3-2017

Neurotensin NTS1 and NTS2 receptor agonists produce anxiolytic-like effects in the 22-kHz ultrasonic vocalization model in rats

Floyd F. Steele III
Virginia Commonwealth University, fsteele@vcu.edu

Shannon C. Whitehouse
Northern Michigan University, swhiteho@nmu.edu

Jacob S. Aday
Northern Michigan University, jaday@nmu.edu

Adam J. Prus
Northern Michigan University, aprus@nmu.edu

RUNNING HEAD: Neurotensin reduces 22-kHz USVs

Neurotensin NTS_1 and NTS_2 receptor agonists produce anxiolytic-like effects in the 22-kHz ultrasonic vocalization model in rats

Floyd F. Steele III[1], Shannon C. Whitehouse[2], Jacob S. Aday[2], and Adam J. Prus[2]*

1-Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA

2-Psychology Department, Northern Michigan University, Marquette, MI

*Corresponding author

Adam J. Prus, Ph.D.
Psychology Department
Northern Michigan University
Marquette, MI 49855
p. 906-227-2941
f. 906-227-2954
e. aprus@nmu.edu

Abbreviations: ultrasonic vocalizations (USV); basolateral amygdala (BLA); central nucleus of the amygdala (CeA)

ABSTRACT

Neurotensin is a neuropeptide neurotransmitter that interacts with multiple neurotransmitter systems, including those regulating amygdalar function, via NTS_1 and NTS_2 receptors. Both receptors are expressed in the amygdala and agonists for NTS_1 or NTS_2 receptors have exhibited anxiolytic effects in animal models. Systemic adminstration of NTS_1 receptor agonist PD149163 was recently shown to reduce footshock conditioned 22-kHz ultrasonic vocalizations in rats, suggesting that PD149163 produced an anxiolytic effect. The effects that neurotensin may have or a selective NTS_2 receptor agonist may have on 22-kHz vocalizations has yet to be examined. The current study evaluated the effects of intracerebroventricularly administered neurotensin (0.1 - 10.0μg), PD149163 (0.1-10.0 ng), or the NTS_2 receptor agonist JMV-431 (0.1 - 1.0 μg) on footshock conditioned 22-kHz vocalizations in male Wistar rats. Neurotensin, PD149163, and JMV-431 all significantly reduced the number 22-kHz calls. No changes in call duration were found, suggesting that non-specific drug effects do not account for the reductions in 22-kHz calls. These data support anxiolytic effects produced by activation of NTS_1 or NTS_2 receptors, and suggest that neurotensin plays a natural role in the expression of conditioned USVs. These data suggest that both receptor subtypes are putative pharmacologic targets.

Keywords: anxiety; ultrasonic vocalization; neurotensin; NTS_1 receptor; NTS_2 receptor

1. Introduction

Neurotensin is a neuropeptide neurotransmitter distributed throughout much of the CNS and that interacts with a number of neurotransmitter systems through NTS_1 or NTS_2 receptors (Kleczkowska and Lipkowski, 2013; Vincent et al., 1999). Soon after its discovery by Carraway and Leeman (Carraway and Leeman, 1973), studies found that intracranial administration of neurotensin produced some physiological and behavioral effects similar to those produced by antipsychotic drugs (Jolicoeur et al., 1993; Nemeroff, 1980). These findings formed the basis for developing a number of synthetic neurotensin analogs capable of crossing the blood-brain barrier, such as NT69L (Tyler et al., 1999) and PD149163 (Wustrow et al., 1995).

There is an emerging line of research examining the role of neurotensin in anxiety and the potential of neurotensin receptor agonists to treat anxiety disorders. In post traumatic stress disorder, Maes et al. (1999) reported high levels of prolyl endopeptidase, a degradation enzyme of neurotensin, in blood plasma, suggesting lower levels of neurotensin in these patients (Maes et al., 1999). Corresponding with these findings, Ruiz et al. (1992) found lower levels of neurotensin in blood plasma of those with either an anxiety disorder or depression, which were raised upon recovery. Both NTS_1 and NTS_2 receptors are also found in the amygdala, including basolateral amygdala and central nucleus (Boudin et al., 1996; Lantos et al., 1996; Sarret et al., 2003).

An early suggestion that neurotensin might exhibit anxiolytic-like effects was demonstrated by Elliot et al. (1986) who found that intracranial neurotensin administration led rats to spend more time in the center versus the perimeter of an open field. Shilling & Feifel (2008) found that the highly selective NTS_1 receptor agonist PD149163 (Petrie et al., 2004;

Table 1) attenuated fear-potentiated startle in rats. The peptide ß-lactotensin, which has a preferential affinity as an agonist for NTS_2 receptors over NTS_1 receptors (Yamauchi et al., 2003), has also been shown to weaken fear conditioning and attenuate the effects of restraint stress measured during a hole-board test (Yamauchi et al., 2006), increase the time spent in open elevated plus maze arms, and time spent in the center of an open field (Hou et al., 2011).

A model becoming increasingly used to screen anxiolytic drugs is the footshock conditioned 22-kHz ultrasonic vocalization (USV) model in rats (Molewijk et al., 1995; Sánchez, 2003; Simola, 2015). Calls at this frequency are produced when rats are confronted with natural predators (Brudzynski, 2015) and increase as the distance to a predator decreases (Litvin, Blanchard, & Blanchard, 2007). Audio playback of rat 22-kHz calls to non-conditioned rats produces increased c-fos activity in brain regions associated with fear conditioning, such as the amygdala and periaqueductal grey area. These calls also correlate with defensive behaviors such as conditioned freezing and staying in the closed arms of an elevated plus maze (Borta et al., 2006). In a previous study from this laboratory, Prus et al. (2014) demonstrated that PD149163 significantly reduced footshock conditioned 22-kHz USVs in rats, which was a result typical of anxiolytic drugs such as the $5\text{-}HT_{1A}$ partial agonist buspirone (Molewijk et al., 1995; Prus et al., 2014) and some benzodiazepines (Molewijk et al., 1995). Chou et al. (2015) measured 22-kHz USVs in rats performing a conditioned avoidance response task, and in these animals, PD149163 also reduced 22-kHz USVs.

The aim of the current study was to add to past findings on the effects of neurotensin ligands on anxiety by testing the CNS effects of PD149163, neurotensin and the NTS_2 receptor agonist Boc-[Ψ11,12]NT(8-13) (JMV-431) (Dubuc et al., 1999; Richard et al., 2001; Table 1) in the conditioned foot-shock induced 22-kHz USV model. Past findings have shown PD149163 to

reduce USVs in this model, but an assessment of neurotensin has not been evaluated. Studies have yet to be conducted using a selective NTS_2 receptor agonist in this model. In order to closely compare the effects of these selective ligands with neurotensin, which does not cross the blood-brain barrier, all compounds were microinjected into the lateral ventricle.

2. Results

Animals that did not emit at least 80 vocalizations during a test after conditioning were eliminated prior to surgery and data were removed for rats with incorrect cannula placements. Based on this, data were analyzed from 19 rats [PD149169 (N=7), JMV-431 (N=7) and NT (N=5)]. There was not a statistically significant difference ($p > 0.05$) between the first and second vehicle assessments on the number of USV calls for either neurotensin [$t(4)=0.95$, $p > 0.05$], PD149163 [$t(6)=0.12$, $p > 0.05$], or JMV-431[$t(6)=0.02$, $p > 0.05$]; moreover, the vehicle data for neurotensin, PD149163 or JMV-431 did not differ significantly between these groups, $F(2, 16) = 1.13$, $p > 0.05$. Neurotensin (0.1, 1.0, and 10.0μg) significantly reduced the number of USV calls, $F(3,12) = 6.69$, $p < 0.05$, and post hoc testing found significantly fewer calls for only 10 μg of neurotensin compared to vehicle (figure 1). PD149163 (0.1, 1.0, & 10.0 ng) also significantly reduced USV calls, $F(3,18) = 17.33$, $p < 0.001$, which occurred as a reduction in calls following administration of 10 ng of PD149163 compared to vehicle (figure 2, top panel). JMV-431 (0.1 and 1.0μg) significantly reduced the number of calls $F(2,12) = 19.08$, $p < 0.001$, following administration of 1.0μg compared to vehicle (figure 2, bottom panel).

There was not a significant difference between the USV call durations of the first and second vehicle tests for any of the drugs evaluated (data not shown), nor did USV call durations for vehicle differ between the treatment groups. None of the compounds tested produced statistical differences in USV call duration (all p values > 0.05, see Table 2).

3. Discussion

In the present study, intracerebroventricular administration of the neuropeptide neurotensin, the selective NTS_1 receptor agonist PD149163, and the selective NTS_2 receptor agonist JMV-431 produced a significant reduction in footshock conditioned 22-kHz USV calls in rats, an effect considered predictive of anxiolytic efficacy (e.g., Molewijk et al., 1995; Simola, 2015). These reductions were shown for the "long" type of 22-kHz USVs (approx. 1s), which are the length of calls emitted from rats presented with threatening stimuli (Simola, 2015). As addressed in the introduction, several past studies suggest an anxiolytic profile for neurotensin and a role for anxiolytic effects via activation of NTS_1 or NTS_2 receptors. In particular, a past study in this laboratory found that systemic administration of PD149163 significantly reduced 22-kHz USVs using procedures similar to those in the present study (Prus et al., 2014). In the present study, PD149163 was 1,000 fold more potent than neurotensin (0.01 vs 10.0 ug, respectively) and 100 fold more potent than JMV-431 (1.0 ug) in reducing the number of 22-kHz calls emitted. Graded dose response curves were not shown for these compounds, likely due to using 10-fold differences between each dose.

Brain neurotensin systems may have specific relationships with mechanisms mediating conditioned 22-kHz USV production, but it seems more likely that neurotensin and neurotensin receptor agonists reduce 22-kHz USVs by acting to reduce anxiety. Numerous pharmacological studies link cholinergic activity and cholinergic muscarinic receptor activation to the production of 22-kHz USVs (for review see Brudzynski, 2015); neurotensin's interactions with cholinergic systems (Szigethy et al., 1988) enhances the activity of cholinergic neurons (Alonso et al., 1994; Gully et al., 1997) leading to acetylcholine efflux (e.g., Gully et al., 1997; Kitabgi and Freychet, 1979; Lapchak et al., 1991; Prus et al., 2007; Rakovska et al., 1998). Thus, from these actions

alone, neurotensin might be predicted to enhance 22-kHZ USVs, which is the opposite of what is found.

Likely, the ability of neurotensin and its agonists to reduce the emission of 22-kHz USVs occurs through processing and conditioning of fear responses, as has been demonstrated by neurotensin, NTS_1 receptor agonists and NTS_2 receptor agonists in past studies noted earlier. Dense neurotensin-containing fibers terminate in the central nucleus of the amygdala (CeA) (Jennes et al., 1982), and the basolateral amygdala (BLA) and CeA contain high levels of NTS_1 and NTS_2 receptors (Boudin et al., 1996; Lantos et al., 1996; Sarret et al., 2003). Neurotensin receptors may alter glutamatergic neurotransmission in the amygdala, which may alter fear conditioning processes. Neurotensin has been shown to facilitate NMDA-elicited increases in glutamate (Ferraro et al., 2011) and NTS_1 receptor activation may disinhibit BLA pyramidal neurons, thereby promoting long-term potentiation (Kroner et al., 2005). Beyond glutamate interactions, there is evidence that neurotensin receptors interact with neurotransmitters with terminals in the amygdala, including dopamine (Asan, 1998) and serotonin (Boudin et al., 1996; Jolas and Aghajanian, 1996; Sarret et al., 2003).

The present study provided further evidence that neurotensin receptor mechanisms appear involved in fear conditioning processes and that agonists for neurotensin receptors may be pursued as novel anxiolytic drugs. Neurotensin, like the NTS_1 receptor agonist PD148163 in a previous study (Prus et al., 2014), exhibited an attenuation on footshock-conditioned USVs, suggesting that neurotensin plays a natural role in reducing anxiety. The findings with JMV-431 also support anxiolytic effects ocurring through NTS_2 receptor activation. These receptors, in particular, are worth pursuing as a novel pharmacologic strategy for treating anxiety as NTS_1, but not NTS_2, receptors have been associated with malignant tumor growth in human cancer cells

(Dal Farra et al., 2001). JMV-431 does not readily cross the blood-brain barrier (unpublished findings), and therefore, there is a need for highly selective and brain penetrant NTS_2 receptor agonists in order to assess the clinical utility of such compounds in animal models.

4. Experimental Procedure

4.1 Subjects

Thirty male Wistar rats (Charles River Laboratories, Portage, MI) were used in this study and housed in a temperature and humidity controlled vivarium set on a 12 hr light/dark cycle. Rats were 10-12 weeks old when experiments began. All procedures were approved by the Institutional Animal Care and Use Committee at Northern Michigan University and followed the Guide for the Care and Use of Laboratory Animals (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals et al., 2011).

4.2 Pharmacological compounds

The test compounds consisted of neurotensin (0.1, 1.0, & 10.0µg doses, AnaSpec, Fremont, CA, USA), PD149163 HCl (0.1, 1.0, & 10ng doses) and JMV-431 (0.1 & 1.0µg) (both generously provided by the NIMH Drug Repository, Bethesda, MD, USA). All compounds were dissolved in a phosphate buffering solution (Sigma-Aldrich, St. Louis, MO) with 1.2 mM CaCl added. The salt form of PD149163 was used.

An Equithesin solution was used for as an injectable (i.p.) surgical anesthesia for the majority of the animals; later in the study, during experiments to test JMV-431, an isoflurane vaporizer became available for gas surgical anesthesia and was used instead. An injection (s.c.) of Penicillin G (Butler Schein Animal Health, Dublin, OH, USA) was given after an animal was sedated and prior to beginning surgery. Buprenorphine (0.05 mg/kg, s.c.; Sigma Aldrich) was

given twice a day as an analgesic for two days following surgery. Animals were given one week to recover before testing began.

4.3 Apparatus and materials

Each test compound for this study was injected into the right lateral ventricle via a 26 ga. guide cannulae (C315G-SPC; PlasticsOne, Roanoke, VA, USA) that was surgically installed using a rodent stereotaxic device (Stoelting Co., Wood Dale, IL, USA). A matching 33 ga. dummy cannula (C315DC-SPC, PlasticsOne) was implanted into the guide immediately following surgery and the dummy cannula was replaced by a 33 ga. injection cannula (C315I-SPC, PlasticsOne) for drug injection prior to a test session. A syringe pump (MD-1001, Bioanalytical Systems, Inc., Lafayette, IN, USA) injected a drug solution through an injection cannula at a rate of 2μL per minute.

Conditioning and testing sessions were conducted using an ultrasonic vocalization chamber equipped with a shock grid floor and USV detector (Med Associates, St. Albans, VT, USA). Two key lights positioned at the front of the chamber were used for illumination during experimental sessions. All experimental events and data recording were conducted using MED PC version 4 software (Med Associates).

4.4 Surgical and behavioral procedures

Prior to the cannula implantation surgery, rats were first footshock conditioned to the USV chamber and then pre-tested to determine if conditioning produced 22-kHz USVs. The pre-training/testing procedures were modified from those described previously by this laboratory (Prus et al., 2014). Conditioning was conducted during two consecutive daily sessions. During these sessions, each rat was exposed to 6 floor grid shocks (0.8 mA, 8s duration) pseudo-randomly distributed during a 7 min session. Inter-shock intervals of 27, 40, 50, 75, and 100

seconds were randomly selected after a shock was presented 60 seconds after the beginning of the training session. A pre-test session was conducted on the following day.

The pre-test session consisted of two trials. The first trial was 2 min long and one shock (0.8 mA, 8 s duration) was given approximately halfway through the trial. The rat was removed from the chamber at the end of this trial and then returned to the chamber 30 min later for the second trial. The second trial was 10 min long. During this trial 22-kHz USVs were recorded and no shocks were administered. Rats that failed to emit at least 80 USVs were excluded from the study prior to surgery. This training requirement was used in Molewijk et al. (1995).

A single guide cannula was installed in the right lateral ventricle at anterior/posterior = -1.0 mm, medial/lateral = +2.0 mm relative to bregma and at a depth of 2 mm from the skull surface according to stereotaxic coordinates (Paxinos and Watson, 2005) and fixed using cranioplastic cement. The injection cannula, when implanted prior to a test session, extended 2 mm beyond the tip of the guide cannula to reach the lateral ventricle. Rats were given one week to recover from surgery before testing sessions were conducted.

The testing procedure was conducted over the course of consecutive daily sessions. The first session consisted of a conditioned session as described above. The remaining sessions were test sessions as described earlier, except that the dummy cannula was replaced with an injection cannula immediately after the 2 min trial. After implanting the injection cannula, 2µL of drug or vehicle solution (i.e., the phosphate buffering solution) was perfused through the cannula for one minute. The syringe pump was then deactivated and remained connected to the injection cannula for an additional minute before disconnecting it. The dummy cannula was replaced after the injection cannula was removed. The 10 min test trial was conducted 30 min after the injection. For each test compound, the phosphate buffering solution vehicle was injected on the first test

day and drug doses were then escalated by 10 fold for each subsequent daily test session. The final test day for each compound consisted of a second administration of phosphate buffering solution to assess if changes in the number of USVs occurred to baseline responding after drug testing. Daily doses were increased instead of counterbalanced due to evidence of PD149163 producing behavioral tolerance in the USV preparation (Prus, Hillhouse, & LaCrosse, 2014). Following testing, the probe placements were verified using histology and data were excluded for incorrect placements.

Data Analysis

The number and duration (s) of 22-kHz calls were reported as means (+/- standard error of the mean [SEM]). As noted earlier, a vehicle test was conducted before drug testing and after drug testing; a paired samples t test was subsequently conducted to determine if these before and after vehicle data differed significantly. Means for each animal of the before and after tests were calculated and used as the vehicle condition for subsequent analyses. A one-way repeated measures analysis of variance was used to assess differences across doses and vehicle for each compound. Dunnett's post hoc tests were used to compare doses to vehicle, when appropriate, at the $p < 0.05$ level. Statistical analyses were conducted using Graph Pad Prism 6 software (GraphPad Software, Inc., La Jolla, CA).

References

Alonso A, Faure MP, Beaudet A. Neurotensin promotes oscillatory bursting behavior and is internalized in basal forebrain cholinergic neurons. J Neurosci. 1994;14:5778-92.

Asan E. The catecholaminergic innervation of the rat amygdala. Adv Anat Embryol Cell Biol. 1998;142:1-118.

Borta A, Wohr M, Schwarting RK. Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxiety-related behavior. Behavioural brain research. 2006;166:271-80.

Boudin H, Pelaprat D, Rostene W, Beaudet A. Cellular distribution of neurotensin receptors in rat brain: immunohistochemical study using an antipeptide antibody against the cloned high affinity receptor. J Comp Neurol. 1996;373:76-89.

Brudzynski SM. Pharmacology of ultrasonic vocalizations in adult rats: significance, call classification and neural substrate. Curr Neuropharmacol. 2015;13:1801-192.

Carraway R, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. Journal of Biological Chemistry. 1973;248:6854-61.

Dal Farra C, Sarret P, Navarro V, Botto JM, Mazella J, Vincent JP. Involvement of the neurotensin receptor subtype NTR3 in the growth effect of neurotensin on cancer cell lines. Int J Cancer. 2001;92:503-9.

Dubuc I, Sarret P, Labbe-Jullie C, Botto JM, Honore E, Bourdel E, et al. Identification of the receptor subtype involved in the analgesic effect of neurotensin. J Neurosci. 1999;19:503-10.

Elliott PJ, Chan J, Parker YM, Nemeroff CB. Behavioral effects of neurotensin in the open field: structure-activity studies. Brain research. 1986;381:259-65.

Ferraro L, Beggiato S, Tomasini MC, Fuxe K, Tanganelli S, Antonelli T. Neurotensin regulates cortical glutamate transmission by modulating N-methyl-D-aspartate receptor functional activity: An in vivo microdialysis study. Journal of Neuroscience Research. 2011;89:1618-26.

Gully D, Labeeuw B, Boigegrain R, Oury-Donat F, Bachy A, Poncelet M, et al. Biochemical and pharmacological activities of SR 142948A, a new potent neurotensin receptor antagonist. The Journal of pharmacology and experimental therapeutics. 1997;280:802-12.

Hou I, Suzuki C, Kanegawa N, Oda A, Yamada A, Yoshikawa M, et al. β-Lactotensin derived from bovine β-lactoglobulin exhibits anxiolytic-like activity as an agonist for neurotensin NTS_2 receptor via activation of dopamine D1 receptor in mice. Journal of neurochemistry. 2011;119:785-90.

Jennes L, Stumpf WE, Kalivas PW. Neurotensin: topographical distribution in rat brain by immunohistochemistry. J Comp Neurol. 1982;210:211-24.

Jolas T, Aghajanian GK. Neurotensin excitation of serotonergic neurons in the dorsal raphe nucleus of the rat in vitro. The European journal of neuroscience. 1996;8:153-61.

Jolicoeur FB, Gagne MA, Rivest R, Drumheller A, St-Pierre S. Atypical neuroleptic-like behavioral effects of neurotensin. Brain research bulletin. 1993;32:487-91.

Kitabgi P, Freychet P. Neurotensin contracts the guinea-pig longitudinal ileal smooth muscle by inducing acetylcholine release. European journal of pharmacology. 1979;56:403-6.

Kleczkowska P, Lipkowski AW. Neurotensin and neurotensin receptors: characteristic, structure-activity relationship and pain modulation--a review. European journal of pharmacology. 2013;716:54-60.

Kroner S, Rosenkranz JA, Grace AA, Barrionuevo G. Dopamine modulates excitability of basolateral amygdala neurons in vitro. J Neurophysiol. 2005;93:1598-610.

Lantos TÁ, Palkovits M, Rostène W, Bérod A. Neurotensin receptors in the human amygdaloid complex. Topographical and quantitative autoradiographic study. Journal of Chemical Neuroanatomy. 1996;11:209-17.

Lapchak PA, Araujo DM, Quirion R, Beaudet A. Neurotensin regulation of endogenous acetylcholine release from rat striatal slices is independent of dopaminergic tone. Journal of neurochemistry. 1991;56:651-7.

Maes M, Lin A-H, Bonaccorso S, Goossens F, Van Gastel A, Pioli R, et al. Higher serum prolyl endopeptidase activity in patients with post-traumatic stress disorder. Journal of affective disorders. 1999;53:27-34.

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. Psychopharmacology. 1995;117:32-40.

National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, National Academies Press. Guide for the care and use of laboratory animals. 8th ed. Washington, D.C.: National Academies Press,; 2011. p. xxv, 220 p.

Nemeroff CB. Neurotensin: perchance an endogenous neuroleptic? Biological psychiatry. 1980;15:283-302.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 5th ed. Amsterdam ; Boston: Elsevier Academic Press; 2005.

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. Neuropsychopharmacology. 2004;29:1878-88.

Prus AJ, Hillhouse TM, LaCrosse AL. Acute, but not repeated, administration of the neurotensin NTS1 receptor agonist PD149163 decreases conditioned footshock-induced

ultrasonic vocalizations in rats. Progress in neuro-psychopharmacology & biological psychiatry. 2014;49:78-84.

Prus AJ, Huang M, Li Z, Dai J, Meltzer HY. The neurotensin analog NT69L enhances medial prefrontal cortical dopamine and acetylcholine efflux: Potentiation of risperidone-, but not haloperidol-, induced dopamine efflux. Brain research. 2007;1184:354-64.

Rakovska A, Giovannini MG, Della Corte L, Kalfin R, Bianchi L, Pepeu G. Neurotensin modulation of acetylcholine and GABA release from the rat hippocampus: an in vivo microdialysis study. Neurochemistry international. 1998;33:335-40.

Richard F, Barroso S, Martinez J, Labbe-Jullie C, Kitabgi P. Agonism, inverse agonism, and neutral antagonism at the constitutively active human neurotensin receptor 2. Mol Pharmacol. 2001;60:1392-8.

Ruiz JS, Perera JLC, Hernanz A. Neuropéptidos plasmáticos en los trastornos afectivos y de ansiedad. Archivos de Neurobiologica. 1992;55:1-5.

Sarret P, Perron A, Stroh T, Beaudet A. Immunohistochemical distribution of NTS2 neurotensin receptors in the rat central nervous system. J Comp Neurol. 2003;461:520-38.

Sánchez, C. Stress-induced vocalisation in adult animals. A valid model of anxiety? Eur J Pharmacol. 2003;463:133-143.

Simola N. Rat ultrasonic vocalizations and behavioral neuropharmacology: from the screening of drugs to the study of disease. Curr Neuropharmacol. 2015;13:164-79.

Szigethy E, Wenk GL, Beaudet A. Anatomical substrate for neurotensin-acetylcholine interactions in the rat basal forebrain. Peptides. 1988;9:1227-34.

Tyler BM, Douglas CL, Fauq A, Pang YP, Stewart JA, Cusack B, et al. In vitro binding and CNS effects of novel neurotensin agonists that cross the blood-brain barrier. Neuropharmacology. 1999;38:1027-34.

Vincent JP, Mazella J, Kitabgi P. Neurotensin and neurotensin receptors. Trends Pharmacol Sci. 1999;20:302-9.

Wustrow DJ, Davis MD, Akunne HC, Corbin AE, Wiley JN, Wise LD, et al. Reduced amide bond neurotensin 8-13 mimetics with potent in vivo activity. Bioorganic & Medicinal Chemistry Letters. 1995;5:997-1002.

Yamauchi R, Usui H, Yunden J, Takenaka Y, Tani F, Yoshikawa M. Characterization of beta-lactotensin, a bioactive peptide derived from bovine beta-lactoglobulin, as a neurotensin agonist. Biosci Biotechnol Biochem. 2003;67:940-3.

Yamauchi R, Wada E, Kamichi S, Yamada D, Maeno H, Delawary M, et al. Neurotensin type 2 receptor is involved in fear memory in mice. Journal of neurochemistry. 2007;102:1669-76.

Yamauchi R, Wada E, Yamada D, Yoshikawa M, Wada K. Effect of beta-lactotensin on acute stress and fear memory. Peptides. 2006;27:3176-82.

Figure Captions

Figure 1. The effects of the neuropeptide neurotensin on USV calls in male Wistar rats (N=5). USV calls (means +/- SEM) refer to 22-kHz vocalizations. The abscissa refers to amounts of the compounds administered icv. * $P < 0.05$ compared to 0.0 μg (i.e., vehicle).

Figure 2. The effects of the NTS_1 receptor agonist PD149163 (N=7) (top) and the NTS_2 receptor agonist JMV-431 (N=7) (bottom) on USV calls. ***$P < 0.001$ compared to 0.0 μg or 0.0 ng (i.e., vehicle). See figure 1 for further details.

Figure 1

Click here to download Figure: Figure 1.pdf

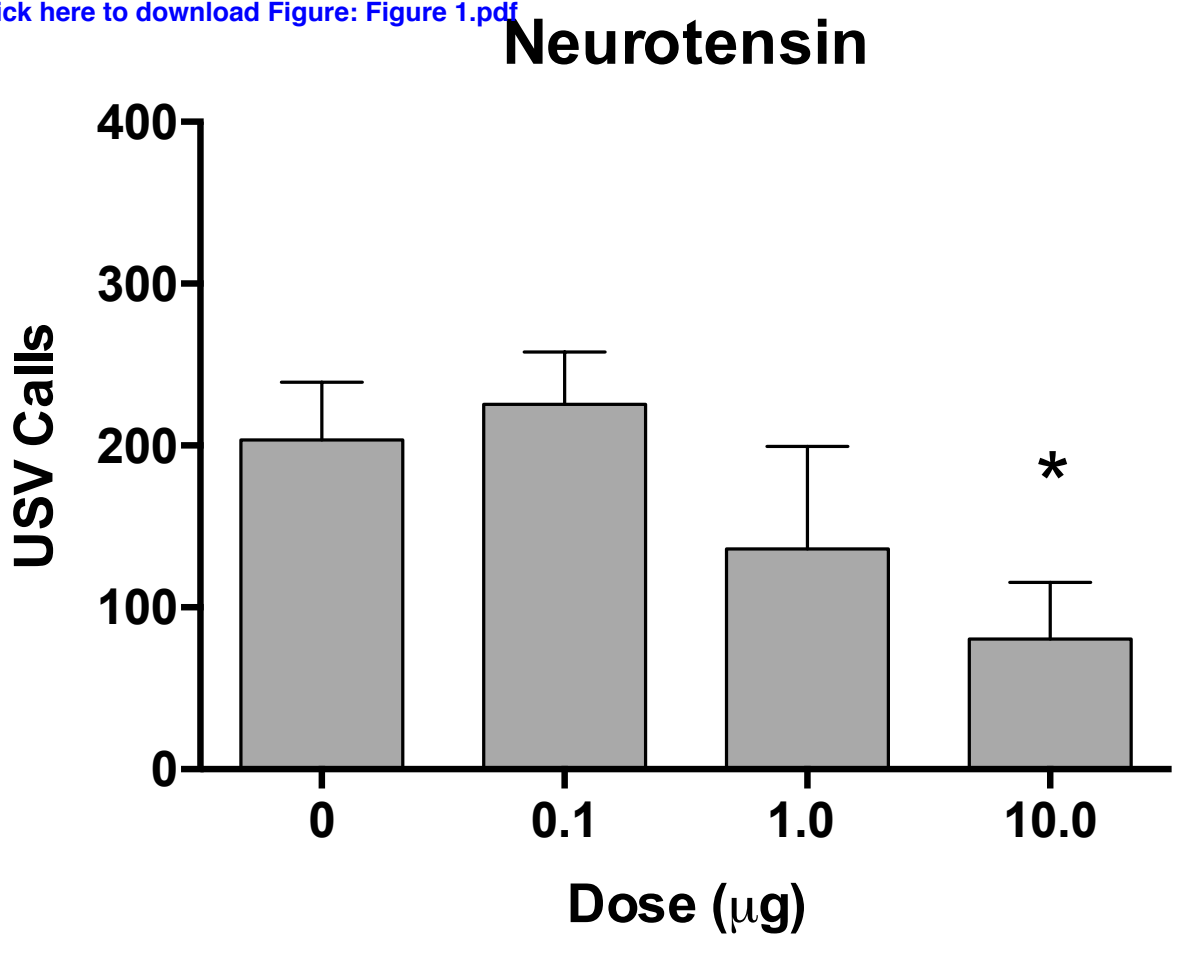
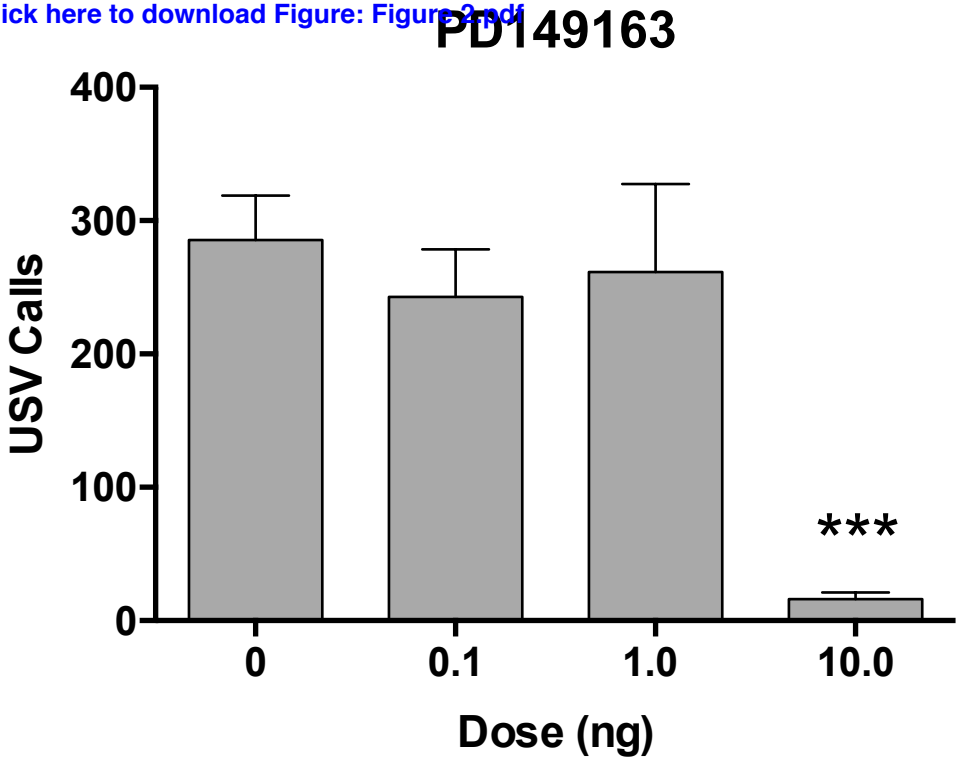


Figure 2

Click here to download Figure: Figure2.pdf



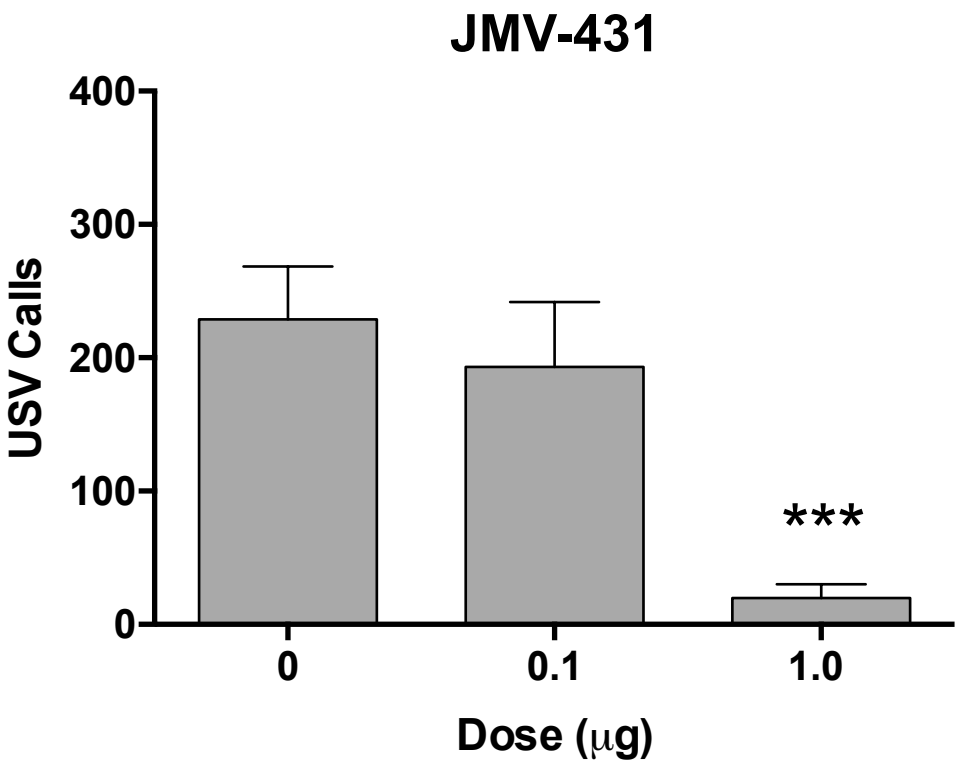


Table 1. Binding affinities for NTS_1 and NTS_2 receptors

Compound	**NTS_1**	**NTS_2**	**Reference**
Neurotensin	0.2 nM (Kd)	4nM (Kd)	Vincent et al. 1999
PD149163	159 nM (Ki)	No affinity	Petrie et al. 2004
JMV-431	3315 nM (Ki)	38 nM (Ki)	Dubuc et al. 1999

Table 2. USV call durations

Compound	Dose	Mean (SEM) USV Call Duration (s)
Neurotensin	Vehicle	1.10 (0.12)
	0.1 µg	1.08 (0.14)
	1.0 µg	0.88 (0.15)
	10.0 µg	1.08 (0.05)
PD149163	Vehicle	1.14 (0.08)
	0.1 ng	1.15 (0.12)
	1.0 ng	1.09 (0.09)
	10.0 ng	1.02 (0.05)
JMV-431	Vehicle	1.12 (0.10)
	1.0 µg	1.09 (0.09)
	10.0 µg	0.95 (0.05)