Published in final edited form as:
Prog Neuropsychopharmacol Biol Psychiatry. 2014 March 3; 49: 78–84. doi:10.1016/j.pnpbp.2013.11.011.

Acute, but not repeated, administration of the neurotensin NTS_1 receptor agonist PD149163 decreases conditioned footshock-induced ultrasonic vocalizations in rats

Adam J. Prus[1,*], **Todd M. Hillhouse**[2], and **Amber L. LaCrosse**[3]

[1]Psychology Department, Northern Michigan University, Marquette, MI USA

[2]Department of Psychology, Virginia Commonwealth University, Richmond VA, USA

[3]School of Psychology, Arizona State University, Phoenix, AZ USA

Abstract

Neurotensin is an endogenous neuropeptide that has significant interactions with monoamine neurotransmitter systems. To date, neurotensin NTS_1 receptor agonists, such as PD149163, have been primarily evaluated for the treatment for schizophrenia, drug addiction, and pain. Recently, PD149163 was found to attenuate fear-potentiated startle in rats, an experimental procedure used for screening anxiolytic drugs. The present study sought to extend these findings through testing PD149163 in a conditioned footshock-induced ultrasonic vocalization (USV) model. Conditioning was conducted in Male Wistar rats using chambers equipped with shock grid floors and an ultrasonic vocalization detector. PD149163 and the 5-HT_{1A} receptor partial agonist buspirone produced a statistically significant reduction of 22 kHz USV counts. The typical antipsychotic haloperidol also reduced 22 kHz USV counts, but did so at cataleptic doses. Ten days of repeated administration of PD149163 abolished the inhibitory effects of PD149163 on 22 kHz USVs. These findings further support an anxiolytic profile for PD149163. However, tolerance to these effects may limit the utility of these drugs for the treatment of anxiety.

Keywords

neurotensin; PD149163; buspirone; ultrasonic vocalization; anxiety

Neurotensin is an endogenous neuropeptide that is an emerging target for the treatment of anxiety. Neurotensin interacts closely with monoamine neurotransmitter systems (Binder et al., 2001; Jolas and Aghajanian, 1997), and neurotensin receptors are densely located in structures important for anxiety and depression, including the amygdala, hippocampus, and raphe nuclei (Alexander and Leeman, 1998). Stress-induced increases in median raphe nucleus 5-hydroxytryptophan levels (Dilts and Boadle-Biber, 1995) have been attenuated by intracerebroventricular administration of neurotensin (Dilts et al., 1996) and potentiated by systemic administration of the neurotensin NTS_1 receptor antagonist SR48692 (Corley et al., 2002). Further, central neurotensin administration has reversed decreases in stress-related

*Corresponding author, Adam J. Prus, Ph.D., Associate Professor, Psychology Department, Northern Michigan University, 1401 Presque Isle Ave., Marquette, MI 49855 USA, aprus@nmu.edu, Phone: 906-227-2941, Fax: 906-227-2954.

foraging in rats with lesioned serotonin neurons in the dorsal raphe nucleus (Shugalev et al., 2005). NTS_1 receptor knockout mice have exhibited anxiety-like responses in an open field, including less time spent in the center and more time spent in the corners of the field compared to wild type mice, although differences were not shown between NTS_1 knockout and wild type mice in an elevated plus maze (Fitzpatrick et al., 2012). Shilling and Feifel (2008) demonstrated that systemic administration of the brain penetrant neurotensin NTS_1 receptor agonist PD149163 (Petrie et al., 2004) significantly decreased fear potentiated startle in rats.

Another method for studying anxiety in rats is to record ultrasonic vocalizations (USVs) during states of fear or stress. In adult rats, 22 kHz USVs occur during fear-like postures (e.g., freezing) (Brudzynski and Chiu, 1995), avoidance behavior, and the presence of an intruder (Tornatzky and Miczek, 1994). Twenty-two kHz USVs are also emitted immediately after footshock stimulation (Tonoue et al., 1986) and when placed in an environment previously paired with footshock (Tonoue et al., 1987; Molewijk et al., 1995). Conditioned footshock-induced 22 kHz USVs are also suppressed by benzodiazepines (e.g., Millan et al., 2001; Molewijk et al., 1995), 5-HT_{1A} receptor agonists (De Vry et al., 1993; Molewijk et al., 1995; Remy et al., 1996), 5-HT reuptake inhibitors (Molewijk et al., 1995; Sanchez et al., 2003; Sanchez and Meier, 1997), and antipsychotic drugs (Sun et al., 2010).

The present study sought to further evaluate the putative anxiolytic effects of the neurotensin by testing the NTS_1 receptor agonist PD149163 on conditioned footshock-induced USVs. In addition, the effects of the 5-HT_{1A} receptor agonist and anxiolytic buspirone, which has been demonstrated to inhibit conditioned footshock-induced 22 kHz USVs (Molewijk et al., 1995; Brodkin et al., 2002), was assessed for comparison. Further, the D_2 receptor-preferring antagonist (Schotte et al., 1996) and typical antipsychotic drug haloperidol was also studied for comparison given that NTS_1 receptor agonists may functionally antagonize dopamine D_2 receptors (for review, see Binder et al., 2001 and St. Galais et al., 2006) and produce antipsychotic-like effects in animals (Boules et al., 2001; Feifel et al., 2008; Holly et al., 2011). Finally, the effects of PD149163 on 22 kHz USVs were assessed after 10 days of repeated administration given that tolerance to the effects of NT_1 receptor agonists has been shown in some behavioral studies (see discussion).

Methods

Subjects

Adult male Wistar rats (N=127; Charles River Laboratories, Portage, MI, USA), weighing approximately 250g when purchased, were group housed in standard plastic cages with free access to water and food (Mazuri Rodent Chow, #5663, Brentwood, MO USA). The vivarium was kept on a 12 hr light/dark schedule under constant temperature and humidity. All procedures were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at Northern Michigan University.

Apparatus

Six rat experimental chambers equipped with a stainless steel grid floor, with each bar of the floor attached to a footshock scrambler, were housed in sound-attenuating cubicles (Med-Associates Inc., St. Albans, VT, USA). Each chamber was equipped with a ultrasonic vocalization detector that responded to sound pressure changes (ANL-937-1, Med-Associates Inc., St. Albans, VT, USA) and was set to detect USVs occurring between 20 and 30 kHz (samplings every 30 ms). Fast-Fourier transform analysis was conducted by the Med-Associates USV software to collect USVs into 1kHz bins, and spectrographs were

produced to verify that USVs were only occurring at a 22 kHz frequency. The decibel level cut-off was adjusted to slightly above surrounding background noise (approximately 30 dB). Data collection for USV experiments were computer controlled by Med-State software (Med PC, Version 4.1, Med-Associates) running on a Windows XP operating system. Catalepsy was assessed using a wire grid fastened to a frame that was inclined 60°.

Drugs

The neurotensin NTS_1 receptor agonist PD149163 (0.01–3.0 mg/kg; NIMH Drug Repository, Bethesda, MD, USA), and the 5-HT_{1A} receptor partial agonist buspirone HCl (0.05–2.0 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 0.9% physiological saline. The typical antipsychotic drug haloperidol (0.03–3.0 mg/kg; Sigma-Aldrich) was dissolved in sterile water with the aid of a few drops of 85% lactic acid. All of the drugs were administered subcutaneously in a volume of 1 ml/kg 30 min prior to each session. All drugs were in a salt form. The doses of these drugs were chosen based upon preliminary studies in this laboratory and published findings from other laboratories (e.g., Molewijk et al., 1995; Shilling and Feifel, 2008).

USV procedures

These procedures were conducted over the course of three consecutive days during the light cycle, according to a standard protocol (Molewijk et al., 1995). Twenty-two kHz USVs were only recorded during the 10 min trial on days 2 and 3. On day 1, each rat was placed in an experimental chamber and exposed to 6 randomly distributed footshocks (0.8 mA, 8 s duration) over the course of a 7 minute session. On day 2, each rat was first placed in a chamber for a 2 min trial, and halfway through this trial, was exposed to a single footshock (0.8 mA, 8 s duration). After this 2 min trial, the rats were returned to their home cages. A 10 min trial was conducted thirty min later. No shocks were delivered during this trial and 22 kHz USV counts were recorded for the entire duration of the trial.

Based upon the number of 22 kHz USVs emitted, rats were then assigned in rank order to each dose of the drug, plus vehicle, to be tested on day 3 (i.e., a balance-block design). Thus, each of the groups were matched for number of baseline 22 kHz USV counts. Rats were removed from further experimentation on day 3 if fewer than 50 USVs were emitted on day 2. On day 3, the procedures for both trials were identical to day 2 except that an injection of drug or vehicle was given immediately after the 2 min trial (i.e., 30 min prior to test session).

Catalepsy assessment

During the 5 minutes preceding the 10 min trials on “day 2” and “day 3,” animals were placed on an inclined wire grid in order to measure catalepsy (Ahlenius and Hillegaart, 1986). The catalepsy assessment was conducted on day 2 so that, aside from injections, all procedures would be identical to day 3. However, the day 2 catalepsy data were not recorded. To measure catalepsy, rats were gently placed on a wire grid and the time to completely remove one paw was measured, after excluding the first 30 s during which time cataleptic animals may still exhibit movement on the grid as an artifact of having been handled by the researcher.

Experiments

Experiment 1. Acute administration testing with PD149163—For the first experiment, 40 rats were used to study the effects of PD149163 (0.3, 1.0, and 3.0 mg/kg) and vehicle on 22 kHz USVs, according to the procedures described above. However, catalepsy measures were not originally planned for this experiment, as PD149163 has not

produced catalepsy in previous studies. Catalepsy tests were added for experiment 2, primarily due to the inclusion of haloperidol as a comparator, and catalepsy tests were then conducted in experiment 3, per the revised protocol.

Experiment 2. Acute administration testing with PD149163, buspirone, and haloperidol—Experiment 2 was conducted in order to evaluate a wider dose range of PD149163 (0.01, 0.1, and 1.0 mg/kg) and compare the compound to buspirone (0.05, 0.5, 1.0, and 2.0 mg/kg) and haloperidol (0.03, 0.3, and 0.03 mg/kg).

Acute administration testing occurred every 10 days in the same subjects (N=47, with 8–10 per groups), following the treatment design employed by Molewijk et al. (1995). During the 2 days prior to each subsequent test, the methods were conducted for day 1 and then day 2, as described earlier. Further, attempts were made to prevent rats from consistently receiving the same dose level for each drug (e.g., preventing a particular rat from always receiving the highest dose of a drug). The order of testing was assigned in a counter-balanced and led to a total four tests for each rat.

For the first two drugs tested, PD149163 and buspirone, the order of testing was counterbalanced so that half of the subjects were tested first with PD149163 and the other half of the subjects were tested first with buspirone. On the second test session conducted 10 days later, the drug testing assignment was switched. All doses, plus vehicles, for each drug were represented on each test day. After testing PD149163 and buspirone, a test was then conducted 10 days later in the same animals with the typical antipsychotic drug haloperidol (0.03, 0.3, and 3.0 mg/kg). Finally, another test with buspirone was conducted in order to examine a lower dose (0.05 mg/kg) than was used in the original assessment.

Experiment 3. Repeated administration of PD149163—After assessing the effects of PD149163 on 22 kHz USV counts from experiments 1 and 2, the most effective dose of PD149163 (1.0 mg/kg) was chosen in order to determine if tolerance to these effects may occur after repeated daily administrations. This study was conducted in a separate group of 40 rats. These 40 rats were equally subdivided into four groups, during the following procedures.

First, the acute effects of PD149163 or vehicle on 22 kHz USV counts was assessed, after conducting the same “day 1” and “day 2” procedures that were described earlier. Half of the animals were tested with PD149163 (1.0 mg/kg) and the remaining half was tested with vehicle. These results were statistically similar to those found for PD149163 in experiments 1 and 2. After this acute test, subjects were given a 3 day washout period before the repeated administration procedures began.

Repeated administration consisted of a single injection of either PD149163 (1.0 mg/kg) or vehicle given at the same time each day for 10 consecutive days. In order to remain consistent with the USV methods used in experiment one, a second “day 1” and “day 2” session was conducted prior to the post-treatment “day 3” test session. The “day 1” and “day 2” procedures, in this case, corresponded to day 9 and day 10 of repeated administration, respectively. The daily administration of PD149163 or vehicle on these last two days occurred 3 hours after the procedures on day 9 and 10 had ended, in order to avoid any cognitive or physiological effects that might interfere with the post-repeated-treatment test occurring on the following day.

The results from day 10 were not used, in this case, for determining treatment assignments for the post-repeated-treatment test. Instead, half of the rats that were repeatedly treated with PD149163 were administered PD149163 as a post-repeated-treatment, whereas the

remaining half of the rats were administered vehicle as a post-repeated-treatment. The same balancing was used for the rats repeatedly treated with vehicle.

Data analysis

The dependent variables analyzed were 1) number of 22 kHz USV counts and 2) seconds till first paw movement (catalepsy). Data were expressed as means [+/− standard error of the mean (SEM)]. For experiments 1 and 2, data were analyzed using a one way between groups analysis of variance (ANOVA). For experiment 3, data were analyzed using a two factor between groups ANOVA using “repeated treatment condition” and “post-repeated-treatment” as factors. Tukey post hoc multiple comparisons tests were conducted after all ANOVAs, as appropriate. Data were analyzed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Experiment 1

PD149163 produced a statistically significant inhibition of 22 kHz USVs, $F(3, 36)=18.67$, $p<0.0001$, figure 1. All doses of PD149163 produced significantly fewer 22 kHz USV counts compared to vehicle (VEH).

Experiment 2

Vehicle comparisons—No statistically significant differences were found between the VEH groups for PD149163, buspirone (both tests), or haloperidol for the number of 22 kHz counts or paw movement times (data not shown).

PD149163—Using a lower and wider dose range for PD149163 (0.01, 0.1, and 1.0 mg/kg) than was used in experiment 1, PD149163 produced a statistically significant reduction in the number 22 kHz USVs emitted, $F(3, 31)=9.16$, $p<0.001$, figure 2a). Only the 1.0 mg/kg dose produced a significantly lower number of 22 kHz USVs compared to VEH. No statistically significant effects were found on inclined grid paw movement times (figure 2b).

Buspirone—A statistically significant decrease on the number of 22 kHz USVs during the first assessment of the 5-HT_{1A} partial agonist buspirone (0.5, 1.0, and 2.0 mg/kg) was found, $F(3, 29)=23.73$, $p<0.0001$, figure 3a). All three doses of buspirone produced a significantly lower number of 22 kHz USVs compared to VEH controls. No statistically significant effects were shown on paw movement times (figure3c).

A statistically significant decrease in the number of 22 kHz USVs during the second assessment of buspirone (0.05 and 0.5 mg/kg) was also found $F(2, 18)=6.97$, $p<0.01$, figure 3b). Both doses of buspirone produced a significantly lower number of 22 kHz USVs compared to VEH. No statistically significant effects were shown on paw movement times (figure 3d).

Haloperidol—The typical antipsychotic drug haloperidol (0.03, 0.3, and 3.0 mg/kg) produced a statistically significant decrease in the number of 22 kHz counts, $F(3,27)=4.71$, $p<0.01$, figure 4a). These decreases were found for the 0.3 and 3.0 mg/kg doses compared to VEH. Haloperidol produced a statistically significant increase on paw movement times, $F(3,27)=15.34$, $p<0.0001$, figure 4b,, which occurred at the 0.3 and 3.0 mg/kg doses compared to VEH.

Experiment 3

Statistically significant effects of repeated PD149163 or VEH administration and PD149163 or VEH post-treatment administration were shown in the number of 22 kHz USVs emitted [main effect of repeated treatment, $F(1, 35)=2.71$, $p>0.05$; main effect of post repeated-treatment administration, $F(1, 35)=10.36$, $p<0.01$; interaction effect, $F(1, 35)=5.36$, $p<0.05$; figure 5a]. For the main effect of post-repeated-treatment administration, PD149163 produced a significantly lower number of 22 kHz USV counts compared to VEH. The interaction effect revealed that rats in the repeated-treatment PD149163+post-repeated-treatment PD149163 group exhibited a significantly greater number of 22 kHz USVs compared to rats in the repeated-treatment VEH+post-repeated-treatment PD149163 group. No statistically significant effects on paw movement times were found (figure 5b).

Discussion

The present study is the first to report on the effects of an NTS_1 receptor agonist on conditioned stress-induced 22 kHz USVs, a preclinical model for anxiety. In two separate experiments, acute administration of PD149163 produced a robust reduction in the number of 22 kHz USVs. In these same experiments, PD149163 did not produce catalepsy, an effect exhibited by typical antipsychotic drugs at therapeutic doses. Acute administration of the anxiolytic drug buspirone also engendered a robust suppression of 22 kHz USVs, and like PD149163, did not produce catalepsy. The D_2 receptor-preferring antagonist and typical antipsychotic drug haloperidol also inhibited 22 kHz USVs, but did so at doses that produced significant increases in paw movement latency, an index of catalepsy.

While this is the first report on the effects of PD149163 on conditioned footshock-induced USVs, the potential anxiolytic effects of PD149163 have been demonstrated previously in a fear potentiated startle (FPS) model (Shilling and Feifel, 2008) that used doses identical to those used in experiment 2 of the current study (0.01–1.0 mg/kg). In this previous study, Shilling and Feifel (2008) found that a 1.0 mg/kg dose of PD149163 significantly reduced fear potentiated startle, which was demonstrated in two separate experiments. The second of these two experiments revealed a significant reduction of startle magnitude as well, suggesting that motor inhibition may have provided an alternative explanation for reduced fear-potentiated startle. However, the reduction of 22 kHz USV counts by PD149163 in the current study generally support these fear potentiated startle effects as being due to a reduction in fear or anxiety rather than motor suppression. Moreover, reductions in USVs occurred from administration of a 0.3 mg/kg dose of PD149163, which generally is not found to be a response-suppressing dose in models where aversive stimulation is used (Grimond-Billa et al., 2008; Holly et al., 2011; Shilling and Feifel, 2008). Thus, the findings from the present study and the study conducted by Shilling and Feifel (2008) appear to be due to an attenuated stress response after PD149163 administration rather than a suppression of motor activity.

The 5-HT_{1A} partial agonist buspirone produced a significant inhibition of 22 kHz USVs. These findings are in line with those reported by Molewijk et al. (1995), who reported a dose dependent reduction of 22 kHz USV counts at a 1.0 and 3.0 mg/kg dose, but not at a 0.3 mg/kg dose of buspirone, and by Brodkin et al. (2002) who also reported a dose-dependent reduction in 22 kHz USV counts after adminstration of buspirone (0.3 – 3.0 mg/kg). Buspirone proved more potent in our study, with our first assessment revealing a substantial reduction of 22 kHz counts at a 0.5 mg/kg dose, while the number of 22 kHz USV counts emitted after VEH administration (mean = 378.1 +/−68.4 SEM) were similar to the study by Molewijk et al., (1995) (approximately 325 USV counts for buspirone VEH administration), which employed methods similar to those used in the present study. While the potency differences between buspirone in the present study and buspirone used in other

studies that recorded 22 kHz USVs appears substantial, lower doses of buspirone have proven effective in other anxiety models, including punished responding (effective at 0.1 mg/kg) (Brodkin et al., 2002). A similar inhibition of 22 kHz USV counts has been observed for other 5-HT_{1A} receptor agonists as well, including 8-OH-DPAT, flesinoxan, and BMY7378 (Molewijk et al., 1995).

Haloperidol produced a dose-dependent suppression of 22 kHz USVs in the present study. Haloperidol is not considered a treatment for anxiety disorders, and generally lacks anxiolytic activity in preclinical models (Sun et al., 2010). The reduction in the number of 22 kHz USVs occurring after haloperidol administration in the present study and in a previous study (Molewijk et al., 1995) occured at cateleptic doses, suggesting that this reduction is due to motor effects. Activation of NTS_1 receptors has been shown in previous studies to functionally antagonize dopamine D_2 receptors possibly due to allosteric mechanisms (for review, see Binder et al., 2001 and St. Galais et al., 2006). Yet, the lack of catalepsy elicited by PD149163 here and in previous studies suggests that the decreased number of 22 kHz USVs that occurred after PD149163 administration is not due to functional antagonism of D_2 receptors, but rather, by activation of NTS_1 receptors.

While PD149163 produced a robust decrease in the number of 22 kHz USVs after acute administration, these effects were abolished after 10 consecutive days of administration. This is the first study to assess tolerance to the putative anxiolytic effects of a NTS receptor ligand, although tolerance to the effects of neurotensin receptor ligands in other behavioral models has been demonstrated. For example, five days of repeated administration of the NTS_1 receptor agonist NT69L was shown to reduce [^{3}H]NTS receptor levels, using autoradiography, in the ventral tegmental area, substantia nigra, and hypothalamus (Wang et al., 2005). The ability of acute intracerebroventricular administration of neurotensin to reduce locomotor activity in an open field, which appears to be limbic-system mediated (Kelly et al., 1975), is reduced after repeated administration in rats (Rinkel et al., 1983) and mice (Meisenberg and Simmons, 1985). Similarly, Hertel et al. (2001) reported that the ability of systematically administered NT69L to inhibit spontaneous locomotor activity is significantly weakened after six days of administration. This same study found that repeated NT69L administration produced tolerance to the ability of NT69L to reduce amphetamine-induced locomotor activity increases. In a later study from this group, the ability of NT69L to inhibit conditioned avoidance responding, which is considered a predictive response for antipsychotic efficacy, was lost after seven days of NT69L administration (Hertel et al., 2002). Further, Norman et al. (2008) found that seven days of continuous subcutaneous infusion of PD14963 did not reverse amphetamine-induced increases in locomotor activity. However, Boules et al. (2003) did not observe tolerance to the ability of NT69L, after five days of repeated administration, to reverse apomorphine-, d-amphetamine, or cocaine-induced increases in locomotor activity. Similarly, the NTS receptor agonists KK28, after four days of administration, (Hadden et al., 2005) and PD149163, after eight days of repeated administration (Feifel et al., 2008), also did not to exhibit tolerance when assessed for reversal of amphetamine-induced increases in locomotor activity.

While there remains some reported differences between studies for tolerance to limbic-system mediated behaviors (e.g., psychostimulant-induced hyperactivity), studies have consistently revealed tolerance to the effects of striatal- and hypothalamic-mediated behaviors. Boules et al. (2003) reported that tolerance occurs to NT69L's hypothermic and anti-cataleptic effects after five days of repeated administration. Tolerance also occurred to PD149163's hypothermic effects after ten days of repeated administration (the same duration and dosing regimen of PD149163 used in the present study) (Feifel et al. 2010).

The tolerance studies cited above generally involved dopamine-related behaviors, including hyperactivity, stereotypy, catalepsy, and thermoregulation, whereas anxiolytic effects of drugs on conditioned stress-induced 22 kHz USVs may involve other neurotransmitter systems, including GABA and serotonin. Indeed, neurotensin receptors are located on GABAergic neurons in the central amygdala (Batten et al., 2002), which are important for the anxiolytic effects of benzodiazepines. Neurotensin-containing neurons are also located in the medial raphe nuclei (Jennes et al., 1982) and serotonin neurons in the medial raphe nuclei are activated during stress. Thus, the tolerance affects predominantly characterized for neurotensin ligands may be unrelated to the same mechanisms of action important for reducing conditioned footshock-induced 22 kHz USVs.

Taken together, studies on the effects of PD149163 on the number of conditioned footshock-induced 22 kHz USV counts in the present study and a reduction of fear-potentiated startle in a previous study (Shilling and Feifel, 2008) provide an emerging preclinical profile for the putative anxiolytic effects of NTS_1 receptor agonists. However, the tolerance exhibited to currently available NTS_1 receptor agonists questions the utility of this pharmacologic strategy for the treatment of anxiety. Nonetheless, further studies of this pharmacologic target may reveal information about the role of neurotensin receptors play in anxiety and possibly aid in the development of novel pharmacotherapeutic, and tolerance-resistant, strategies involving neurotensin receptors.

Acknowledgments

This study was funded by an NIMH grant to AJP (1R15MH083241-01). PD149163 was generously provided by the NIMH Drug Repository. We are grateful for the technical assistance of Alexis Bench, Bree-Ona Ebrecht, and Mary Ross. All experiments complied with federal regulations and policies for institutional animal care and use.

Abbreviations

USV	Ultrasonic vocalization
VEH	vehicle

References

Ahlenius S, Hillegaart V. Involvement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: a compareison between the effects produced by pre- and post-symaptic inhibition of dopaminergic neurotransmission. Pharmacol. Biochem. Behav. 1986; 24:1409–1415. [PubMed: 2873588]

Alexander MJ, Leeman SE. Widespread expression in adult rat forebrain of mRNA encoding high-affinity neurotensin receptor. J. Comp. Neurol. 1998; 402:475–500. [PubMed: 9862322]

Batten TFC, Gamboa-Esteves FO, Saha S. Evidence for peptide co-transmission in retrograde- and anterograde-labelled central nucleus of amygdala neurons projecting to NTS. Autonomic Neuroscience. 2002; 98:28–32. [PubMed: 12144035]

Binder EB, Kinkead B, Owens MJ, Nemeroff CB. Neurotensin and dopamine interactions. Pharmacol. Rev. 2001; 53:453–486. [PubMed: 11734615]

Boules M, McMahon B, Wang R, Warrington L, Stewart J, Yerbury S, Fauq A, McCormick D, Richelson E. Selective tolerance to the hypothermic and anticataleptic effects of a neurotensin analog that crosses the blood-brain barrier. Brain Res. 2003; 987:39–48. [PubMed: 14499944]

Boules M, McMahon B, Warrington L, Stewart J, Jackson J, Fauq A, et al. Neurotensin analog selective for hypothermia over antinociception and exhibiting atypical neuroleptic-like properties. Brain research. 2001; 919:1–11. [PubMed: 11689157]

Brodkin J, Busse C, Sukoff SJ, Varney MA. Anxiolytic-like activity of the mGluR5 antagonist MPEP: A comparison with diazepam and buspirone. Pharmacology Biochemistry and Behavior. 2002; 73:359–366.

Brudzynski SM, Chiu EM. Behavioural responses of laboratory rats to playback of 22 kHz ultrasonic calls. Physiol. Behav. 1995; 57:1039–1044. [PubMed: 7652022]

Corley KC, Phan TH, Daugherty WP, Boadle-Biber MC. Stress-induced activation of median raphe serotonergic neurons in rats is potentiated by the neurotensin antagonist, SR 48692. Neurosci. Lett. 2002; 319:1–4. [PubMed: 11814639]

De Vry J, Benz U, Schreiber R, Traber J. Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. European journal of pharmacology. 1993; 249:331–339. [PubMed: 7904565]

Dilts RP, Boadle-Biber MC. Differential activation of the 5-hydroxytryptamine-containing neurons of the midbrain raphe of the rat in response to randomly presented inescapable sound. Neurosci. Lett. 1995; 199:78–80. [PubMed: 8584232]

Dilts RP, Novitzki MR, Phan TH, Corley KC, Boadle-Biber MC. Neurotensin inhibits the activation of midbrain serotonergic neurons produced by random inescapable sound. Brain Res. 1996; 742:294–298. [PubMed: 9117407]

Feifel D, Goldenberg J, Melendez G, Shilling PD. The acute and subchronic effects of a brain-penetrating, neurotensin-1 receptor agonist on feeding, body weight and temperature. Neuropharmacology. 2010; 58:195–198. [PubMed: 19596358]

Feifel D, Melendez G, Murray RJ, Tina Tran DN, Rullan MA, Shilling PD. The reversal of amphetamine-induced locomotor activation by a selective neurotensin-1 receptor agonist does not exhibit tolerance. Psychopharmacol. 2008; 200:197–203.

Fitzpatrick K, Winrow CJ, Gotter AL, Millstein J, Arbuzova J, Brunner J, et al. Altered sleep and affect in the neurotensin receptor 1 knockout mouse. Sleep. 2012; 35:949–956. [PubMed: 22754041]

Grimond-Billa SK, Norman C, G WB, Cassaday HJ. Selectively increased trace conditioning under the neurotensin agonist PD 149163 in an aversive procedure in which SR 142948A was without intrinsic effect. J. Psychopharmacol. 2008; 22:290–299. [PubMed: 18308776]

Hadden MK, Orwig KS, Kokko KP, Mazella J, Dix TA. Design, synthesis, and evaluation of the antipsychotic potential of orally bioavailable neurotensin (8–13) analogues containing non-natural arginine and lysine residues. Neuropharmacology. 2005; 49:1149–1159. [PubMed: 16095636]

Hertel P, Byskov L, Didriksen M, Arnt J. Induction of tolerance to the suppressant effect of the neurotensin analogue NT69L on amphetamine-induced hyperactivity. Eur. J. Pharmacol. 2001; 422:77–81. [PubMed: 11430916]

Hertel P, Olsen CK, Arnt J. Repeated administration of the neurotensin analogue NT69L induces tolerance to its suppressant effect on conditioned avoidance behaviour. Eur. J. Pharmacol. 2002; 439:107–111. [PubMed: 11937099]

Holly EN, Ebrecht BE, Prus AJ. The neurotensin-1 receptor agonist PD149163 inhibits conditioned avoidance responding without producing catalepsy in rats. Eur Neuropsychopharmacol. 2011; 21:526–531. [PubMed: 21277173]

Jennes L, Stumpf WE, Kalivas PW. Neurotensin: topographical distribution in rat brain by immunohistochemistry. J. Comp. Neurol. 1982; 210:211–224. [PubMed: 6754769]

Jolas T, Aghajanian GK. Neurotensin and the serotonergic system. Prog. Neurobiol. 1997; 52:455–468. [PubMed: 9316156]

Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 1975; 94:507–522. [PubMed: 1171714]

Meisenberg G, Simmons WH. Motor hypoactivity induced by neurotensin and related peptides in mice. Pharmacol. Biochem. & Behav. 1985; 22:189–193. [PubMed: 3983212]

Millan MJ, Brocco M, Gobert A, Dorey G, Casara P, Dekeyne A. Anxiolytic properties of the selective, non-peptidergic CRF(1) antagonists, CP154,526 and DMP695: a comparison to other classes of anxiolytic agent. Neuropsychopharmacol. 2001; 25:585–600.

Molewijk HE, van der Poel AM, Mos J, van der Heyden JA, Olivier B. Conditioned ultrasonic distress vocalizations in adult male rats as a behavioural paradigm for screening anti-panic drugs. Psychopharmacol. 1995; 117:32–40.

National Research Council. Guide for laboratory animal facilities and care. 8th ed.. Washington, D.C.: National Research Council; 2011.

Norman C, Beckett SR, Spicer CH, Ashton D, Langlois X, Bennett GW. Effects of chronic infusion of neurotensin and a neurotensin NT1 selective analogue PD149163 on amphetamine-induced hyperlocomotion. J. Psychopharmacol. 2008; 22:300–307. [PubMed: 18208905]

Petrie KA, Bubser M, Casey CD, Davis MD, Roth BL, Deutch AY. The neurotensin agonist PD149163 increases Fos expression in the prefrontal cortex of the rat. Neuropsychopharmacol. 2004; 29:1878–1888.

Remy SM, Schreiber R, Dalmus M, De Vry J. Somatodendritic 5-HT1A receptors are critically involved in the anxiolytic effects of 8-OH-DPAT. Psychopharmacol. 1996; 125:89–91.

Rinkel GJE, Hoeke EC, van Wimersma, Greidanus TB. Elective tolerance to behavioral effects of neurotensin. Physiology & Behavior. 1983; 31:467–470. [PubMed: 6657768]

Sanchez C, Bergqvist PB, Brennum LT, Gupta S, Hogg S, Larsen A, Wiborg O. Escitalopram, the S-(+)-enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. Psychopharmacol. 2003; 167:353–362.

Sanchez C, Meier E. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? Psychopharmacol. 1997; 129:197–205.

Schotte A, Janssen PF, Gommeren W, Luyten WH, Van Gompel P, Lesage AS, et al. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology. 1996; 124:57–73. [PubMed: 8935801]

Shilling PD, Feifel D. The neurotensin-1 receptor agonist PD149163 blocks fear-potentiated startle. Pharmacol. Biochem. Behav. 2008; 90:748–752. [PubMed: 18577396]

Shugalev NP, Stavrovskaia AV, Iamshchikova NG. [Effect of neurotensin on the behavior of rats with damaged serotoninergic neurons under immobilization stress]. Zh Vyssh Nerv Deiat Im I P Pavlova. 2005; 55:671–676. [PubMed: 16316028]

Sun T, He W, Hu G, Li M. Anxiolytic-like property of risperidone and olanzapine as examined in multiple measures of fear in rats. Pharmacol. Biochem. Behav. 2010; 95:298–307. [PubMed: 20167232]

Tornatzky W, Miczek KA. Behavioral and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol. Psychopharmacol. 1994; 116:346–356.

Tonoue T, Ashida Y, Makino H, Hata H. Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: A psychotropic effect. Psychoneuroendocrinology. 1986; 11:177–184. [PubMed: 2428074]

Tonoue T, Iwasawa H, Naito H. Diazepam and endorphin independently inhibit ultrasonic distress calls in rats. Eur. J. Pharmacol. 1987; 142:133–136. [PubMed: 3121362]

Wang R, Boules M, Gollatz E, Williams K, Tiner W, Richelson E. Effects of 5 daily injections of the neurotensin-mimetic NT69L on the expression of neurotensin receptors in rat brain. Mol. Brain Res. 2005; 138:24–34. [PubMed: 15878217]

Highlights

- The neurotensin NTS_1 receptor agonist PD149163 significantly reduced stress-induced 22-kHZ vocalizations in rats
- The typical antipsychotic drug and dopamine D_2 receptor-preferring antagonist haloperidol significantly reduced stress-induced 22-kHz, but this was likely due to cataleptic effects
- The serotonin $5\text{-}HT_{1A}$ receptor partial agonist buspirone significantly reduced stress-induced 22-kHz vocalizations in rats
- Tolerance occurred to the inhibitory effects of PD149163 (10 days repeated administration) on 22-kHz vocalizations

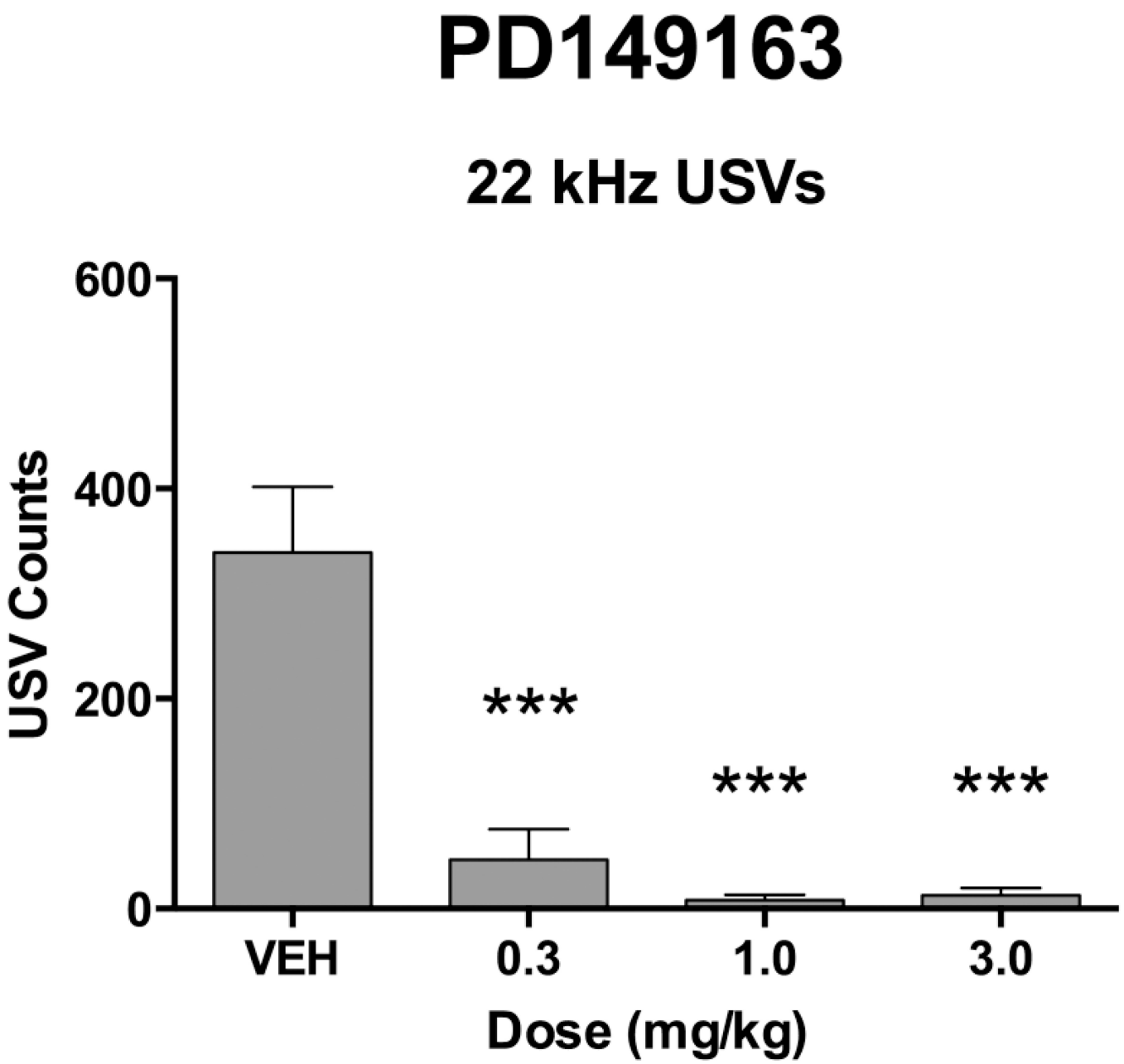

Figure 1.
Effects of the neurotensin NT_1 receptor agonist PD149163 on the mean (+/− SEM) number of 22 kHz ultrasonic vocalization (USV) counts (n= 10 per dose). ***p<0.001 versus vehicle (VEH)

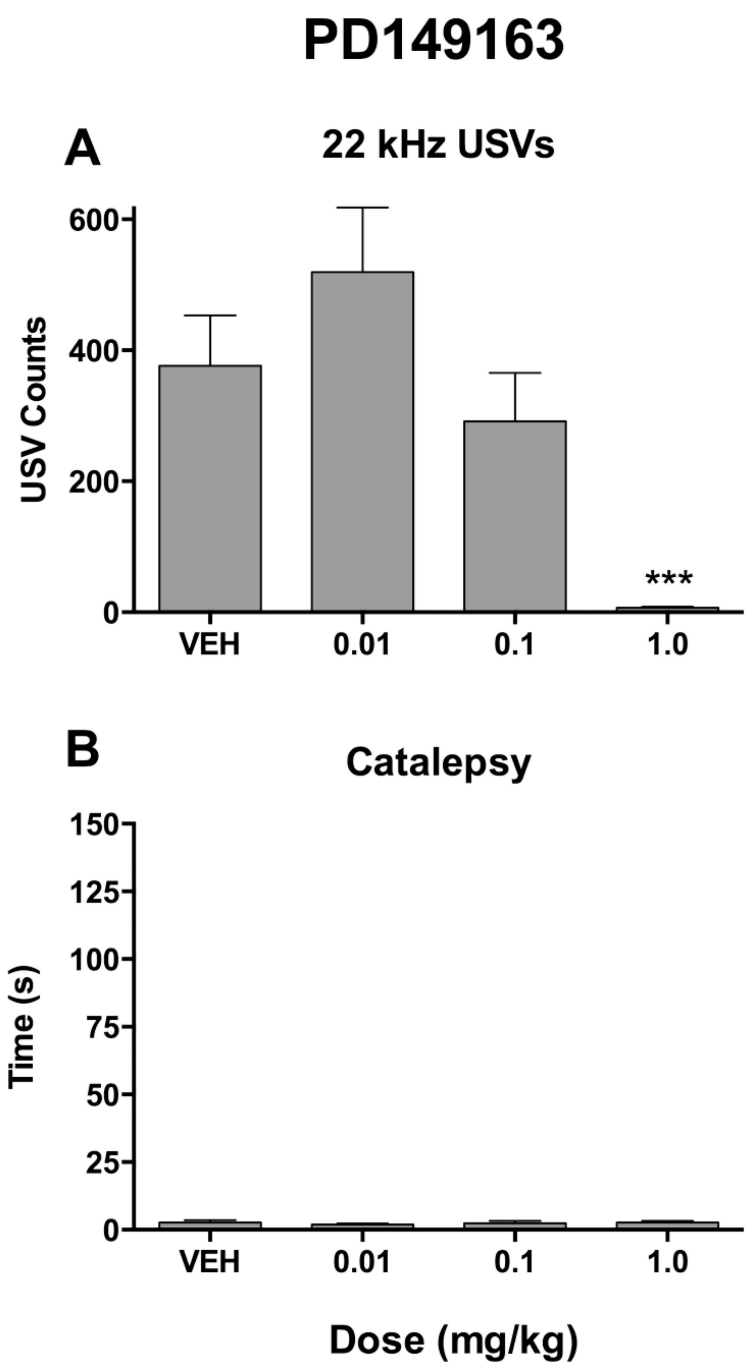

Figure 2.
Effects of PD194163 on the mean (+/− SEM) A) number of 22 kHz USV counts and B) time till first paw movement on the inclined grid (catalepsy (n=8–9 per dose). ***p<0.001 versus VEH

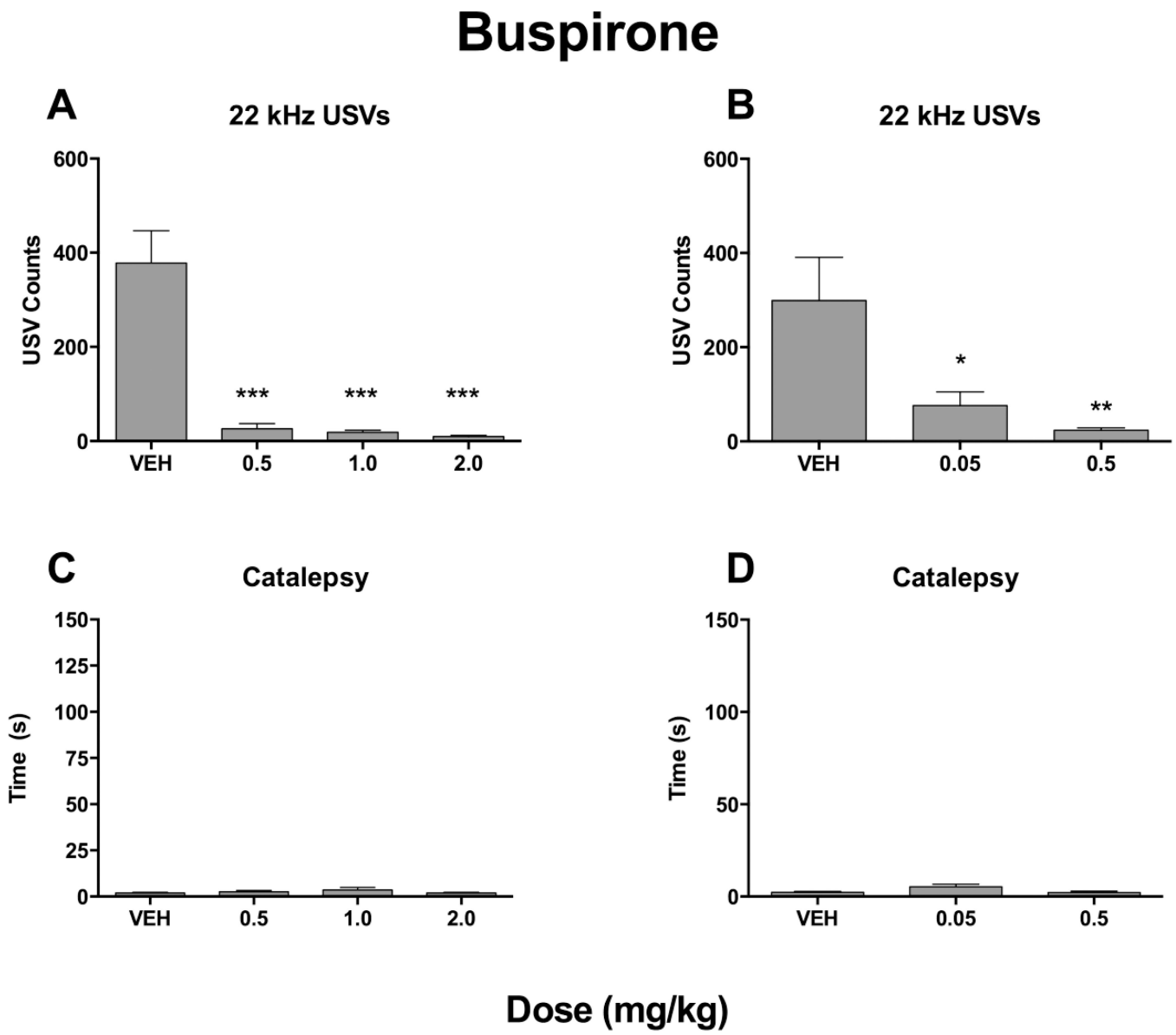

Figure 3.

Effects of the 5-HT_{1A} receptor partial agonist buspirone on the mean (+/− SEM) number of 22 kHz USV counts (panels A and C) and time till first movement on the inclined grid (panels B and D) (n=8–9 per dose). *p<0.05, **p<0.01, and ***p<0.001 versus VEH

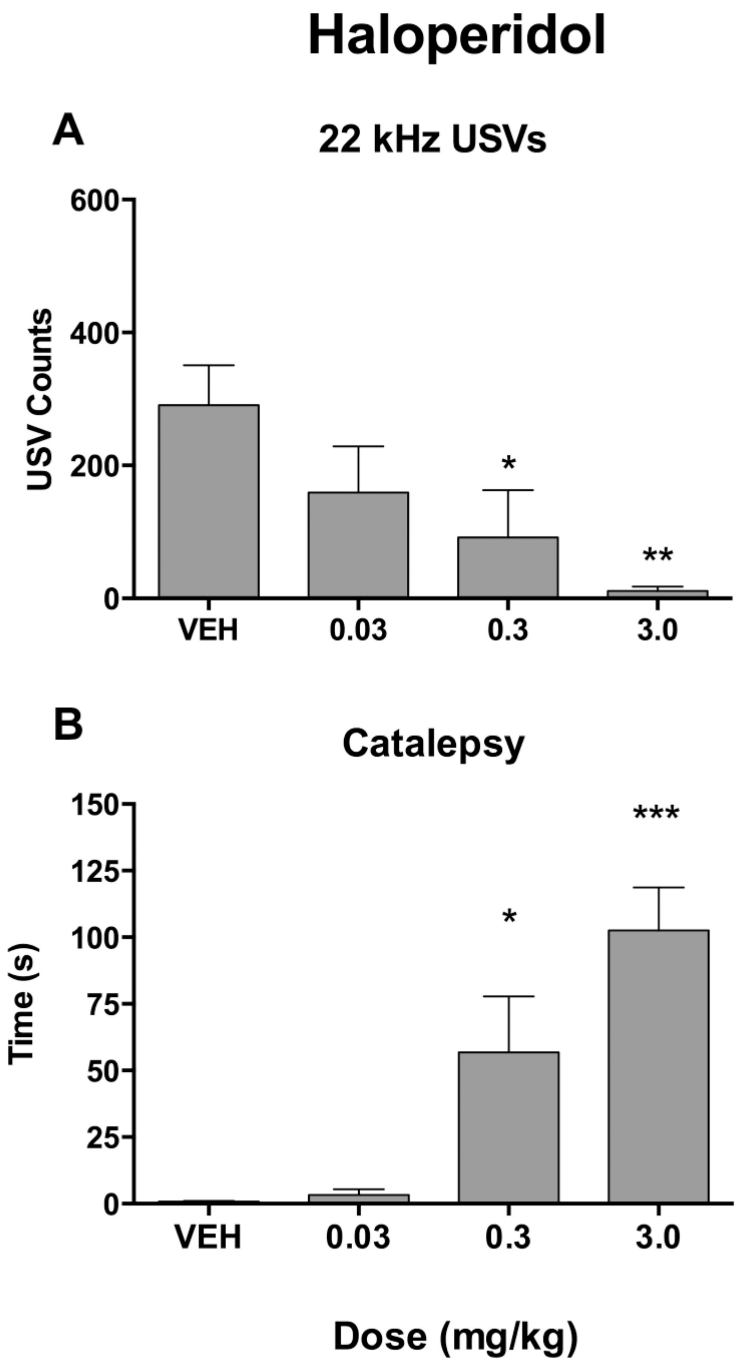

Figure 4.

Effects of the antipsychotic drug haloperidol on the mean (+/− SEM) A) number of 22 kHz USV counts and B) time till first movement on the inclined grid (n=7–9 per dose). *p<0.05, **p<0.01, and ***p<0.001 versus VEH

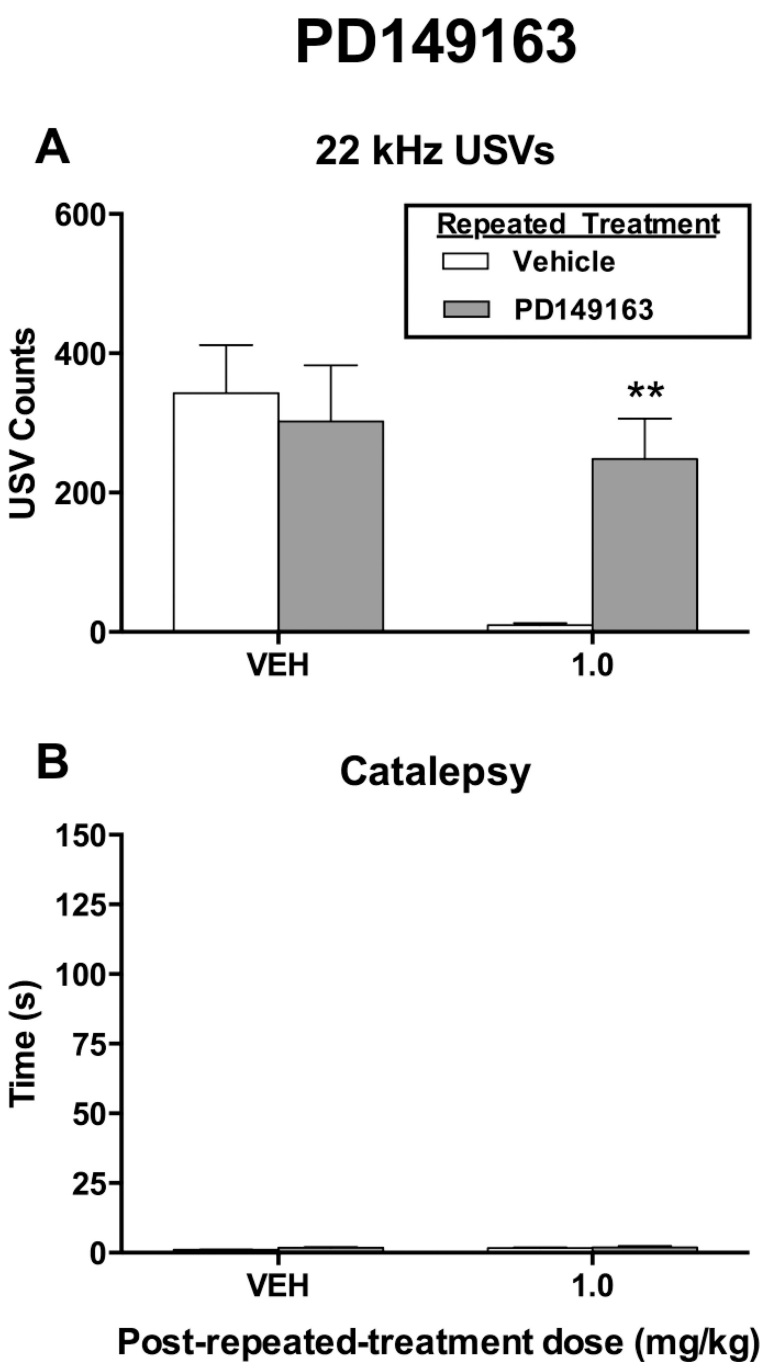

Figure 5.

Effects of repeated and post-repeated-treatment administration of PD149163 (1.0 mg/kg) or vehicle (VEH) on A) the mean (+/− SEM) number of 22 kHz USV counts and B) time till first movement on the inclined grid. The empty bars refer to groups repeatedly administered with vehicle and the filled bars refer to groups repeatedly administered with PD149163. The post-repeated-treatment administration groups are shown on the abscissa (n=10 per group). **p<0.01 for rats given repeated administration with PD149163 and then given PD149163 for the post-repeated treatment test PD149163 versus rats given repeated administration with vehicle and then given PD149163 for the post-repeated treatment test.