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The effects of serotonin reuptake inhibitors on locomotor activity in gerbils

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Abstract

In the current study we examined the effects of serotonin reuptake inhibitors on the locomotor activity of gerbils, and undertook experiments to understand the mechanisms involved in their effects. The selective serotonin reuptake inhibitors (SSRIs) fluoxetine (1–30 mg/kg, i.p.) and escitalopram (0.03–10 mg/kg, i.p.) dose-dependently increased locomotor activity, whereas the serotonin and noradrenaline reuptake inhibitor duloxetine (0.3–30 mg/kg, i.p.) did not. The noradrenaline reuptake inhibitor (NRI) reboxetine, which alone did not significantly affect locomotion (1–30 mg/kg, i.p.), markedly reduced the effects of escitalopram. The locomotor effects of fluoxetine and escitalopram were dependent on novelty since both compounds showed rapid habituation in novel cages and were inactive when tested in home cages. Both diazepam (0.3–10 mg/kg, i.p.) and D-amphetamine (0.3–10 mg/kg, s.c.) increased locomotor activity but only the effects of diazepam were novelty-dependent. The finding that SSRIs increased locomotion, with a negative modulatory role for NRI, in a novelty-dependent manner, similar to diazepam, suggests that anxiety plays an important role in the present paradigm. The increase in locomotion as observed in our test conditions can be readily used as a selective and sensitive *in vivo* assay for serotonin transport inhibition in gerbils.
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Keywords: Locomotor activity; Locomotion; Anxiety; Serotonin reuptake inhibitors; Diazepam; Gerbils

1. Introduction

The Mongolian gerbil has been increasingly used to assess the effect of pharmacological treatment on behaviour in a variety of anxiety and depression tests (Ballard et al., 2001; Cheeta et al., 2001; File et al., 2001; Varty et al., 2002a,b). The interest in the gerbil initially arose from the first report of positive clinical trial data in anxiety and depression with an NK_1 receptor antagonist (Kramer et al., 1998) and the knowledge that the pharmacology of the human NK_1 receptor was closer in homology to gerbil and guinea pig than rat and mouse (Beresford et al., 1991; Gitter et al., 1991) which may be due to similarities in the amino acid sequences of gerbil and human receptors (Steward et al., 2005). It has been suggested that compounds which are dual NK_1 antagonists and selective serotonin reuptake inhibitors (SSRIs) may represent a new class of antidepressants with better efficacy, fewer side effects and potentially faster onset of action (Ryckmans et al., 2002a). One of these compounds was shown to have *in vivo* activity in the isolation-induced guinea pig pup vocalisation test (Ryckmans et al., 2002b), which is sensitive to both SSRI and NK_1 antagonism (Kramer et al., 1998). In gerbils, such interactions have not been studied, and the effects of SSRIs are conflicting depending on the paradigm used. For example, acute fluoxetine administration was found to increase foot tapping induced by a foot shock (Ballard et al., 2001; Rupniak et al., 2003), yet increase time spent on the open arms of an elevated plus maze (Varty et al., 2002b) which is indicative of an anxiogenic and anxiolytic effect respectively. Since the effect of SSRIs may differ depending on the test conditions used to induce anxiety in gerbils, the purpose of the current study was to set up an *in vivo* assay preferably having simple, automated and objective readouts, not necessarily linked to anxiety, to investigate potential interaction between SSRIs and compounds acting on receptors that show species differences in pharmacology, such as NK_1 antagonists.

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The effects on locomotor activity of SSRIs have been extensively examined in mice and rats. For a recent update and novel findings see a paper by Brocco et al. (2002). In brief, SSRIs increase locomotor activity in mice when exposed to a novel environment but not when they are habituated to the environment. In contrast, SSRIs do not generally increase locomotion in rats under any condition (Brocco et al., 2002). The underlying mechanism for the locomotion-increasing effects of SSRIs in mice has been extensively discussed in Brocco et al. (2002). Among the most plausible hypotheses is a role for anxiety, although they conclude that the predictive validity in their conditions seems to be limited.

In the present study, we first examined whether two SSRIs (i.e. fluoxetine and escitalopram) would increase locomotion in gerbils when exposed to a novel environment, as was reported in mice (Brocco et al., 2002). We found marked increases in locomotion with both compounds but did not find a clear effect of the dual NE and 5-HT reuptake inhibitor duloxetine. Since the SSRIs increased locomotion whereas duloxetine did not, we next determined whether this could be due to noradrenaline reuptake properties inhibiting 5-HT reuptake properties by testing combinations of the noradrenaline reuptake inhibitor (NRI) reboxetine and escitalopram. We then assessed whether the effects of fluoxetine and escitalopram on locomotion were dependent on novelty by comparing their effects in the novel cage to those in a home cage, and by measuring locomotion for a longer period to examine the habituation curves. Even though not anticipated (see above), these data clearly suggest a possible role of anxiety which is why we assessed the effect of diazepam on locomotion and compared its effect to D-amphetamine. Again, the role of novelty in the effects of the latter compounds was examined.

2. Materials and methods

2.1. Animals and housing

Naïve male Mongolian gerbils (Charles River Laboratories, Germany) weighing between 25 and 30 g were used in all experiments. On arrival, gerbils were housed in groups of 4 for at least 5 days (type III transparent polycarbonate cage, $L \times W \times H$, $42 \times 26 \times 15$ cm). Then they were isolated in a type II transparent polycarbonate cage ($L \times W \times H$, $26 \times 21 \times 15$ cm) on a 5 cm layer of sawdust 24 h prior to behavioural testing. All animals were housed with sawdust bedding under standard maintenance conditions (12:12 h light–dark cycle; 21–23 °C; 55–65% relative humidity). Food and water were given ad libitum in the home cage. All testing was performed during the light portion of the day–night cycle (lights on at 6:00 AM). The present testing procedure received prior approval from local committee based on adherence to Swiss federal regulations and guidelines on animal experimentation provided by the Swiss Academy of Sciences and Swiss Academy of Medical Sciences (1995).

2.2. Locomotor activity measures

All behavioural tests were performed under a dim white ceiling light (15 lux, measured at the level of the arena). During the pre-treatment time period of 30 min for all compounds (except D-amphetamine: 10 min), gerbils were placed back in their home cage.

Following the pre-treatment time each gerbil treated with either vehicle or compound was either placed in a novel cage (type II, see above) containing a 5 cm layer of sawdust or maintained in its home cage (without food and water) which was covered with a Plexiglas top in order to allow the recording of total distance travelled by a video tracking system (Videotrack, View Point-Behaviour Technology, France) for 15 or 60 min.

2.3. Drugs

Except D-amphetamine sulphate (Sigma-Aldrich) which was administered s.c., fluoxetine HCl, diazepam (synthesized at F. Hoffmann-La Roche Ltd.; Basel, Switzerland), escitalopram HCl, duloxetine HCl (extracted from commercially available capsules at F. Hoffmann-La Roche Ltd.; Palo Alto, USA) and reboxetine mesylate (TRC inc.) were administered i.p. in 0.3% (v/v) Tween-80 in physiological saline (0.9%) in a volume of 10 ml/kg body weight.

2.4. Statistical analysis

Effects of treatments (dose–responses) on locomotor activity were analyzed by one-way analysis of variance (ANOVA). Planned Dunnett's tests were used to determine significant differences between drug-treated groups and the vehicle group. The effects of reboxetine on the increase in locomotion induced by escitalopram were analyzed by two-way ANOVA (factors Reboxetine and Escitalopram) followed by planned Newman–Keuls tests. Comparison of the effects of drug treatments on locomotion measured in novel and home cages was analyzed by two-way ANOVA (factors Cage and Treatment; note that fluoxetine and escitalopram were tested and analyzed together) followed by planned Newman–Keuls tests. Finally, time-dependent effects (six intervals of 10 min) of treatments on locomotor activity measured in novel and home cages were analyzed with two-way ANOVAs (with between factor Cage, and within factor Time interval) followed by planned Newman–Keuls tests, only for the drug-treated groups (the curves of all the vehicle groups were similar to those of the home cage groups in Figs. 3B, D and 5B, D and for clarity were not included in the graphs). For all comparisons, significance was set at $p < 0.05$. All data are expressed as mean ± SEM ($n = 7$–8 per group).

3. Results

3.1. Effects of antidepressants on locomotion measured in an unfamiliar environment

As shown in Fig. 1, the SSRIs fluoxetine and escitalopram significantly increased locomotor activity in gerbils tested in novel cages [$F(4,35) = 7.8$, $p < 0.001$ and $F(6,49) = 23.53$, $p < 0.001$, respectively] and significance was reached for the doses of 10 and 30 mg/kg of fluoxetine and 0.3, 1, 3 and 10 mg/kg of escitalopram. In contrast, in the same test conditions, neither duloxetine nor reboxetine significantly modified locomotor activity [$F(5,42) = 1.78$, $p = 0.14$ and $F(4,34) = 0.84$, $p = 0.5$, respectively].

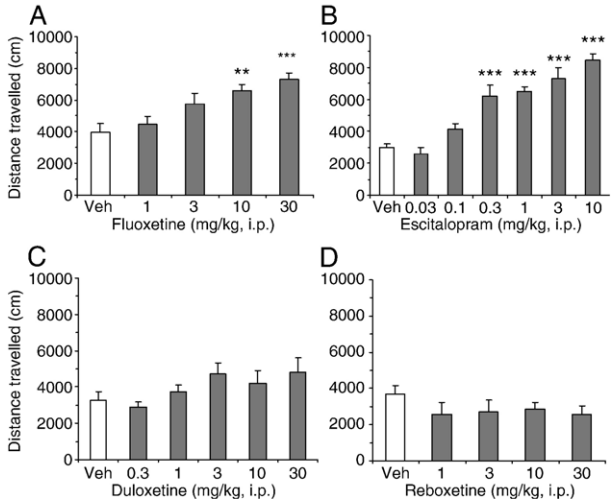

Fig. 1. Effects of antidepressants on locomotor activity in gerbils tested in novel cages for 15 min. A) Fluoxetine, B) escitalopram, C) duloxetine, and D) reboxetine. Data represent mean±SEM (n=7–8). **p<0.01, ***p<0.001 compared to vehicle-treated (Veh) animals, based on a 1-way ANOVA followed by a planned Dunnett's test.

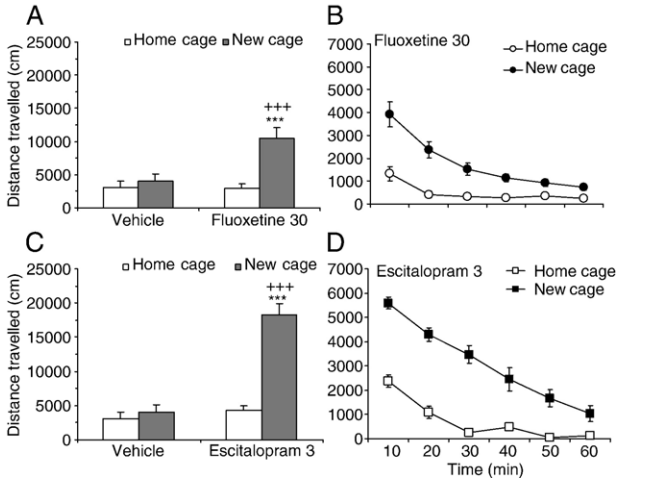

Fig. 3. Effects of compounds on locomotor activity in gerbils tested in home or novel cages. A) Fluoxetine, mean values for total of 60 min, B) fluoxetine, mean values per 10 minute time bins, C) escitalopram, mean values for total of 60 min, and D) escitalopram, mean values per 10 minute time bins. Doses are expressed in mg/kg, i.p. Data represent mean±SEM (n=7–8). *p<0.05, **p<0.01, ***p<0.001 compared to vehicle-treated animals tested in the same condition, based on a 2-way ANOVA followed by planned Newman–Keuls tests. ^{+++}p<0.001 compared to animals receiving the same treatment and tested in home cages, based on a 2-way ANOVA followed by planned Newman–Keuls test.

3.2. Interaction between reboxetine and escitalopram: effects on locomotion measured in an unfamiliar environment

Escitalopram alone significantly increased locomotor activity (Fig. 2) [effects of Escitalopram, $F(2,40)=13.3$, $p<0.001$] and significance was reached for both doses tested. This effect was significantly reduced by pre-treatment with reboxetine [effects of Reboxetine, $F(1,40)=25.75$, $p<0.001$; interaction between Escitalopram and Reboxetine, $F(2,40)=8.5$, $p<0.001$].

3.3. Impact of test condition (novel versus home cages) on the effects of fluoxetine and escitalopram on locomotion

As shown in Fig. 3 (A, C), both SSRIs increased locomotor activity only in novel cages [effect of Treatment $F(2,42)=24.3$, $p<0.001$; effect of Cage $F(1,42)=67.9$, $p<0.001$; interaction of test Cage with Treatment $F(2,42)=16.9$, $p<0.001$]. In addition, there was a time-dependent reduction of locomotor activity in animals treated with the SSRIs (Fig. 3B, D) [fluoxetine: effect of Cage $F(1,14)=20.36$, $p<0.001$; effect of Time $F(5,70)=52.7$, $p<0.001$; interaction between Cage and Time $F(5,70)=14.35$, $p<0.001$; escitalopram: effect of Cage $F(1,14)=67.4$, $p<0.001$; effect of Time $F(5,70)=83.7$, $p<0.001$; interaction between Cage and Time $F(5,70)=13.1$, $p<0.001$].

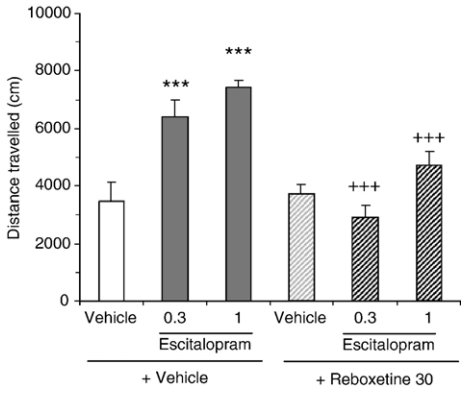

Fig. 2. Effects of reboxetine on increases in locomotor activity induced by escitalopram in gerbils tested in novel cages for 15 min. Doses are expressed in mg/kg, i.p. Data represent mean±SEM (n=7–8). ***p<0.001 compared to vehicle/vehicle-treated animals, based on a 2-way ANOVA followed by planned Newman–Keuls test. ^{+++}p<0.001 compared to the respective escitalopram/vehicle-treated animals, based on a 2-way ANOVA followed by planned Newman–Keuls tests.

3.4. Effects of diazepam and D-amphetamine on locomotion measured in an unfamiliar environment

As shown in Fig. 4, locomotor activity was significantly and dose-dependently increased by diazepam [$F(4,29)=8.9$, $p<0.001$] and D-amphetamine [$F(4,30)=4.25$, $p<0.01$] in gerbils exposed to novel cages. Doses of 1 and 3 mg/kg for diazepam and

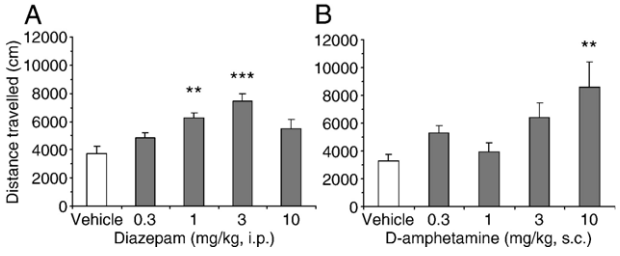

Fig. 4. Effects of diazepam (A) and D-amphetamine (B) on locomotor activity in gerbils tested in novel cages for 15 min. Doses are expressed in mg/kg. Treatments were administered i.p for diazepam and s.c. for D-amphetamine. Data represent mean±SEM (n=7–8). **p<0.01, ***p<0.001 compared to vehicle-treated animals, based on a 1-way ANOVA followed by a planned Dunnett's test.

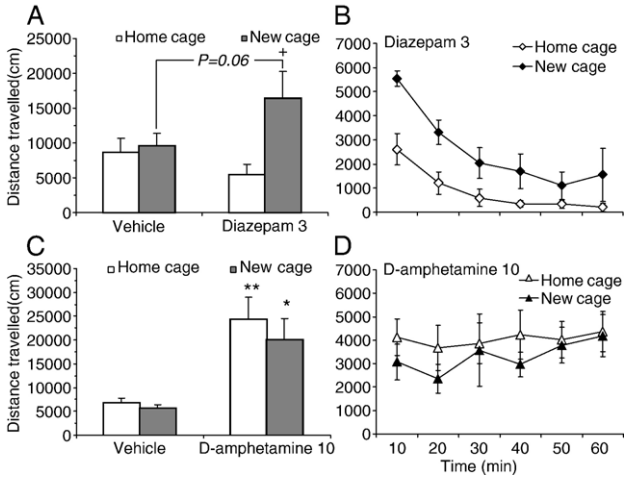

Fig. 5. Effects of compounds on locomotor activity in gerbils tested in home or novel cages. A) Diazepam, mean values for total of 60 min, B) diazepam, mean values per 10 minute time bins, C) D-amphetamine, mean values for total of 60 min, and D) D-amphetamine, mean values per 10 minute time bins. Doses are expressed in mg/kg. Treatments were administered i.p for diazepam and s.c. for D-amphetamine. Data represent mean±SEM (n=7–8). *p<0.05, **p<0.01, compared to vehicle-treated animals tested in the same condition, based on a 2-way ANOVA followed by planned Newman–Keuls tests. ^{+}p<0.05, compared to animals receiving the same treatment and tested in home cages, based on a 2-way ANOVA followed by planned Newman–Keuls tests.

10 mg/kg for D-amphetamine were significantly different from their respective vehicle groups.

3.5. Impact of test condition (novel versus home cages) on the effects of diazepam and D-amphetamine on locomotion

As shown in Fig. 5A, diazepam (3 mg/kg) enhanced locomotor activity only in novel cages [effect of Treatment F(1,28)=0.55, p=0.46; effect of Cage F(1,28)=5.9, p<0.05; interaction between Cage and Treatment F(1,28)=4.2, p<0.05]. In addition, there was a time-dependent reduction of locomotor activity in animals treated with diazepam (Fig. 5B) [effect of Cage F(1,14)=6.7, p<0.05; effect of Time F(5,70)=28.34, p<0.001; interaction between test Cage and Time F(5,70)=2.45, p<0.05].

As shown in Fig. 5C, D-amphetamine invariably increased locomotor activity both in novel and home cage conditions [effect of Cage F(1,28)=0.7, p=0.4; effect of Treatment F(1,28)=23, p<0.001; interaction between Cage and Treatment F(1,28)=0.22, p=0.64]. The time–effect graphs did not show a marked difference over time (Fig. 5D) and none of the factors reached significance [effect of Cage F(1,14)=0.44, p=0.51; effect of Time F(5,70)=1.28, p=0.27; interaction between Cage and Time F(5,70)=0.57, p=0.72].

4. Discussion

The main findings in the present study are that: 1) Mongolian gerbils exposed to a novel cage increased locomotion when they were treated with the SSRIs fluoxetine and escitalopram, 2) the SNRI duloxetine did not significantly increase locomotion whereas the NRI reboxetine reversed the increase in locomotion induced by escitalopram, 3) the effects of the SSRIs were absent when the animals were tested in their home cages, and 4) diazepam produced effects similar to those of SSRIs whereas D-amphetamine produced a different – psychostimulant – profile. This data suggests that the present paradigm can be used to study the acute effects of SSRIs, effects that appear to be related to their anxiolytic properties.

Fluoxetine increased locomotion in gerbils, suggesting a role for 5-HT reuptake. However, the selectivity of fluoxetine is limited and other mechanisms could play a role (Owens et al., 2001). Therefore, we tested escitalopram, which is the most selective SSRI with respect to biogenic amine systems (Owens et al., 2001; Chen et al., 2005). Similar to fluoxetine, escitalopram dose-dependently and monotonically increased locomotor activity suggesting a role for 5-HT reuptake. This is supported by the fact that the doses that increase locomotion in the present study are similar to those reported to increase 5-HT levels in rodents (e.g., Malagie et al., 2002; Ceglia et al., 2004; Pinna et al., 2006). Over the last ten years it has become clear that apart from their effects on transporters, SSRIs may also affect enzyme activities leading to increases in neuroactive steroids, which modulate GABA activity (e.g., Uzunov et al., 1996). However, regarding the evidence above and given that the increase in neuroactive steroids occurs at lower doses than the increase in 5-HT levels (Pinna et al., 2006), this does not seem to play a major role in the present study. The increase in locomotion by SSRIs likely depends on experimental conditions as Varty et al. (2002b) in control experiments for the elevated plus maze (see Introduction) observed no such effects in female gerbils. The difference between that and the present study does not seem to be related to test condition (novel cages in both studies) or gender (we observed similar increases in female gerbils; data not shown); one possible explanation is the presence of sawdust during the present studies but not in those by Varty et al. (2002b).

Duloxetine did not significantly increase locomotion. This was surprising because one of its main properties is to inhibit 5-HT reuptake (e.g. Vaishnavi et al., 2004). In fact, its affinity for the serotonin transporter is in the same range as that of fluoxetine and escitalopram (Owens et al., 2001; Vaishnavi et al., 2004). Such a difference between the SSRIs and duloxetine appears to be specific to gerbils since in mice, dual reuptake inhibitors were found to increase locomotion like SSRIs (e.g., Brocco et al., 2002). The most likely explanation of the relative lack of effect of duloxetine in the present study was that NE reuptake inhibiting properties, which are marked for duloxetine but not for fluoxetine or escitalopram (Owens et al., 2001; Vaishnavi et al., 2004), could mask its stimulatory effects. This hypothesis was tested by studying the interaction between the most selective NRI and SSRI, reboxetine (Wong et al., 2000) and escitalopram (Owens et al., 2001; Chen et al., 2005), respectively. Reboxetine alone did not significantly affect locomotor activity. However, when combined with escitalopram, it markedly reduced the locomotor activity effects of the latter compound. Drug–drug interactions are unlikely to explain the observed interaction since this would lead to increases not decreases in levels of escitalopram. Moreover, both escitalopram and reboxetine are weak substrates of

P450 enzymes and are not expected to have such interactions (Burke, 2002; Hajos et al., 2004). The pharmacodynamic interaction between reboxetine and escitalopram supports the hypothesis that for dual reuptake inhibitors such as duloxetine, activation of the NE system can mask the behavioural consequences of activation of the 5-HT system. Similar interactions are observed in certain anxiety models such as the four-plate test (Hascoet et al., 2000), and conditioned fear stress paradigm (Miyamoto et al., 2004) where the anxiolytic effects of 5-HT reuptake properties can be modulated negatively by NE reuptake properties.

Given the foregoing, one could speculate that the effects of the SSRIs were due to their anxiolytic-like properties. If so, we would expect these effects to be due to modulation of novelty stress-induced inhibition of exploration behaviour – also known as behavioural inhibition (cf. McNaughton and Corr, 2004) – and, consequently, would expect the effects to habituate rather quickly and to be reduced or absent when animals were tested in their home cage. We tested the influence of novelty by comparing the effects of SSRIs in novel cages to those in home cages. We also measured the locomotor activity for a longer period so that we could study possible habituation. Whereas we replicated the effects of the SSRIs in novel cages, we did not observe any increase in locomotion when gerbils were tested in their home cages. Also, in novel cages the increase in locomotor activity was high at the beginning of the test and then showed clear habituation. The latter could not be explained by the half-life of fluoxetine and escitalopram which is reported to be relatively long (Caccia et al., 1990; Cheer and Goa, 2001; Burke, 2002; Kugelberg et al., 2003). Taken together, this data strongly suggests that the increase in locomotor activity by the SSRIs is fully dependent on novelty, and together with the observation that the vehicle-treated animals did not show more locomotion when tested in novel cages as compared to home cages, these effects may be interpreted as a disinhibition of exploration behaviour.

To further test the hypothesis that anxiety could play a role in the effects of the SSRIs, we tested diazepam, a prototypical benzodiazepine receptor agonist and rapidly-acting anxiolytic in the clinic and in most preclinical anxiety models. Diazepam increased locomotor activity in a bell-shaped manner under conditions of novelty, whereas it did not affect locomotor activity when animals were tested in their home cage. The relevance of these novelty-specific findings was further underlined by finding that the psychostimulant D-amphetamine produced effects independent of novelty. The active doses of diazepam were very similar to those observed in our laboratory in another anxiety model in gerbils, fear conditioning (Ballard et al., 2001). Together with the foregoing this strongly suggests that anxiolytic-like effects play a role in the effects of the SSRIs. This would imply that a compound like fluoxetine after acute administration – in gerbils – may have anxiolytic and anxiogenic effects depending on the paradigm (Ballard et al., 2001; Varty et al., 2002b; Rupniak et al., 2003). Indeed, these apparent opposite effects could be due to effects of 5-HT in different brain areas and/or on different 5-HT receptor subtypes (for review, see Graeff et al., 1996). The present data suggesting that the total distance travelled under the present conditions in gerbils is related to anxiety is somewhat unexpected in that in mice, the thigmotaxic behaviour rather than total distance travelled is thought to be related to anxiety (cf. Prut and Belzung, 2003). In general, it appears that the present paradigm has relevance for anxiety; nevertheless further pharmacological validation is necessary.

In conclusion, the present results suggest that this novel paradigm in gerbils can be added to the existing ones and may provide an *in vivo* model to further characterize interactions between SSRIs and compounds acting on other mechanisms. This could be of particular interest for compounds acting at receptors showing species differences in pharmacology between gerbils and rats/mice (e.g. NK_1 antagonists).

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