treated with L-760635 exhibit normalized marking behavior of up to 80% of the pre-stress baseline level, while in the animals treated with clomipramine, marking remained significantly suppressed, despite the three weeks of treatment.

The decreased testis weight in the stressed animals was completely reversed by pharmacological intervention. The trend towards a decreased epididymis weight after chronic stress was also counteracted by antidepressant treatment. These observations are in accordance with the observation that marking behavior is restored by L-760735 treatment.

In this experiment chronic psychosocial stress had no effect on the duration of self-grooming behavior. In our previous experiments with tree shrews, the duration of grooming behavior was reduced by chronic psychosocial stress. Apparently, the time of the observation may influence the outcome of the analysis of self-grooming.
behavior. Directly after the physical confrontation with the dominant tree shrew, the stressed animal does not groom differently from controls (Kramer et al., 1999). It seems that due to the constant social presence of the dominant animal in the adjacent cage, the duration of self-grooming was not altered in the subordinates. Furthermore, the individual differences within tree shrews are considerable, since decreases as well as increases of self-grooming behavior were observed during the stress period (van Kampen et al., 2002a). Interestingly, drug treated animals demonstrated an increase of grooming up to 350% above baseline levels after three weeks. It is not known whether the increased grooming activity after chronic treatment is due to the pharmacological intervention itself, or due to the combination of chronic psychosocial stress and chronic treatment. From earlier studies however, it is known that chronic clomipramine treatment does not increase grooming activity in non-stressed animals (Fuchs et al., 1996).

In an earlier study we could demonstrate that the anxiolytic diazepam was not able to normalize the stress-induced behavioral and neuroendocrine changes in tree shrews (van Kampen et al., 2000). This indicates that the observed alterations in stressed tree shrews are not a result of anxiety behavior (van Kampen et al., 2000). Furthermore, we could previously demonstrate that longer treatment with clomipramine could counteract the stress-induced behavioral alterations in this paradigm (Fuchs et al., 1996).

Most of the antidepressant drugs exert their effect on the monoamine system. In this study we could demonstrate that L-760735, a compound that blocks the NK receptor, was able to counteract certain behavioral changes seen after chronic stress in tree shrews. This could predict a potential antidepressant-like activity for NK receptor antagonists, however clinical studies have shown ambiguous results concerning its anti-depressant effects. Further research is needed to gain an insight into the working mechanisms of NK receptor antagonists.

Acknowledgements:
We thank Andreas Heutz for the analysis of the urine samples. This study was partially supported by Merck Research Laboratories.

2.4 Reference List

Substance P and the Neurokinin 1 Receptor: from behavior to bioanalysis


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