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Anxiolytic-like Effects of the Neurokinin 1 Receptor Antagonist GR-205171 in the Elevated Plus-Maze and Contextual Fear-Potentiated Startle Model of Anxiety in Gerbils

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Abstract

Gerbils show a NK1 receptor pharmacological profile which is similar to that seen in humans and thus have become a commonly used species to test efficacy of NK1 receptor antagonists. The aim of the present study was to determine whether systemic administration of the NK1 receptor antagonist GR-205171 produced anxiolytic-like effects in the elevated plus maze and in a novel contextual conditioned fear test using fear-potentiated startle (FPS). On the elevated plus maze, treatment with GR-205171 at 0, 0.3, 1.0 and 5.0 mg/kg doses 30 min before testing produced anxiolytic-like effects in an increasing dose-response fashion as measured by the percentage of open arm time and percentage of open arm entries. For contextual fear conditioning, gerbils were given 10 unsignaled footshocks (0.6 mA) at a 2 min variable interstimulus interval in a distinctive training context. Twenty-four hours after training gerbils received treatment of GR-205171 at 0, 0.3, 1.0 and 5.0 mg/kg doses, 30 min before testing in which startle was elicited in the same context in which they were trained. Contextual FPS was defined as an increase in startle over pre-training baseline values. All drug doses levels (0.3, 1.0 and 5.0 mg/kg) significantly attenuated contextual FPS when compared to the vehicle control group. A control group which received testing in a different context, showed little FPS. These findings support other evidence for anxiolytic activity of NK1 receptor antagonists and provide a novel conditioned fear test that may be an appropriate procedure to test other NK1 antagonists for preclinical anxiolytic activity in gerbils.

Keywords

Anxiety; Substance P; context conditioning; plus maze; gerbil

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Introduction

The mammalian tachykinins include substance P (SP), neurokinin (NK) A, and NKB, the effects of which are preferentially mediated by the G protein-coupled receptors NK1, NK2, and NK3, respectively (Pennefather et al. 2004; Regoli et al. 1994). In the nervous system, tachykinins operate as neurotransmitters and neuromodulators and have historically been implicated in a wide variety of biological actions including pain transmission, inflammation, smooth muscle contraction, vasodilation, gland secretion, and activation of the immune system (Kramer et al. 1998; Quartara and Maggi 1998; Severini et al. 2002). More recent evidence suggests that the central SP-NK1 system is also involved in various stress-related pathologies, including anxiety and depression (for review see Ebner and Singewald 2006). In mammals, SP is the most abundant tachykinin in the central nervous system, where, along with NK1, it is widely distributed in brain regions involved in the regulation of affective behavior and mediation of stress responses, such as the amygdala, septum, hippocampus, hypothalamus, and periaqueductal gray (Barbaresi 1998; Commons and Valentino 2002; Hietala et al. 2005; Hokfelt et al. 1985; Maeno et al. 1993; Nagano et al. 2006; Rigby et al. 2005; Szeide mann et al. 1995).

Previous animal studies have shown that exposure to a variety of aversive and stressful situations alter SP transmission in various brain regions (Bannon et al. 1986; Brodin et al. 1994; Ebner et al. 2004; Kramer et al. 1998; Rosen et al. 1992; Siegel et al. 1987). In addition, both systemic and central injections of SP agonists elicit a variety of anxiety-like behaviors in animals, including conditioned place aversion (Aguiar and Brandao 1994; Elliott 1988), more time in the closed arms of the elevated plus-maze (Aguiar and Brandao 1996; Bassi et al. 2007; De Araujo et al. 2001; Duarte et al. 2004; Teixeira et al. 1996) and enhanced inhibitory avoidance learning (Hasenohrl et al. 1990; Pelley mounter et al. 1986). In contrast, pharmacological blockade of SP with NK1 receptor antagonists reduced depressant- and anxiety-like behaviors in different animal species (Dableh et al. 2005; Kramer et al. 1998; Rupniak et al. 2001, 2003b; Varty et al. 2002a; Woolley et al. 2006). NK1 antagonists also have a robust anxiolytic effect in various conditions of the social interaction test of anxiety (Cheeta et al. 2001; File 1997; Gentsch et al. 2002).

These findings have led pharmaceutical companies to develop a variety of selective and potent NK1 antagonists to offer new options for the treatment of anxiety and depression (for recent review see Quartara and Altamura 2006). Unfortunately, assessing the preclinical efficacy of these NK1 antagonists has been complicated by marked differences in the pharmacology between human and rat or mouse NK1 receptors. Most NK1 receptor antagonists that display high affinity at human receptors show low affinity, selectivity, and brain penetration in rats and mice, due to differences in the amino acid sequence of the NK1 receptor (Beresford et al. 1991; Fong et al. 1992), thus necessitating the administration of high doses that typically results in unspecific pharmacological effects in these rodents (Rupniak et al. 2001, 2003a; Rupniak and Jackson 1994; Smith et al. 1994). The NK1 receptor antagonist GR-205171 has high binding selectivity for rat NK1 receptors; however, as seen with other NK1 receptor antagonists, the ability of GR-205171 to inhibit SP binding in rat NK1 receptors is low, with K_i values approximately 40 fold higher than those obtained using human NK1 receptors (Gitter et al. 1991; Saria 1999a). Furthermore, GR-205171 causes only a moderate rightward shift in SP-induced inositol-1-phosphate accumulation and acidification rate in cells expressing rat NK1 receptors relative to human NK1 receptors.

In contrast to mice and rats, gerbils show a NK1 receptor pharmacological profile which is very similar to that seen in humans (Griffante et al. 2006; Saria 1999b), and thus the gerbil has become a commonly used preclinical model to test efficacy of NK1 receptor antagonists. *In vivo* studies reveal that the doses of GR-205171 needed for central NK1 receptor

occupancy are several hundred-fold higher for rats than gerbils. For example, in gerbils the ID_{50} for inhibition of NK1 agonist-induced foot drumming is 0.02 mg/kg; however, in rats, 10–30 mg/kg of GR-205171 are needed to significantly reduce SP-induced sniffing and agonist-induced hypertension (Rupniak et al. 2003a).

In clinical trials, several studies have revealed that NK1 antagonists, including GR-205171, show promise as treatment options for individuals suffering with pathological anxiety (Kramer et al. 2003). For example, as a treatment for symptoms of social phobia, the efficacy of GR-205171 is reportedly similar to that of citalopram (Furmark et al. 2005). The NK1 receptor antagonist GW597599 is effective against CO₂-induced panic attack with an efficacy similar to alprazolam (McLean 2005). In a more recent study, George, et al. (2008) have shown that the NK1 receptor antagonist LY686017 suppresses spontaneous alcohol cravings and blunts craving and cortisol responses induced by alcohol-cue challenges in alcohol-dependent subjects with high trait anxiety. Currently, phase II clinical trials are also evaluating the effectiveness of GR205171 in decreasing symptoms of post-traumatic stress disorder.

At present, relatively few studies have examined the effects of NK1 antagonists on conditioned fear. The fear-potentiated startle (FPS) paradigm may be particularly useful for examining conditioned fear in gerbils, because fear induces foot drumming in this species, which inhibits the expression of conditioned freezing - a behavior used to index levels of fear and anxiety in many conditioning paradigms (Rupniak et al. 2003a; Woolley et al. 2006). Furthermore, because much is known about the neurocircuits mediating startle and the augmentation of startle by states of fear and anxiety, the FPS paradigm may be particularly useful in future studies design to identify central mechanisms responsible for anxiolytic-like actions of NK1 antagonists. Thus, the purpose of the current study was to determine whether the NK1 receptor antagonist GR-205171 inhibits contextual conditioned fear using the FPS paradigm. Because past research has shown that a number of NK1 receptor antagonists produce an anxiolytic-like profile in gerbils as assessed by the elevated plus maze (Varty et al. 2002a), we also examined whether GR-205171 produces anxiolytic-like effects in the elevated plus maze.

Methods

Subjects

Male Mongolian gerbils (Charles River, USA) weighing between 60 and 75 g at the onset of testing were used in all experiments. Gerbils were housed 4 per cage with food and water freely available in a room maintained under constant temperature (22°C). Gerbils were allowed 2 weeks to acclimate to the housing conditions prior to the start of experiments. All testing was done in the light phase of a 12 h light/dark cycle (lights on: 07:00h). All experimental procedures used in the present investigation were carried out in accordance with the National Institute of Health 'Guide for the Care and Use of Laboratory Animals' and approved by our Institutional Protocol Approval Committee in accordance with Yerkes Primate Research Center Regulations.

Startle Apparatus

Four SR-LAB startle response systems (SR-LAB, San Diego Instruments, San Diego, California, USA) were used for training and testing. Each startle device consisted of a clear Plexiglas cylinder (8.8 cm in diameter and 20.5 cm in length) mounted on a Plexiglas base and placed in a ventilated, sound- and vibration-attenuated chamber. Each chamber was equipped with a horn Radio Shack speaker mounted 24 cm above each cylinder, which was used to present a background white-noise stimulus and 50-ms noise-burst startle stimuli at

intensities ranging from 90–120 dB. A 15-W light bulb attached 24 cm above each cylinder was used to provide the light stimulus. The footshock unconditioned stimulus (US) was delivered through a removable stainless steel grid floor using one of four LeHigh Valley shock generators (SGS-004; LeHigh Valley, Beltsville, MD) located outside the sound-attenuating chamber. Movements within the cylinder created changes in voltage as detected by a piezoelectric accelerometer attached to the Plexiglas base. Voltage output signals were rectified, amplified, and digitized on a 0–4096 unit scale. Startle amplitude was defined as the peak accelerometer voltage that occurred during a 200-ms period beginning at the onset of the startle stimulus. Data acquisition and stimuli deliveries were controlled by a computer using SR-LAB software designed by San Diego Instruments.

To establish a second context used to evaluate the context-specificity of FPS, the SR-LAB startle response system was modified in terms of odor, somatosensory, auditory and visual cues. The stainless steel grid floors were removed and 2 chains of 2 cm in length were hung from the top of each cylinder to provide distinctive somatosensory environments. A jar containing a lavender odor was placed inside the sound -attenuated chamber to provide a distinctive olfactory environment. To further differentiate environments, the chamber was supplied with an ambient white-noise stimulus that raised the overall ambient background to 70 dB. In addition, testing and/or training were performed in the chamber illuminated with a light bulb (15 W) located inside the sound-attenuated chamber.

Elevated plus-maze

The elevated plus maze consisted of two open arms (50×6.5 cm) and two closed arms with a wall ($50 \times 6.5 \times 15$ cm) attached to a common central platform (6.5×6.5 cm) to form a cross. The maze was elevated 65 cm above the floor. Test sessions were conducted under standard room lighting (100 lux) where behaviors were continuously videotaped by a video camera placed over the apparatus. Before each test, the plus maze was cleaned with Quatricide (Pharmacal, Waterbury, CT).

Handling and animal assignment

Gerbils used in all experiments were accustomed to repeated handling prior to initiating experimental testing. On each of 3 days prior to testing, animals were transported to an experimental room and each animal was handled for approximately 1 min until they appeared to show reduced indicators of stress when handled (e.g., absence of vocalization and struggling). Following this handling period, each animal was weighed and the tail was inked with permanent marker to designate subject number before returning to colony housing.

Gerbils used to determine the floor and ceiling effects to various startle intensities were also used to examine the effects of GR-205171 on baseline startle responses. Naïve animals were used to characterize contextual FPS, evaluate the effects of GR-205171 contextual FPS, and evaluate the effects of GR-205171 on elevated plus-maze testing.

Evaluation of the effects of GR-205171 on baseline startle responses

For two consecutive days, each gerbil was acclimated to the startle cylinder for 20 min during which time no startle stimuli were presented and then they were returned to their home cage. The following day gerbils were placed in the cylinder and after 5 min given 10 startle stimuli at each of four different startle stimulus intensities (90, 100, 110, 120 dB) with an interstimulus interval (ISI) of 30 s. All startle stimuli were presented in a pseudorandom order with the constraint that each stimulus intensity occurred only once in each consecutive four-trial block. Ten blocks were presented for a total of 40 trials. This 40-trial startle-stimulus session was used to determine the floor and ceiling effects to various

startle intensities, to enable selection of startle intensities which produced reliable yet submaximal startle responses for subsequent testing (i.e. 110- and 115-dB).

Gerbils were subsequently matched into 4 groups with similar mean startle amplitudes to test the effects of GR-205171 on startle responses. The mean startle amplitudes were calculated by averaging the startle amplitude across all previous 40 test trials. The following day gerbils were injected i.p. with GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg) 30 min before receiving a drug startle test. During the drug startle test, animals received a total of 24 startle stimuli at two different intensities (110- and 115-dB) starting after a 5-min acclimation period. The interstimulus interval was 30 s and the test session was 20 min in duration.

Characterization of contextual FPS in gerbils

The characterization of contextual FPS in gerbils was conducted with a separate cohort of animals using a modified procedure originally designed to validate contextual FPS in rats as described by McNish et al. (1997). After handling, 16 animals received a pre-training startle session and were subsequently matched into groups with similar baseline startle amplitudes. During the pre-training startle session, animals received a total of 24 startle stimuli at two different intensities (110- and 115-dB) starting after a 5-min acclimation period (identical to the drug startle test session described above). The interstimulus interval was 30 s and the test session was 20 min in duration. Half of the animals were tested in chamber A, the other half in chamber B.

Animals in the “Same Group” (A–A and B–B) were trained and tested in the same chamber, whereas animals in the “Different Group” (A–B and B–A) were trained and tested in a different chamber. The assignment of animals to groups and chambers (context A or B) was counterbalanced so that 4 animals were represented in each of the four possible training and testing conditions. In the training session, gerbils were placed in the startle cylinder and after 5 min received the first of 10 unsignaled foot shocks (0.6 mA) at a 2-min variable interstimulus interval (range, 1–3 min). The training session was 25 min in duration, after which gerbils were returned to their home cages. Twenty-four hours after training, animals received a post-training test that was identical to the pre-training startle session.

Evaluation of the effects of GR-205171 contextual FPS in gerbils

After handling, naïve gerbils were first given a pre-training startle session in Context A and matched into groups with similar startle amplitudes. One day later gerbils were given unsignaled footshock training sessions on each of two consecutive days. Two days of training sessions were given to increase levels of FPS. The following day gerbils were injected i.p. with GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg) 30 min before they were tested for startle in Context A. This test was identical to the pre-training startle session.

Evaluation of the effects of GR-205171 on elevated plus-maze testing

After handling, naïve gerbils were injected i.p. with GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg) 30 min before elevated plus-maze testing. At the start of each session, one gerbil was placed at the distal end of a closed arm with their heads facing the wall - rather than the central portion of the maze - to prevent possible ambiguous or biased arm entries. Animals were allowed to explore freely for 5 min. The percentage of open arm entries [open arm/(open + closed arm) entries] x 100 and percentage time in open arms [time in open arms/(time in open + closed arms)] x 100 were computed. Both of these parameters are indicators of anxiolytic-like activity (Hogg 1996; Pellow and File 1986). The total number of closed arm entries was used as an indicator of locomotor activity (Rodgers and Dalvi 1997). Arm entry was considered complete if all four paws entered a closed or open arm from the central platform. In addition to these standard parameters, we also measured the number of entries

and amount of time spent by the animals in the center platform. Center time and entries accumulated when least one paw was placed out of an arm.

Drug preparation

The NK1 antagonist GR-205171 (GlaxoSmithKline Pharmaceuticals) was administered intraperitoneally (i.p.) in phosphate buffered saline (PBS) at a volume of 5 ml/kg 30 min before testing. Past research has shown GR-205171 to have high affinity and selectivity for NK1 receptors (Gardner et al. 1996). Doses and pretreatment times were based on data from autoradiography and behavioral experiments demonstrating that the doses selected for the current study, 0.3, 1.0, and 5.0 mg/kg, are sufficient to linearly inhibit ^{125}I -SP binding approximately 25–75% in striatal homogenates of gerbils ($\text{IC}_{50}=1.25\text{mg/kg}$) and significantly inhibit NK1 agonist-induced foot drumming in gerbils (Duffy et al. 2002).

Data reduction and analyses

Mean startle response elicited at increasing noise-burst intensities were calculated by averaging the startle amplitude at each intensity (90, 100, 110, 120 dB). Mean startle amplitudes for the pre-training and post-training test sessions were calculated by averaging the startle amplitude across all test trials. In cases where analyses were conducted on the first and/or second halves of the post-training test, mean startle responses were calculated by averaging the startle amplitude across Trials 1–12 or Trials 13–24, respectively. Block values represented in figures denote the mean startle response of two sequential test trials of different intensity (110, 115 dB). Fear-potentiated startle was detected by comparing pre-training mean startle amplitudes to post-training mean startle amplitudes. Significant fear-potentiated startle was defined as a reliable increase from pre- to post-training startle. Therefore, group differences were examined using repeated-measures ANOVAs, with session (pre-training, post-training) as the within-subject variable, or paired t-tests. Group differences were also evaluated with simple effects at each level of session. Each elevated plus-maze dependent measure was examined using a one-way ANOVA. Significant mean groups differences were detected by use of using Bonferroni t-tests to control the experimentwise error rate at $\alpha = 0.05$.

Results

Effects of GR-205171 on baseline startle response

Figure 1A displays the mean startle responses elicited at increasing noise-burst intensities in gerbils. Startle amplitudes increased as a function of stimulus intensity, as confirmed by a repeated measures ANOVA which revealed a significant intensity effect, $F(4, 95)=20.78$, $p<0.001$. Bonferroni *t*-tests revealed significant differences among all intensities ($ps<0.001$), and a trend analysis indicated that the linear component accounted for a largest and significant proportion of the variance, $\eta^2=0.73$, $p<0.001$. As seen in Figure 1B, none of the doses of GR-205171 altered mean baseline startle responses when gerbils were subsequently given a test in which startle was elicited with two different noise-burst intensities (110- and 115-dB). The lack of drug effect on startle was verified by a one-way ANOVA which yielded no significant main effect of drug dose, $F(3, 19)=0.04$, *NS*.

Contextual FPS in gerbils

Figure 2A displays the mean pre-training and post-training startle response of gerbils in the same (A–A and B–B) and different (A–B and B–A) context groups. Animals trained and tested in the same context displayed greater mean startle responses after training relative to before training, indicating significant FPS to the context; whereas, gerbils trained and tested in different contexts showed no FPS. Figure 2B displays the mean startle responses of

gerbils across blocks during the post-training test. The significant difference in startle responses between groups gradually declined across the test session, and comparatively little differences in startle amplitudes could be detected during the second half of the test session. Together, these findings suggest that gerbils tested in the same context, but not in a different context, displayed significant contextual FPS, and within-session extinction of contextual FPS.

An overall ANOVA using group (same, different) as a between-subjects factor and session (pre-training, post-training) as a within-subjects factor revealed a significant main effect of group, $F(1,14)=4.73$; $p<0.05$, and a significant Group by Session interaction, $F(1,14)=10.96$; $p<0.01$. Subsequent paired *t*-tests with Bonferroni corrections indicated that the same-context group displayed greater mean startle amplitudes after training relative to before training, $t(7)=2.99$; $p<0.02$, whereas the different context group showed no differences in startle between sessions, $t(7)=1.69$; *NS*. Same- and different-context groups displayed similar startle amplitude during the pre-training startle sessions, $t(14)=0.92$; *NS*.

A closer examination of the post-training test session revealed that during the first half of the test session (Blocks 1–6), the gerbils in the same-context group showed greater mean startle responses, $t(14)=5.07$; $p<0.05$ (Figure 2B inset). However, during the second half of the test session (Blocks 7–12), no reliable differences in mean startle responses were detected, $t(14)=0.32$; *NS*. Thus, while the same-context group showed a greater overall FPS than the different-context group, this effect was mainly mediated by differences during the first half of the post-training test session.

Effects of GR-205171 on contextual FPS

The effects of administration of GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg, i.p) on contextual FPS are shown in Figure 3. Because greater contextual FPS was seen during the first half of the post-training test session, we evaluated the effects of GR-205171 during the first half of the test session, in addition to an analysis of the post-training session as a whole. Figure 3 displays the mean startle response of groups before training, as well as their mean startle responses during the first and second halves of the post-training test (the inset displays post-training session as a whole). Overall, all groups displayed greater mean startle responses during the post-training test session relative to pre-training session, indicating contextual FPS in all groups. However, relative to the vehicle control group (0 mg/kg), groups of animals that received pretreatment of GR-205171 (0.3, 1.0 or 5.0 mg/kg) prior to the post-training test displayed less FPS. The reduction of contextual FPS was greatest in the group treated with 5 mg/kg of GR-205171.

An ANOVA using group (0, 0.3, 1.0 or 5.0 mg/kg) and session (pre-training, post-training) as factors yielded significant main effects of dose, $F(3,23)=4.05$; $p<0.05$, and session, $F(1,23)=151.80$; $p<0.01$. More importantly, there was a significant Dose x Session interaction, $F(3,23)=5.45$; $p<0.01$, signifying that test performance was differentially affected by drug dose. Simple one-way ANOVAs at each session level revealed a reliable post-training dose effect, $F(3,23)=5.46$; $p<0.01$, but no pre-training dose effect, $F(3,23)=0.64$; $p<0.05$. Follow-up Bonferroni *t*-tests revealed that FPS in the group treated with 5 mg/kg of GR-205171 was significantly less ($p<0.01$) than in the vehicle control group (0 mg/kg; Figure 3, inset). No other *t*-test was statistically significant.

The planned analysis of FPS levels during the first half of the test session (Blocks 1–6) also yielded a reliable post-training dose effect, $F(3,23)=8.06$; $p<0.01$. Follow-up Bonferroni *t*-tests indicated that all groups that received GR-205171 (0.3, 1.0 and 5.0 mg/kg) displayed less FPS than the vehicle control group ($p<0.05$; Figure 3). Thus, while the highest dose of GR-205171 (5mg/kg) reduced overall FPS (Blocks 1–12), lower doses (0.3 and 1.0mg/kg)

also induced a reduction of FPS, which could be detected during the first half of the post-training test session when FPS expression is greatest (Blocks 1–6).

The effects of GR-205171 on elevated plus-maze testing

An evaluation of the percentage time in open arms revealed a significant treatment effect $F(3,30)=5.14$, $p<0.01$. As seen in Figure 4A, there was a significant relationship between drug dose and percentage time in open arms, $F(1,30)=14.78$, $p<0.01$. Individual contrasts revealed that treatment with 5 mg/kg of GR-205171 increased percentage time in open arms, $t(19)=3.86$, $p<0.01$. The comparison between vehicle (0 mg/kg) and 1.0 mg/kg groups was marginally nonsignificant, $t(19)=1.79$, $p=0.08$, and the difference between vehicle and 0.3 mg/kg groups was nonsignificant, $t(19)=1.01$, *NS*.

Assessment of total closed arm entries indicated significant differences in locomotor activity among treatments groups, $F(3,30)=8.33$, $p<0.01$. Individual contrasts comparing the vehicle group to each GR-205171 treatment group indicated that gerbils treated with the high dose of GR-205171 (5.0mg/kg) made significantly more closed arm entries than the vehicle group, indicating a drug-induced increase in exploratory/motor-stimulant activity, $t(19)=4.39$, $p<0.01$. No significant differences in number of closed arm entries were observed between the vehicle, 0.3 mg/kg, and 1.0 mg/kg treatment groups (all *NS*, Figure 4B). To factor out this increase in locomotor activity, the data were analyzed using percentage open arm entries. At doses of 0.3, 1.0, and 5.0 mg/kg, GR-205171 caused a significant anxiolytic-like effect as revealed by significant increases in the percentage open arm entries. As seen in Figure 4C, all three treatment levels of GR-205171 reliably increased the percentage open arm entries as revealed by a significant one-way ANOVA, $F(3,30)=8.45$, $p<0.01$, and individual contrasts comparing the vehicle group to subsequent treatments groups were all significant ($ps<0.01$). No other *t*-test was statistically significant. Group means for the number of open, total, and center entries, as well as center arm time are presented in Table 1.

Discussion

The present study sought to determine whether systemic administration of the NK1 receptor antagonist GR-205171 in gerbils produced anxiolytic-like effects in the elevated plus maze and a novel contextual conditioned fear test using the FPS paradigm. In the contextual FPS test, gerbils displayed reliable increases in startle when tested in a context previously associated with unsignaled footshocks. In contrast, previous unsignaled footshock training did not augment startle if gerbils were tested in a different context, indicating context-specific conditioning. When gerbils were tested for FPS after GR-205171 administration, all drug doses (0.3, 1.0 and 5.0 mg/kg) significantly attenuated contextual FPS when compared to the vehicle control group.

Data from previous studies conducted with laboratory mice and rat demonstrate that reference anxiolytics, such as benzodiazepine agonists, effectively reduce the expression of both cue-specific (Guscott et al. 2000; Risbrough et al. 2003) and contextual FPS (Guscott et al. 2000; Joordens et al. 1997; Young et al. 1991), thus supporting the use of the FPS paradigm to investigate anxiolytic properties of drugs. Independent of the anxiolytic-like effects, benzodiazepine agonists also attenuate baseline startle amplitudes in a dose-dependent manner (Joordens et al. 1998). In contrast, none of the doses of GR-205171 tested altered baseline startle amplitude in the present study. Future studies will need to explore the behavioral profile of anxiolytics such as benzodiazepines, serotonin agonists, and other NK1 antagonists in gerbils using the FPS paradigm.

In the elevated plus maze, GR-205171 produced an anxiolytic-like effect as indexed by an increase in the percent time spent in the open arms and percent of open arms entries. In the case of percentage open arms entries, all 3 doses of GR-205171 produced significant increases as compared with the control vehicle group. GR-205171 administration increased percentage open arm time in a dose-dependent manner, causing a significant increase above the control group at the highest dose (5.0 mg/kg). The highest dose of GR-205171, however, also increased locomotor activity as reflected by the total number of closed arms entries (Cruz et al. 1994; Rodgers et al. 1997; Rodgers and Johnson 1995). It is often assumed that increases in open arm indices reflect a specific effect on fear/anxiety, provided there is no simultaneous change in locomotor activity. The increase in closed arm entries could reflect a non-specific arousal/locomotor stimulation resulting in potential “false positives.” However, the increase in open arms entries seen at lower doses levels (0.3 and 1.0 mg/kg) were not accompanied by significant locomotor effects. Furthermore, no group differences were detected in center hub behaviors, which have been used as indices of exploratory motivation (Lee and Rodgers 1991; Rodgers et al. 1992). Thus, whether changes in open arm time reflect an anxiolytic-like effect or some other processes independent of the fear, such as general arousal or an enhanced motivation to explore is unclear. However, reference anxiolytics such as benzodiazepine agonists, which robustly increase open arm indices, also increase locomotion in the elevated plus-maze (Dawson et al. 1995; Derrien et al. 1994; File and Aranko 1988; Lister 1987; Moser 1989; Rodgers et al. 1992; Varty et al. 2002b). In contrast, locomotor stimulants, such as amphetamine and caffeine have inconsistent effects on locomotion in the elevated plus-maze (Pellow et al. 1985). Such evidence leads one to question the rationale for excluding compounds that increase closed-area entries.

Varty et al. (2002a) have previously demonstrated that a number of NK1 receptor antagonists produce anxiolytic-like effects in a novel gerbil elevated plus maze. In that study, investigators reported that antagonists produced no enhanced locomotor activity. Additionally, open arm indices in vehicle-treated control animals were higher than the present study. Because the plus-maze is very sensitive to methodological factors, it is likely that differences in maze construction and test procedures account for the observed discrepancies. For example, testing in our study was carried out on a plus-maze elevated higher (65 cm vs. 35 cm) and with narrower arms (6.5 cm vs. 8 cm). Furthermore, the maze used by in the study of Varty et al (2002a) had clear closed arms to allow for constant illumination in all parts of the maze and holes incorporated into the Plexiglas floor, to allow gerbils to grip the surface. That study also used female gerbils as opposed to the males gerbils used in the current study. In addition to the above factors, animals in our study were tested under higher light conditions (100-Lux vs. 5-Lux), which have been shown to reduce open arm entries in gerbils (Varty et al. 2002b). It is possible that any one or combination of these variables could account for the baseline differences observed in our vehicle-treated animals.

In recent years a number of different behavioral models have been used to assess the anxiolytic-like properties of NK1 receptor antagonists in gerbils. Varty et al. (2002b) demonstrated that the elevated plus-maze induces an anxiety-like profile and has predictive validity for anxiolytics like benzodiazepines in gerbils. Past research indicates that the NK1 antagonists MK-869, L-742,694, L-733,060, CP-99,994, and CP-122,721 produced anxiolytic-like effects in the gerbil elevated plus-maze (Varty et al. 2002a). Consistent with these findings, we have shown that the NK1 antagonist GR-205171 also induces an anxiolytic-like profile in the elevated plus-maze without accompanying sedative effects often observed with anxiolytic drugs like benzodiazepines (Rupniak et al., 2001). NK1 antagonists have also been tested against behavioral conditioning paradigms that induce foot drumming, which is seen as a species-specific alarm or fear response in gerbils (Randall 2001). Ballard et al. (2001) have shown that pairing a light-tone conditioned stimulus (CS)

with a footshock US produces a robust foot-drumming response during both the conditioning period and following presentation of the conditioned stimulus in a retest 24 h later. Moreover, gerbils treated with the NK1 antagonist MK-869 prior to conditioning show less shock-induced foot drumming and a significant inhibition of CS-induced foot drumming during subsequent testing. In the same study, Ballard et al. (2001) showed that pretreatment with the NK1 antagonist CP-99,994 also produced a significant reduction in foot drumming induced by a 2-mA footshock US. Similarly, exposure to the aversive four-plate fear conditioning apparatus induced foot drumming in gerbils, which could be abolished by either diazepam and the NK1 receptor antagonist L-760735 given before testing (Rupniak 2003).

Our findings support evidence from other laboratories for anxiolytic activity of NK1 receptor antagonists and provide a novel Pavlovian conditioned fear test that may be an appropriate procedure to test other NK1 antagonists for preclinical anxiolytic activity in gerbils. The FPS paradigm is widely recognized as a valid measure of fear and anxiety because: (1) humans demonstrate FPS (Ameli et al. 2001; Grillon and Davis 1997; Grillon et al. 1999), (2) anxiolytic drugs decrease FPS (Davis 1979; Grillon et al. 2006; Patrick et al. 1996; Riba et al. 2001; Winslow et al. 2007), and (3) lesions of brain structures critical for the expression of fear eliminate the expression of FPS (Campeau and Davis 1995; Falls and Davis 1995; Heldt et al. 2000; Sananes and Davis 1992). Fear-potentiated startle may be particularly useful for examining conditioned fear in gerbils because fear-induced foot drumming can inhibit the expression of conditioned freezing (Woolley et al. 2006), which many conditioning paradigms use as a behavioral response to index levels of fear and anxiety. It is worth noting, however, that conditioning procedures that induce foot drumming often use higher footshock US intensity than used in the current study. Although not quantified, we did not provoke noticeable foot drumming, presumably due to the relatively low US intensity (i.e. 0.6mA).

A growing line of evidence indicates that the amygdala is a potential site of action for NK1 receptor antagonists in anxiety. Autoradiographic experiments with NK1 radioligands performed in brain slices of gerbils reveal a distribution profile that is highly homologous to human, and in both species NK1 receptors are widely distributed in brain regions involved in the regulation of affective behavior and the mediation of stress responses, including various nuclei of the amygdala (Caberlotto et al. 2003; Griffante et al. 2006; Nagano et al. 2006; Rigby et al. 2005). Previous studies have also shown that exposure to a variety of aversive stressors such as immobilization and forced swim stress increase substance P release in the amygdala, as indexed by microdialysis and corresponding NK1 receptor internalization (Ebner et al. 2004; Ebner and Singewald 2005; Smith et al. 1999). In contrast, microinjection of NK1 antagonists into the amygdala blocks stress-induced anxiogenic effects (Ebner et al. 2004). Furthermore, the effect of amygdala lesions resembled that seen after administration of the NK1 antagonists. For example, gerbils that had undergone basolateral and lateral amygdala lesions after aversive four-plate fear conditioning exhibited a release of plate crossings and reduced foot drumming when they were returned to the apparatus 7 days after the initial exposure, resembling effects seen after administration of the SPA, L-760735 (Rupniak et al. 2003b). Bilateral amygdala lesions also blocked footshock-induced immobility (Woolley et al. 2006), and drug-induced foot drumming (Rupniak et al. 2003b).

At present, much less is known about the efficacy of NK1 receptor antagonists as a treatment option for individuals suffering with pathological anxiety; however, several studies have revealed that NK1 antagonists, including GR-205171, show promise as anxiolytic and antidepressant drugs in clinical trials (Furmark et al. 2005; Kramer et al. 2003). Recent clinical findings have also shown that SP concentrations are elevated both

during and after the symptom-provoking stimulus in the CSF of PTSD patients (Geraciotti et al. 2006). Similarly, in a recent PET study using a radioactive analog of GR205171, Michelgard et al. (2007) found that patients viewing phobia-inducing pictures had reduced uptake of the labeled GR-205171 in the amygdala during symptom provocation. This reduction reflected a fear-induced increase in the release of endogenous SP and corresponding reduction in NK1 receptor availability. More recently, Fujimoura et al. (2009) have shown that compared with healthy subjects, patients with panic disorder have widespread reduction of NK1 receptor binding in brain. Together, these findings support the notion that the SP-NK1 receptor system might be an important neurochemical target for the development of selective drugs designed to control pathological anxiety.

Within the context of the current study, extensive evidence indicates that the amygdala is critically involved in the acquisition and retrieval of contextual fear conditioning. Amygdala lesions or inactivation block conditioned fear responses to both the context and the explicit cue (Davis 1992; Kapp et al. 1984; LeDoux 1994). Studies using freezing as an index of fear have shown that hippocampal lesions made either before training (Phillips and LeDoux 1992) or after training (Kim and Fanselow 1992) also disrupted fear conditioning to the context but not to a explicit cue. However, as assessed by the FPS, the hippocampus may not be as critical as the amygdala for contextual learning (Gewirtz et al. 2000; McNish et al. 1997). Lastly, there is published evidence supporting the possibility that augmented startle responses associated with FPS may result from the influence of SP on downstream targets of amygdala. Microinjections of SP into the caudal pontine reticular nucleus (PnC), an important part of the primary acoustic startle circuit, increases the amplitude of the startle response (Krase et al. 1994). This effect can be antagonized by local pretreatment with the NK1 antagonist CP-96,345. Furthermore, sensitization of the startle response by footshocks can also be blocked by local microinjections of CP-96,345 into the PnC (Krase et al. 1994).

In conclusion, the current study demonstrates that the NK1 receptor antagonist GR-205171 induced an anxiolytic-like response in the elevated plus maze test. In addition, we have shown that gerbils can display contextual FPS, which can be reduced by pretreatment of GR-205171. These findings support evidence from other laboratories for anxiolytic activity of NK1 antagonists and provide a novel Pavlovian conditioned fear test, which may be an appropriate procedure to test other NK1 antagonists for preclinical anxiolytic activity in gerbils.

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Figure 1.

A) Mean startle responses elicited at increasing noise-burst intensities in gerbils (N=20). Startle responses in gerbils increased as a function of stimulus intensity (90, 100, 110, 120 dB). B) Effects of the NK1 antagonist GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg, i.p) on mean startle responses in gerbils. Gerbils received treatment of GR-205171 at 0, 0.3, 1.0 or 5.0 mg/kg doses (n=5/group) 30 min before testing in which animals received startle stimuli at two different intensities (110- and 115-dB). Error bars denote 1 standard error of the mean. Bonferroni *t*-tests revealed significant startle differences among all intensities. Stars indicate $p < 0.001$.

Figure 2.

A) Mean pre-training and post-training startle response of gerbils trained and tested in the same (SAME) context (n=8; A–A and B–B) or different (DIFF) contexts (n=8; A–B and B–A). B) Mean startle responses of gerbils across blocks during the post-training startle test. Block values represent the mean startle response of two sequential test trials of different intensity (110, 115 dB). The insert panel displays the mean startle responses during the first and second halves of the post-training test. The single star indicates significant fear-potentiated startle as defined as a reliable increase from pre- to post-training startle ($p < 0.05$). The pound sign (#) indicates significant group differences. The error bars denote 1 standard error of the mean.

Figure 3.

Effects of the NK1 antagonist GR-205171 on contextual FPS. Gerbils were administered GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg, i.p) 30 min before the post-training test session. The bar graph presents the mean startle response of groups before training as well as their mean startle responses during the first and second halves of the post-training test. The insert panel displays the startle responses of gerbils across blocks during the post-training startle test. Block values represent the mean startle response of two sequential test trails of different intensity (110, 115 dB). Overall startle response (SEM) collapsed across the post-training test session: 0 mg/kg=1216(73), 0.3 mg/kg=882(78), 1.0 mg/kg=931(103), and 5.0 mg/kg=753 (86). Stars indicate significant difference from vehicle (0mg/kg) group, $p < 0.05$. Error bars denote 1 standard error of the mean.

Figure 4.

Effects of the NK1 antagonist GR-205171 on elevated plus-maze performance. Gerbils were administered GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg, i.p) 30 min before testing. A) Percentage of open arm time. B) Number of closed arm entries. C) Percentage of open arm entries on the elevated plus-maze. Stars indicate significant difference from vehicle (0mg/kg) group, $p < 0.05$. Error bars denote 1 standard error of the mean.

TABLE 1

Effects of GR-205171 (0, 0.3, 1.0 or 5.0 mg/kg) on the behavioral measures from the elevated plus-maze.

Dose (mg/kg)	Number of Open Entries	Number of Total Entries	Number of Center Entries	Center Time (sec)
0.0	0.9(0.2)	12.8(1.3)	14.1(1.1)	102.5(6.1)
0.3	3.1(0.3)	14.6(1.0)	12.7(1.1)	117.4(9.7)
1.0	5.5(1.5)	18.8(2.8)	11.3(1.1)	111.7(9.8)
5.0	8.3(2.5)	28.1(3.0)	12.8(1.9)	82.4(9.9)