The anxiolytic effect of oxytocin is specific to oxytocin receptors in the prelimbic medial prefrontal cortex

Undergraduate Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation "with Honors Research Distinction in Neuroscience" in the undergraduate colleges of The Ohio State University

by

Shirley May Dong

The Ohio State University

April 2015

Project Advisor: Professor Benedetta Leuner, Departments of Psychology/Neuroscience

Abstract

Numerous studies in animals and humans have established that the neuropeptide oxytocin (OT) reduces anxiety. However, the specific brain regions where OT acts to regulate anxiety and the mechanisms by which it does so require further investigation. The medial prefrontal cortex (mPFC) has been shown to play a role in the modulation of anxiety-related behavior. In addition, the mPFC contains OT-sensitive neurons, expresses OT receptors (OTR), and receives long-range axonal projections from OT-producing neurons in the hypothalamus, suggesting that the mPFC may be a target where OT acts to diminish anxiety. In order to address this possibility, we performed five experiments. First we assessed whether the regulation of anxiety by OT is subregion specific within the mPFC. In the second experiment, we determined if the effects of OT on anxiety-like behavior in the prelimbic (PL) mPFC are sex-dependent or neuropeptide specific. The third experiment examined whether OTR activation is required for the anxiolytic action of OT. In the fourth experiment, we examined the importance of endogenous OT in the regulation of anxiety-like behavior using virgin male and female rats that received PL infusions of an OTR antagonist (OTR-A). Finally, given recent studies showing that the mPFC expresses OTR on GABAergic interneurons, we examined the extent to which the anxiolytic effect of exogenous OT in the PL mPFC is mediated via actions on GABAergic neurotransmission using site-specific administration of OT and a GABA-A receptor antagonist, bicuculline. Results show that OT in the PL mPFC decreased anxiety-like behavior regardless of sex. The effect of mPFC OT on anxiety-like behavior was determined to be region specific and neuropeptide specific as anxiety-like behavior was not affected by either OT in other mPFC subregions or by administration of the closely related neuropetide vasopressin into the PL mPFC. Additionally, the anxiolytic actions of OT were shown to be mediated through its binding to the OTR and were

blocked by the GABA-A receptor antagonist. In contrast to exogenous OT, endogenous OT did not influence anxiety as anxiety-like behavior was unaffected by administration of an OTR-A. Taken together, these results demonstrate that the anxiolytic actions of OT are specific to OTR in the PL mPFC and may attenuate anxiety-related behavior through utilization of a GABAergic mechanism. In doing so, our study not only provides a better understanding of the neural circuitry underlying OT's anxiety attenuating actions but also suggests putative targets for the pharmacological treatment of anxiety disorders.

Introduction

Oxytocin (OT) is a neuropeptide synthesized in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus. OT is released peripherally into the bloodstream where it acts as a hormone to regulate partuition and lactation (Gimpl and Fahrenholz, 2001). OT is also released centrally through dendritic release or axonal projections from OT synthesizing neurons (Ludwig and Leng, 2006). OT within the brain acts as a neuromodulator to influence reproductive (Bale et al., 2001), maternal (Bosch and Neumann, 2012) and other social behaviors such as pair bonding (Lonstein and Gammie, 2002; Gammie, 2005; Lim and Young, 2006; Bosch and Neumann, 2012), social recognition and social memory (Engelmann et al., 1998).

In addition to its role in social functions, OT has also been implicated in the regulation of anxiety-like behavior in both rodents and humans. For example, studies involving OT knockouts demonstrate that these rodents present with an anxious phenotype (Mantella et al., 2003). Other studies have shown that administration of exogenous OT either peripherally or centrally is effective in reducing anxiety in rodents (Uvnas-Moberg et al., 1994; McCarthy et al., 1996; Windle et al., 1997; Bale et al., 2001; Ring et al., 2006; Blume et al., 2008; Yoshida et al., 2009; Ayers et al., 2011; Mak et al., 2012; Sabihi et al., 2014b). Similarly, OT is anxiolytic in humans when administered intranasally (Heinrichs et al., 2003; Guastella et al., 2010; de Oliveira et al., 2012). Together, this research clearly demonstrates that OT is anxiolytic; however, the brain regions where OT acts to produce these anxiolytic effects are largely unknown. Previous work in rodents has implicated the hypothalamic paraventricular nucleus (PVN) of males (Waldherr and Neumann, 2007; Blume et al., 2008) and amygdala of females (Bale et al., 2001; Neumann, 2002) as sites which mediate the anxiolytic actions of OT. However, these areas are likely to be part of a larger network that may also include the medial prefrontal cortex (mPFC).

The mPFC is known to contain OT-sensitive neurons (Ninan, 2011), express OT receptors (Liu et al., 2005; Smeltzer et al., 2006), and receive long-range axonal projections from OT producing neurons in the hypothalamus (Sofroniew, 1983; Knobloch et al., 2012). Lesion, inactivation and molecular approaches have shown that the mPFC plays a role in regulating anxiety-like behavior as assessed in a variety of rodent behavioral paradigms including the elevated plus maze (EPM) and and social interaction (SI) test (Maaswinkel et al., 1996; Gonzalez et al., 2000; Lacroix et al., 2000; Sullivan and Gratton, 2002; Shah and Treit, 2003; Shah et al., 2004; Resstel et al., 2008; Stack et al., 2010; Stern et al., 2010). A recent study has also shown that OT receptors (OTR) are present on GABA interneurons in the mPFC (Nakajima et al., 2014), which may influence glutamatergic projections to the CeA/BNST, areas critically involved in anxiety regulation (Peters et al., 2009). Together, these results suggest that the mPFC may be a target for OT's anxiolytic effects and may do so by influencing GABAergic neurotransmission. This hypothesis was tested in a series of experiments that addressed the following questions. First, does OT act in mPFC to reduce anxiety and is this effect sub-region specific? Second, are the anxiolytic effects of OT sex-dependent or neuropeptide specific? Third, are the anxiolytic effects of OT specifically due to actions on the OTR? Fourth, does endogenous OT in the mPFC regulate anxiety-like behavior? Last, are the anxiolytic effects of OT in the mPFC mediated via actions on GABAergic neurotransmission?

Materials and methods

Animals

Age-matched adult (9-12 weeks of age) virgin female (225-250g) and male (300-350g) Sprague-Dawley rats from Taconic (Germantown, NY) were used. Rats were housed individually in a temperature and humidity controlled room and maintained on a 12h/12h

light/dark cycle (lights on at 0600 hr) with access to food and water ad libitum. All procedures were conducted in accordance with The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by The Ohio State University Institutional Animal Care and Use Committee.

When females were used, stages of estrous were monitored through daily vaginal swabs. Samples of cells were obtained with a sterile cotton swab saturated in 0.9% saline and applied to a glass slide. After drying, slides were stained with 1% aqueous Toluidine Blue and cell types characterized under 10X magnification (Everett, 1989). Only those females that had normal 4-5 d estrous cycles were used.

Surgical procedures

After at least 7 d of acclimation to the colony, rats were anesthetized with a 2-4% isoflurane gas/air mixture and aligned on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Body temperature was maintained throughout the surgery with a warming pad. Bilateral cannula guides (pedestal mounted 22-gauge stainless steel tubes with 1.5 mm separation and cut either 1.4 mm (Cg1), 3.5 mm (PL), or 4.6 mm (IL) below the pedestal (Plastics One, Roanoke, VA) were secured in a stereotaxic holder and lowered into one of three regions of the mPFC (Cg1: AP: + 2.7 mm, ML: ± 0.5 mm, DV: -1.4 mm; IL: AP: + 3.2 mm, ML: ± 0.5 mm, DV: -4.6 mm; PL: AP: + 3.2 mm, ML: ± 0.5 mm, DV: -3.5 mm) (Paxinos and Watson, 1998). The cannulae were secured by stainless steel screws and dental cement. A bilateral stainless steel obturator (0.35 mm diameter; Plastics One) extending 0.2 mm beyond the tip of the guide cannula was placed into the guide cannula after surgeries. The scalp was closed around the

protruding portion of the cannula with sutures. Rats were allowed to recover 7 d before behavioral testing.

Central infusions

On days 3 and 5 post-surgery, rats were habituated to the handling and infusion procedures. During habituation, rats were removed from their home cage and handled for 3 min while being lightly restrained in a terrycloth towel. The obturators were then removed and a 28-gauge bilateral injection cannula extending 0.2 mm beyond the tip of the guide cannula was inserted into the guide. The injection cannulas were left in place for 3 min then removed and the obturator replaced. On the day of testing, rats underwent the same procedure as described above except that an injection cannula attached to a 10 µl Hamilton Syringe via PE-10 tubing was inserted into the guide cannula. Infusions were made using a Harvard Apparatus Pico Plus Elite infusion pump (Holliston, MA) which delivered a 0.5 µl volume over 1.5 min. The injector was left in place for an additional 1 min before withdrawal.

Anxiety-like behavior

Anxiety-like behavior was evaluated one week post-surgery using the elevated plus maze (EPM) and/or social interaction (SI) test (Lapiz-Bluhm et al., 2008; Rotzinger et al., 2010). The EPM consisted of a cross-shaped platform (height: 50 cm) with four arms (width: 10 cm, length: 50 cm), two of which were enclosed by walls 50 cm in height. Rats were placed in the center of the platform (10 x 10 cm), facing a junction between an open and closed arm and allowed to explore for 5 min. The number of entries into the open arms and the percentage of time spent in the open arms (time in open arms/time in open and closed arms x 100) were used as measures of

anxiety-like behavior (Pellow et al., 1985; Cruz et al., 1994; Lapiz-Bluhm et al., 2008). An increase in the percentage of time spent in the open arms and a greater number of open arm entries are indicative of reduced anxiety. Closed arm entries were used as a measure of locomotion independent of anxiety (Pellow et al., 1985; Cruz et al., 1994; Lapiz-Bluhm et al., 2008). The EPM measures of anxiety and locomotion analyzed are consistent with numerous other studies investigating anxiety like-behavior including those manipulating OT (Mantella et al., 2003; Waldherr and Neumann, 2007; Mak et al., 2012).

In the SI test, an age, weight (+/- 10 g) and gender matched 'stimulus' rat which was unfamiliar to the test subject was placed in the corner of a 60 x 60 cm Plexiglas arena with walls 40 cm high opposite from the corner in which the test rat was placed. The floor of the arena was covered with gridlines which allowed for measurement of locomotion. The gridlines were spaced 10 cm apart yielding a total of 36 10 x 10 cm squares. The inner area was considered the central 16 squares which covered a 40 x 40 cm area. Stimulus rats were used a maximum of two times and were never used twice in the same day. The assignment of a stimulus rat to a test rat was random and not restricted to a particular drug or dose of drug. During a 5 min test, the time spent in active social behavior (i.e. communal grooming, sniffing, approaching, following, climbing on or under the stimulus rat) initiated by the test rat was scored. The time the experimental rat spent interacting with the stimulus rat was used as a measure of anxiety-like behavior. Increased anxiety is reflected by a decrease in social interaction time (File, 1980).

Beginning on day 7 post-surgery, females underwent behavioral testing upon entering their first diestrus in order to control for fluctuations in anxiety due to hormonal changes across the estrous cycle. Although social anxiety is not affected by the estrous cycle (Stack et al., 2010), other measures of anxiety-like behavior as assessed in the EPM are such that during the stage of

proestrus females exhibit a reduction in anxiety while during estrus and diestrus anxiety is relatively stable (Mora et al., 1996; Marcondes et al., 2001; Walf and Frye, 2007). Studies examining factors which regulate anxiety-like behavior in females also commonly test during diestrus (De Almeida et al., 1998; Marcondes et al., 2001; Hiroi and Neumaier, 2006; Figueira et al., 2008). Males were tested 1 week post-surgery.

All behavioral tests were performed under lighting conditions of ~550 lux within the same time range each day (approximately 0900-1300h), which is sufficiently separated from light-dark transitions (lights on at 0600h, lights off at 1800h) to avoid any potential diurnal variations in exploratory behavior (Lapiz-Bluhm et al., 2008). Tests were digitally recorded and later scored blind by a trained observer using BEST Collection and BEST Analysis software (Education Consulting Inc., Hobe Sound, FL).

Experimental design

Experiment 1:

In the first experiment we investigated the extent to which the anxiolytic effect of OT in the mPFC is subregion specific. Separate groups of male rats received infusions of synthetic OT (cat# O6379; Sigma, St. Louis, MO) into one of the three regions of the mPFC. OT was dissolved in 0.5 µl saline at a dose of 0.1µg (Cg1: n = 6; PL: n = 6; IL: n = 9) or 1.0µg (Cg1: n = 6; PL: n = 6; IL n = 9) (Bale et al., 2001; Ring et al., 2006; Blume et al., 2008; Ayers et al., 2011; Toth et al., 2012; Sabihi et al., 2014a; Sabihi et al., 2014b). Control rats received a 0.5 µl infusion of saline (Cg1: n = 7; PL: n = 6; IL: n = 8). Testing for anxiety-related behavior was done 15 min after infusions. The two tests of anxiety-like behavior (EPM and SI) were done 5 min apart and the order of the two tests was counterbalanced among rats.

Experiment 2:

In the second experiment, we evaluated the specificity of OT as well as whether the effects of OT on anxiety-like behavior are sex dependent. Separate groups of female and male rats received infusions of 1.0µg/1µl OT (n = 18 female; n = 14 male), 1.0µg/1µl of the closely related neuropeptide, arginine vasopressin (AVP; cat# V9879; Sigma, St. Louis, MO) (n = 19 female; n = 15 male), or 1µl of saline vehicle (n = 18 female; n = 15 male) in the PL region of the mPFC. Previous studies that have looked at the effects of AVP versus OT on anxiety-like behavior have used a similar dose of each (Lee et al., 2007; Blume et al., 2008). All subjects in the second experiment underwent testing in the EPM; approximately half of the animals in each group were then tested in the SI test (males: OT n = 8, AVP n = 8, saline n = 8; females: OT n = 8, AVP n = 8, saline n = 8). Testing for anxiety-related behavior was done 15 min after infusions. The two tests of anxiety-like behavior (EPM and SI) were done 5 min apart and the order of the two tests was counterbalanced among rats.

Experiment 3

In experiment 3, we evaluated whether the anxiolytic effect of OT in the PL mPFC is dependent on OTR activation. Separate groups of male rats received one 0.5µl infusion of saline followed by an infusion of 1.0µg OT (S/OT; n = 7), one infusion of a highly specific OTR-A (desGly-NH_2-d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT, courtesy of Dr. Maurice Manning, University of Toledo) at a dose of 0.1µg (Manning et al., 2008) followed by an infusion of 1.0µg OT (OTR-A/OT; n = 7) or one infusion of a highly specific AVPR-A (d[$Tyr(Me)^2$, Dab^5]AVP, courtesy of Dr. Manning) at a dose of 0.1µg (Manning et al., 2012) followed by an infusion of 1.0µg OT

(AVPR-A/OT; n = 7). OT, OTR-A and AVPR-A were all dissolved in 0.5 µl saline. An additional group of rats received two 0.5µl infusions of saline (S/S; n = 7). In each case, the second infusion occurred 10 min after the first (Yosten and Samson, 2010; Sala et al., 2011) and testing for anxiety-related behavior was done 15 min after the second infusion. All subjects in this experiment underwent testing only in the SI test.

Experiment 4:

The importance of endogenous OT in the regulation of anxiety-like behavior was evaluated using an OTR antagonist (OTR-A; d$(CH_2)_5^1$,Tyr(Me)^2, Thr^4,Orn^8,des-Gly-NH_2^9)OVT; cat# H-2098; Bachem, Torrance, CA). Separate groups of male and virgin female rats were infused with 0.1µg/1µl OTR-A (n = 6 female; n = 5 male) (Lukas et al., 2011) or 1µl saline vehicle (n = 6 female; n = 6 male) in the PL region of the mPFC and were tested in both the EPM and SI tests. All rats underwent testing 20 min after OTR-A infusions (Ring et al., 2006; Nyuyki et al., 2011). Tests were done 5 min apart and the order of the two tests was counterbalanced among rats.

Experiment 5:

To evaluate whether the anxiolytic effect of OT in the PL mPFC is dependent on GABA-A receptor activation, separate groups of male rats received either one 0.5µl infusion of saline followed by an infusion of 1.0µg OT 0.5µl OT (S/OT) or 5ng/0.5µl bicuculline (S/GABA-A) or one infusions of GABA-A followed by an infusion of 1.0µg/0.5µl OT (GABA-A/OT). Control animals received two infusions of saline (S/S). The dose of bicuculline used in this study is a subthreshold dose that has been shown to have no effect on anxiety-like behavior (Chodari et al.,

2014). OT and bicuculline were both dissolved in 0.5 µl saline. In each case, the second infusion occurred 10 min after the first (Yosten and Samson, 2010; Sala et al., 2011) and testing for anxiety-related behavior was done 15 min after the second infusion. All subjects in the experiment underwent testing in the both the EPM and the SI test.

Histology

After the completion of anxiety testing, rats were overdosed with Euthasol and transcardially perfused with 4% paraformaldehyde. Brains were removed, postfixed for 24 hr and then sectioned on a Vibratome. 40-µm thick coronal sections were collected throughout the area of the cannula implant and stained with 0.2% cresyl violet for verification of correct placement. Examination under high magnification (40X) revealed limited to no damage at the tip of the cannula. Those animals with cannula placements outside of the intended region of the mPFC were excluded from the study.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software version 5.01 (La Jolla, CA). All anxiety-like data was analyzed using either a one-way or two-way Analysis of Variance (ANOVA). Statistical significance for main effects and interactions were indicated by p values less than 0.05 and when significance was found were followed by Tukey's HSD or Bonferroni post hoc comparison tests.

Results

Experiment 1: OT specifically in the PL mPFC reduces anxiety-like behavior

In the EPM, OT had a subregion specific effect on anxiety-like behavior. For the number of open arm entries (Fig. 1a) there was a significant main effect of infusion type ($F_{2,55} = 4.48$, $p < 0.05$) and brain region ($F_{2,55} = 6.98$, $p < 0.05$) as well as an infusion type X brain region interaction ($F_{4,55} = 3.51$, $p < 0.05$). Post hoc analysis revealed that rats infused with the higher 1.0 µg dose of OT in the PL region of the mPFC made a greater number of entries into the open arms as compared to all other groups (p's < 0.05) which did not differ from one another (p's > 0.05), indicating decreased anxiety-like behavior. For the percentage of time spent in the open arms (Fig. 1b), there were also significant main effects of infusion type ($F_{2,55} = 4.56$, $p < 0.05$) and brain region ($F_{2,55} = 7.55$, $p < 0.05$) and a non-significant trend for an infusion type X brain region interaction ($F_{4,55} = 2.24$, $p = 0.07$). Post hoc analysis revealed that rats infused with the higher 1.0 µg dose of OT spent a greater percentage of time in the open arms as compared to all other groups (p's < 0.05) which did not differ from one another (p's > 0.05), again indicating a decrease in anxiety-like behavior. Locomotor activity, as measured by the number of closed arm entries (Fig. 1c), was not altered by infusion type ($F_{2,55} = 2.21$, $p > 0.05$) or region of infusion ($F_{2,55} = 0.08$, $p > 0.05$) and there was no significant infusion type X brain region interaction ($F_{4,55} = 0.36$, $p > 0.05$).

In the SI test (Fig. 1d), there was a significant main effect of infusion type ($F_{2,55} = 3.47$, $p < 0.05$) and brain region ($F_{2,55} = 8.54$, $p < 0.05$) and a significant infusion type X brain region interaction ($F_{4,55} = 4.33$, $p < 0.05$) on the amount of time spent interacting with an unknown stimulus rat. Post hoc analysis showed that rats infused with the higher 1.0 µg dose of OT in the PL mPFC spent a greater amount of time interacting with an unknown stimulus rat when

compared to all other groups (p's < 0.05) which did not differ from each other (p's > 0.05) indicating decreased anxiety-like behavior.

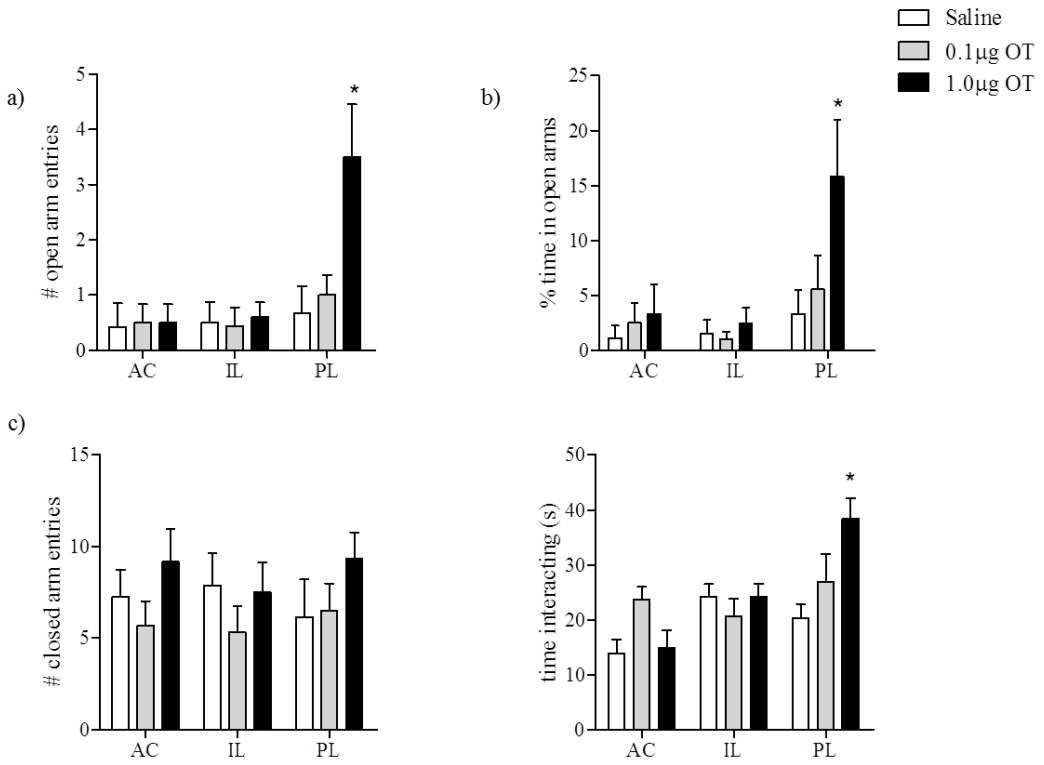

Fig. 1. OT in the PL mPFC decreases anxiety-like behavior. Rats that received the higher 1.0µg dose of OT in the PL mPFC displayed an increase in the number of open arm entries (a) and a greater percentage of time spent in the open arms (b) as compared to rats infused with saline or 0.1µg OT in the PL mPFC, neither of which differed from any other group. Locomotor activity as measured by the number of closed arm entries (c) was not altered by infusion type or region of infusion. Rats that received the higher 1.0µg dose of OT in the PL mPFC spent more time interacting with an unknown stimulus rat as compared to those infused with saline or 0.1µg OT, neither of which differed from any other group.

Experiment 2: OT, but not AVP, infused into the PL region of the mPFC reduces anxiety-like behavior in females and males

For percentage of time spent in the open arms of the EPM (Fig. 2a), there was a significant main effect of infusion type ($F_{2,93} = 18.70$, $p < 0.05$) but no main effect of sex ($F_{1,93} = 1.18$, $p > 0.05$) and no interaction between infusion type and sex ($F_{2,93} = 2.66$, $p > 0.05$). Post hoc

analysis of infusion type showed that overall, animals infused with 1.0μg/μl OT into the PL region of the mPFC were less anxious as demonstrated by a greater percentage of time in the open arms than those infused with saline ($p < 0.05$) and those infused with AVP ($p < 0.05$), which did not differ from one another ($p > 0.05$). For number of open arm entries in the EPM (Fig. 2b), there was also a significant main effect of infusion type ($F_{2,93} = 21.22$, $p < 0.05$) with post hoc analysis revealing a greater number of open arm entries in animals receiving OT infusion into the PL region of the mPFC as compared to saline ($p < 0.05$) and AVP ($p < 0.05$), which did not differ ($p > 0.05$), again indicating an anxiolytic effect of OT. There was also a significant main effect of sex ($F_{1,93} = 4.99$, $p < 0.05$) on the number of open arm entries with females entering the open arms more than males. The interaction between infusion type and sex on the number of open arm entries was not significant ($F_{2,93} = 2.90$, $p > 0.05$). The number of entries into the closed arms (Fig. 2c) showed no significant effects of infusion type ($F_{2,93} = 0.68$, $p > 0.05$) or sex ($F_{2,93} = 1.14$, $p > 0.05$) and no interaction between these factors ($F_{2,93} = 3.67$, $p > 0.05$).

In the SI test (Fig. 2d), there was a main effect of infusion type ($F_{2,42} = 13.05$, $p < 0.05$) but no main effect of sex ($F_{1,42} = 2.41$, $p > 0.05$) and no interaction between infusion type and sex ($F_{1,42} = 0.72$, $p > 0.05$) in time spent interacting with an unknown stimulus rat. Post hoc analysis of infusion type showed that overall, animals infused with OT spent a greater amount of time interacting with an unknown stimulus rat than those infused with AVP ($p < 0.05$) or saline ($p < 0.05$), which did not differ from one another ($p > 0.05$).

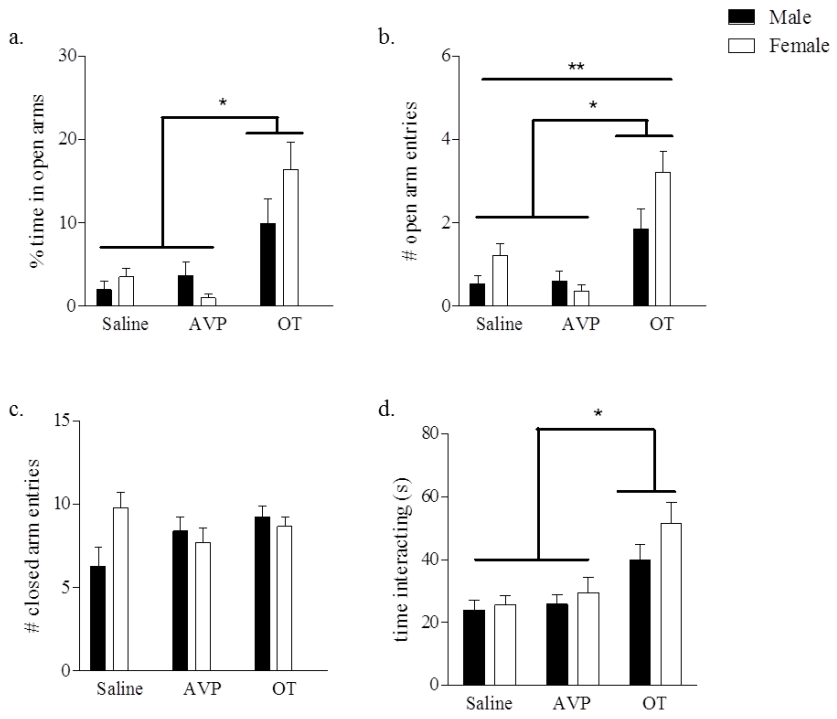

Fig. 2. OT, but not AVP, infused into the PL region of the mPFC reduces anxiety-like behavior in females and males. In the EPM, males and females infused with 1.0µg/µl OT into the PL region of the mPFC spent a greater percentage of time in the open arms (a) and made more open arm entries (b) than rats infused with saline or AVP. Females also entered the open arms of the EPM more than males (b). Locomotor activity (closed arm entries) was not altered by sex or infusion type (c). In the SI test, both males and females infused with OT spent a greater amount of time interacting with an unknown stimulus rat than those infused with AVP or saline (d). Bars represent mean ± SEM; *$p < 0.05$, main effect of dose; **$p < 0.05$, main effect of sex.

Experiment 3: The anxiolytic effect of OT in the PL mPFC is dependent on OTR activation

The anxiolytic effect of OT in the PL mPFC was blocked pretreatment with an OTR-A, but not AVPR-A ($F_{3,23} = 11.62$, $p < 0.0001$; Fig. 3). Post hoc analysis revealed that rats infused with S/OT and AVPR-A/OT spent more time interacting with an unknown conspecific than those infused with S/S (p's < 0.05) while subjects treated with OTR-A/OT did not and were no different than S/S rats (p's > 0.05).

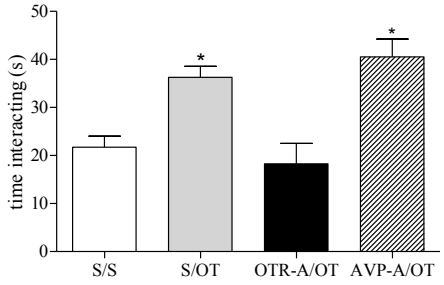

Fig. 3. OT acting on the OTR in the PL mPFC decreases anxiety in the SI test. Rats infused with AVPR-A/OT and those infused with S/OT spent more time interacting with an unknown conspecific than those infused with S/S or OTR-A/OT, which did not differ from one another. Bars represent mean ± SEM; *p < 0.05.

Experiment 4: OTR-A infused into the PL region of the mPFC does not alter anxiety-like behavior in females or males

In the EPM, there were no significant main effects for infusion type ($F_{1,19}$ = 0.62, p > 0.05) or sex ($F_{1,19}$ = 0.002, p > 0.05) and no infusion type X sex interaction ($F_{1,19}$ = 0.09, p > 0.05) in the percentage of time spent in the open arms (Fig. 4a). Similarly, for number of open arm entries (Fig. 4b) there was no significant main effect for infusion type ($F_{1,19}$ = 0.009, p > 0.05) or sex ($F_{1,19}$ =0.41, p > 0.05) and no infusion type X sex interaction ($F_{1,19}$ = 0.02, p > 0.05). Locomotor activity, as measured by the number of closed arm entries in the EPM, was not altered (p's > 0.05; Fig. 4c).

In the SI test (Fig. 4d), there was no significant main effects for either infusion type ($F_{1,19}$ = 0.02, p > 0.05) or sex ($F_{1,19}$ = 1.03, p > 0.05) and no infusion type X sex interaction ($F_{1,19}$ = 0.11, p > 0.05) in the amount of time spent interacting with an unknown stimulus rat.

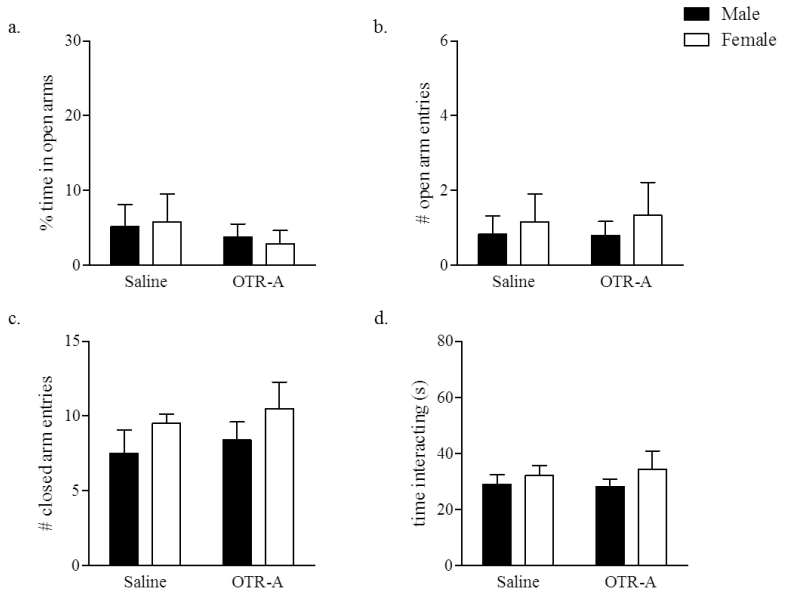

Fig. 4. OTR-A infused into the PL region of the mPFC does not alter anxiety-like behavior in females or males. In both males and females, blockade of OTR in the PL mPFC did not alter the percentage of time spent in the open arms (a) or the number of open arm entries (b) in the EPM. Locomotor activity in the EPM (number of closed arm entries) was unaffected by sex or infusion type (c). OTR-A in the PL region of the mPFC also had no effect on the amount of time spent interacting with an unknown stimulus rat in the SI test (d). Bars represent mean ± SEM.

Experiment 5: The anxiolytic actions of OT are prevented with the GABA-A receptor antagonist bicuculline

For percent time in the open arms in the EPM (Fig. 5a), there were significant main effects of GABA-A infusion ($F_{1,22} = 6.58$, $p < 0.05$) and OT infusion ($F_{1,22} = 7.95$, $p< 0.05$) as well a a significant OT X GABA-A interaction ($F_{1,22} = 6.54$, $p< 0.05$). Post-hoc analysis showed that animals infused with S/OT spent a greater percentage of time in the open arms (p's < 0.05), an effect which was not evident in the OT treated rats that also received the GABA-A ($p > 0.05$). Similarly, for number of open arm entries in the EPM (Fig. 5b), there was a main effect of GABA-A infusion ($F_{1,22} = 7.01$, $p < 0.05$), a main effect of OT infusion ($F_{1,22} = 9.74$, $p< 0.05$) and an OT X GABA-A interaction ($F_{1,22} = 5.81$, $p< 0.05$). Post-hoc analysis reveled that only the

S/OT group displayed reduced anxiety as indicated by a greater number of open arm entries (p's < 0.05). The OT-induced reduction in anxiety was prevented by prior administration of the GABA-A. Both groups treated with the GABA-A spent a similar percentage of time in the open arms and made a similar number of open arm entries as compared to the S/S group (p's > 0.05) indicating that the GABA-A itself had no effect on anxiety-like behavior. The number of entries into the closed arms (Fig. 5c) was not altered (p's > 0.05).

In the SI test (Fig. 5d), there were main effects of GABA-A ($F_{1,22} = 13.72$, $p < 0.05$) and OT ($F_{1,22} = 18.70$, $p < 0.05$) as well as a signficant GABA-A X OT interaction ($F_{1,22} = 29.20$, $p < 0.05$). Post hoc analysis revealed that animals infused with saline followed by OT spent a greater amount of time interacting with an unknown stimulus rat (p's < 0.05), an effect which was not evident in the OT treated rats that also received GABA-A (p > 0.05). Both groups treated with the GABA-A spent a similar amount of time interacting with the unknown stimulus rat as compared to the S/S group (p > 0.05) indicating that the GABA-A itself had no effect on anxiety-like behavior in the SI test.

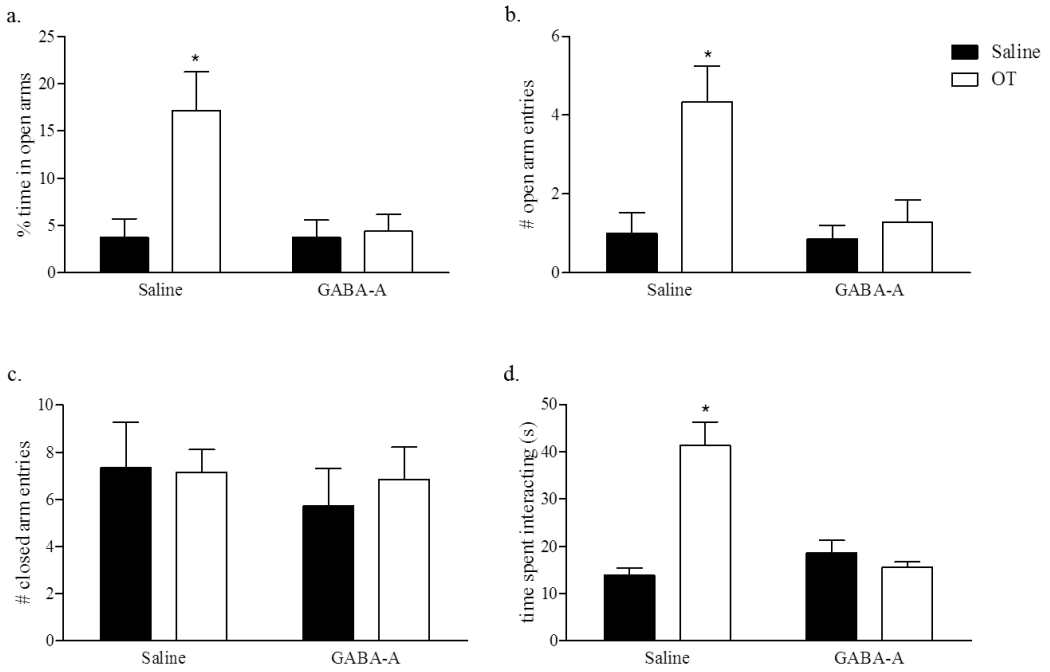

Fig. 5. GABA-A receptor antagonism prevents the anxiolytic effects of OT. Animals were infused with Saline or GABA-A (x-axis) followed by an infusion of saline (white bars) or OT (black bars). In the EPM, males infused with S/OT into the PL region of the mPFC made more open arm entries (a) and spent a greater percentage of time in the open arms (b) than all other groups. Locomotor activity (closed arm entries) was not altered by either infusion (c). In the SI test, males infused with S/OT spent a greater amount of time interacting with an unknown stimulus rat than any other group (d). Bars represent mean ± SEM; *$p < 0.05$.

Discussion

In this series of studies we show that OT infused into the PL region of the mPFC is effective in reducing anxiety-like behavior, regardless of sex. This anxiolytic effect of OT is subregion specific and neuropeptide specific as neither OT in other mPFC subregions nor the closely-related neuropeptide vasopressin in the PL mPFC had any effect on anxiety-like behavior. Our data also show that the anxiolytic effects of OT in the PL mPFC requires activation of the OTR and may work via a GABAergic mechanism. In contrast to exogenous OT, however, endogenous OT in the PL region of the mPFC did not influence anxiety in males or females.

The anxiolytic effect of OT in the mPFC is restricted to the PL region

In the rodent brain, the mPFC can be broken down into 3 subregions - the infralimbic cortex (IL), PL cortex, and anterior cingulate cortex (Cg1) (Heidbreder and Groenewegen, 2003). We found that OT in the PL, bt not IL or Cg1, was effective in reducing anxiety. The PL mPFC has been strongly implicated in the regulation of fear and anxiety. Specifically, enhancing activity in the PL mPFC promotes the expression of learned fear (Vidal-Gonzalez et al. 2006) and innate anxiety (Resstel et al. 2008; Saitoh et al. 2014; Stern et al. 2010) while lesion or inactivation generally has the opposite effect (Blum et al. 2006; Corcoran and Quirk 2007; but see Jinks and McGregor, 1997; de Visser et al., 2011; Gonzalez et al. 2000; Maaswinkel et al. 1996; Shah et al. 2004). The PL mPFC promotes the expression of learned fear via glutamatergic projections to the basolateral amygdala (BLA) which in turn excites the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) (Peters et al. 2009). Although innate anxiety, as was measured here, has been less studied, recent work implicates a similar neural circuit (Yamada et al. 2015). When combined with other studies showing that OTR are present on mPFC GABAergic interneurons (Nakajima et al. 2014), that OT increases GABA levels in the mPFC (Qi et al. 2012) and that activation of GABA-A receptors in the PL mPFC is anxiolytic (Solati et al. 2013), OT in PL mPFC may inhibit glutamatergic output neurons and thus decrease excitatory drive to the CeA and BNST resulting in a decrease in anxiety-like behavior (Figure 6).

Unlike the PL mPFC, OT in the Cg1 and IL subregions of the mPFC had no effect on anxiety-like behavior, even though both have also been implicated in fear and anxiety regulation (Cg1: Bissiere et al., 2008; Albrechet-Souza et al., 2009; but see Bissiere et al., 2006; IL: Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011; Bi et al., 2013). In this regard, the dose of

OT may be an important factor. Since little is known about OTR distribution in the mPFC, it is possible that anxiety-like behavior is differentially sensitive to exogenous OT depending on the mPFC subregion. Thus, while 1 ug, but not 0.1 μg (Sabihi et al. 2014b), OT in the PL mPFC is effective in reducing anxiety, other mPFC subregions may require different doses than tested here for an effect on anxiety to be revealed. Consistent with this possibility are findings showing that OT in the IL mPFC promotes fear extinction when administered at a much lower dose of only 0.01 μg (Lahoud and Maroun 2013), although these results could also suggest that learned fear versus innate anxiety, and the different paradigms used to assess these processes, may not be equally sensitive to OT. It is also important to consider emerging evidence that OT is not solely involved in reducing fear and anxiety but can also have the opposite action and act to increase fear and anxiety (Bartz et al. 2011; Grillon et al. 2012; Guzman et al. 2013). In the current study, such effects would not necessarily be evident because at least in the EPM, anxiety levels in the saline-treated control rats were already fairly high. However, this was not the case in the SI test the results of which not only verify that the anxiolytic actions of OT are restricted to the PL mPFC but also show that OT is not anxiogenic in any of the subregions.

OT in the PL mPFC reduces anxiety-like behavior in females and males

Previous studies have indicated that female rats exhibit less anxiety-related behaviors than males (Johnston and File, 1991; Stack et al., 2010). Some sex differences were observed in the baseline levels of anxiety when comparing males versus females (experiment 2). Despite this, administration of exogenous OT into the PL mPFC was found to be anxiolytic in both males and females and is thus consistent with prior work demonstrating that exogenous OT is anxiolytic in both males and females when delivered peripherally or centrally (Uvnas-Moberg et al., 1994;

McCarthy et al., 1996; Windle et al., 1997; Bale et al., 2001; Ring et al., 2006; Blume et al., 2008; Yoshida et al., 2009; Ayers et al., 2011; Mak et al., 2012).

OT, but not AVP, in the PL mPFC reduces anxiety and this effect is dependent on OTR activation

AVP is also known to regulate anxiety-related behaviors but its effects are often opposite to that of OT and thus act to increase anxiety (Neumann and Landgraf, 2012). Here we found that administration of AVP in the PL region of the mPFC did not significantly alter anxiety-related behavior in males or females. Thus, our results suggest that the PL mPFC is not a site of action for the anxiogenic actions of AVP, and furthermore provide evidence that the anxiolytic effects of PL OT are neuropeptide specific. It is important to keep in mind that OT and AVP show structural similarities, and cross-reactivity at the receptor level has been described (Postina et al., 1998). OT does indeed have a moderate to strong affinity for the AVP 1A (V1aR) receptor (Hicks et al., 2012) and can act as a partial agonist at this site (Chini et al., 1996). Recent studies have shown that some of the prosocial effects of OT may be mediated by the V1aR rather than the OTR (Sala et al., 2011; Ramos et al., 2013). Therefore, exogenous OT may potentially be acting at the V1aR to reduce anxiety. To address this possibility, we co-administered OT with either an OTR-A or an AVPR-A and found that the anxiolytic effects of OT were blocked by the OTR-A and not the AVPR-A. Thus, the anxiolytic actions of OT are specific to the OTR which not only lends support to other work showing that OTR are present within the mPFC (Gould and Zingg 2003; Insel and Shapiro 1992; Liu et al. 2005; Smeltzer et al. 2006) but also indicate that their activation is required in order to have behavioral significance.

Endogenous OT in the PL mPFC does not alter anxiety-like behavior in females or males

In contrast to exogenous OT, endogenous OT in the PL mPFC does not appear to directly be involved in regulating of anxiety since anxiety-like behavior was not affected in males or females infused with OTR-A. Prior studies have found similar results following acute ICV OTR-A administration (Neumann et al., 2000; Neumann, 2002; Slattery and Neumann, 2010). This does not eliminate the possibility that endogenous PL OT could be involved in regulating anxiety during times of elevated OT system activity. Indeed, in other studies we have found that administration of an OTR-A into the PL mPFC of postpartum females (Sabihi et al., 2014a) blocks the anxiolytic properties of high endogenous OT. Whether endogenous PL OT is similary involved in males during elevated OT system activity as occurs after mating (Waldherr and Neumann, 2007), remains to be determined.

Interactions between mPFC OT and GABA in the regulation of anxiety-like behavior

As noted above, recent studies have found that OTR are present on mPFC GABAergic interneurons (Nakajima et al. 2014). When combined with other work showing that OT increases GABA levels in the mPFC (Qi et al. 2012) and that activation of GABA-A receptors in the PL mPFC is anxiolytic (Solati et al. 2013), OT in PL mPFC may inhibit glutamatergic output neurons and thus decrease excitatory drive to the CeA and BNST resulting in a decrease in anxiety-like behavior (Figure 6). To begin exploring this potential mechanism, we co-administered OT with a GABA-A receptor blocker (bicuculline) and found that that the anxiolytic effects of OT were indeed prevented. Thus, our results provide support for the possibility that OT is acting on GABAergic neurons within the PL mPFC to attenuate anxiety.

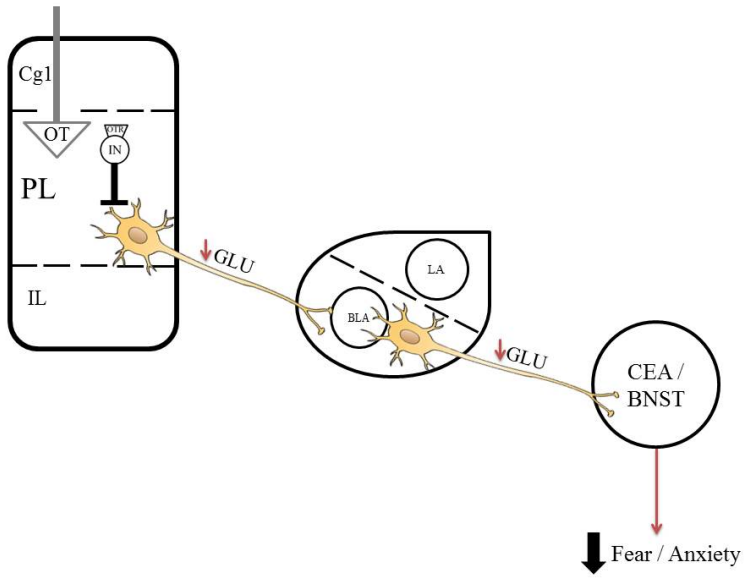

Fig. 6. Proposed circuit in which OT acts in order to have anxiolytic effects. Administration of exogenous OT to the PL mPFC binds to OTR on GABA interneurons and activates them, thereby increasing inhibitory tone in this region. This increase in inhibition leads to a decrease in excitation on glutamatergic projections that are sent from the PL region to downstream areas including the BLA and BNST, which, when activated produce an increase in anxiety-like behavior. Thus, increased inhibition in the PL region by OT administration may prevent excitation of the CEA/BNST and result in a decrease in fear and anxiety.

Implications

Recently in humans, brain neuropeptides, including OT, have received increasing attention as central regulators of anxiety behavior (Heinrichs et al., 2003; Landgraf, 2006; Meyer-Lindenberg et al., 2011; de Oliveira et al., 2012; Benarroch, 2013; Macdonald and Feifel, 2014). Anxiety can be characterized in animals by a temporary behavioral state induced by threatening stimuli such as open spaces and bright lights (Crawley, 1985; Pellow et al., 1985; Sylvers et al., 2011; Adhikari, 2014). In humans, however, anxiety is commonly defined as prolonged hypervigilance in anticipation of, or response to, a threat where the danger is not clearly imminent (Gorman, 2003; Adhikari, 2014). Despite the differences in behavioral outputs, there are many similarities in the neurobiological and neurochemical underpinnings that regulate

anxiety in both rodents and humans (Lang et al. 2000; Leuner and Shors 2013; Maroun 2013; Myers-Schulz and Koenigs 2012). As such, the study of rodent anxiety-like behavior is able to provide useful insights into human anxiety disorders. The results of the current study provide a better understanding of the neural circuitry underlying OT's anxiety attenuating actions, and in doing so may reveal putative targets for the pharmacological treatment of anxiety disorders in humans.

References

Adhikari, A. (2014). Distributed circuits underlying anxiety. *Front Behav Neurosci* 8**,** 112. doi: 10.3389/fnbeh.2014.00112.

Albrechet-Souza L, Borelli KG, Carvalho MC, Brandao ML (2009) The anterior cingulate cortex is a target structure for the anxiolytic-like effects of benzodiazepines assessed by repeated exposure to the elevated plus maze and Fos immunoreactivity. Neuroscience 164: 387-97.

Ayers, L.W., Missig, G., Schulkin, J., Rosen, J.B., 2011. Oxytocin Reduces Background Anxiety in a Fear-Potentiated Startle Paradigm: Peripheral vs Central Administration. Neuropsychopharmacology 36, 2488-2497.

Bale, T.L., Davis, A.M., Auger, A.P., Dorsa, D.M., and Mccarthy, M.M. (2001). CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. *J Neurosci* 21**,** 2546-2552. doi: 21/7/2546 [pii].

Bartz JA, Zaki J, Bolger N, Ochsner KN (2011) Social effects of oxytocin in humans: context and person matter. Trends in cognitive sciences 15: 301-9.

Benarroch, E.E. (2013). Oxytocin and vasopressin: Social neuropeptides with complex neuromodulatory functions. *Neurology* 80**,** 1521-1528. doi: 10.1212/WNL.0b013e31828cfb15 80/16/1521 [pii].

Bi LL, Wang J, Luo ZY, Chen SP, Geng F, Chen YH, Li SJ, Yuan CH, Lin S, Gao TM (2013) Enhanced excitability in the infralimbic cortex produces anxiety-like behaviors. Neuropharmacology 72C: 148-156.

Bissiere, S., Mcallister, K.H., Olpe, H.R., and Cryan, J.F. (2006). The rostral anterior cingulate cortex modulates depression but not anxiety-related behaviour in the rat. *Behav Brain Res* 175**,** 195-199. doi: 10.1016/j.bbr.2006.08.022.

Bissiere S, Plachta N, Hoyer D, McAllister KH, Olpe HR, Grace AA, Cryan JF (2008) The rostral anterior cingulate cortex modulates the efficiency of amygdala-dependent fear learning. Biol Psychiatry 63: 821-31.

Blum S, Hebert AE, Dash PK (2006) A role for the prefrontal cortex in recall of recent and remote memories. Neuroreport 17: 341-4.

Blume, A., Bosch, O.J., Miklos, S., Torner, L., Wales, L., Waldherr, M., Neumann, I.D., 2008. Oxytocin reduces anxiety via ERK1/2 activation: local effect within the rat hypothalamic paraventricular nucleus. Eur J Neurosci 27, 1947-1956.

Bosch, Oliver J., and Inga D. Neumann. "Both Oxytocin and Vasopressin Are Mediators of Maternal Care and Aggression in Rodents: From Central Release to Sites of Action." Hormones and Behavior 61.3 (2012): 293-303.

Chodari, Leila, Oryan, S., Ahmadi, R., Ashabi, G.. Dorsal hippocampus histaminergic and septum GABAergic neurons work in anxiety related behavior: Comparison between GABAA and GABAB receptors. El Mednifico Journal, [S.l.], v. 2, n. 2, p. 64-69, feb. 2014. ISSN 2307-7301.

Chini, B., Mouillac, B., Balestre, M.N., Trumpp-Kallmeyer, S., Hoflack, J., Hibert, M., Andriolo, M., Pupier, S., Jard, S., Barberis, C., 1996. Two aromatic residues regulate the response of the human oxytocin receptor to the partial agonist arginine vasopressin. FEBS Lett 397, 201-206.

Corcoran KA, Quirk GJ (2007) Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci 27: 840-4.

Crawley, J.N. (1985). Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev* 9**,** 37-44.

Cruz, A.P., Frei, F., Graeff, F.G., 1994. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 49, 171-176.

De Almeida, R.M., Giovenardi, M., Charchat, H., Lucion, A.B., 1998. 8-OH-DPAT in the median raphe nucleus decreases while in the medial septal area it may increase anxiety in female rats. Neurosci Biobehav Rev 23, 259-264.

de Visser L, Baars AM, van 't Klooster J, van den Bos R (2011) Transient inactivation of the medial prefrontal cortex affects both anxiety and decision-making in male wistar rats. Front Neurosci 5: 102.

de Oliveira, D.C., Chagas, M.H., Garcia, L.V., Crippa, J.A., Zuardi, A.W., 2012. Oxytocin interference in the effects induced by inhalation of 7.5% CO(2) in healthy volunteers. Hum Psychopharmacol 27, 378-385.

Engelmann, M., Ebner, K., Wotjak, C.T., and Landgraf, R. (1998). Endogenous oxytocin is involved in short-term olfactory memory in female rats. *Behav Brain Res* 90**,** 89-94.

Everett, J.W., 1989. Neurobiology of reproduction in the female rat. A fifty-year perspective. Monogr Endocrinol 32, 1-133.

Figueira, R.J., Peabody, M.F., Lonstein, J.S., 2008. Oxytocin receptor activity in the ventrocaudal periaqueductal gray modulates anxiety-related behavior in postpartum rats. Behav Neurosci 122, 618-628.

File, S.E., 1980. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 2, 219-238.

Gammie, S.C. (2005). Current models and future directions for understanding the neural circuitries of maternal behaviors in rodents. *Behav Cogn Neurosci Rev* 4**,** 119-135. doi: 10.1177/1534582305281086.

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81, 629-683.

Gonzalez, L.E., Rujano, M., Tucci, S., Paredes, D., Silva, E., Alba, G., Hernandez, L., 2000. Medial prefrontal transection enhances social interaction. I: behavioral studies. Brain Res 887, 7-15.

Gorman, J.M. (2003). New molecular targets for antianxiety interventions. *J Clin Psychiatry* 64 Suppl 3**,** 28-35.

Gould, B.R., Zingg, H.H., 2003. Mapping oxytocin receptor gene expression in the mouse brain and mammary gland using an oxytocin receptor-LacZ reporter mouse. Neuroscience122, 155-167.

Grillon C, Krimsky M, Charney DR, Vytal K, Ernst M, Cornwell B (2012) Oxytocin increases anxiety to unpredictable threat. Mol Psychiatry.

Guastella, A.J., Einfeld, S.L., Gray, K.M., Rinehart, N.J., Tonge, B.J., Lambert, T.J., Hickie, I.B., 2010. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry 67, 692-694.

Guzman YF, Tronson NC, Jovasevic V, Sato K, Guedea AL, Mizukami H, Nishimori K, Radulovic J (2013) Fear-enhancing effects of septal oxytocin receptors. Nat Neurosci 16: 1185-7.

Heidbreder, C.A., Groenewegen, H.J., 2003. The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27, 555-579.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 54, 1389-1398.

Hicks, C., Jorgensen, W., Brown, C., Fardell, J., Koehbach, J., Gruber, C.W., Kassiou, M., Hunt, G.E., McGregor, I.S., 2012. The nonpeptide oxytocin receptor agonist WAY 267,464: receptor-binding profile, prosocial effects and distribution of c-Fos expression in adolescent rats. J Neuroendocrinol 24, 1012-1029.

Hiroi, R., Neumaier, J.F., 2006. Differential effects of ovarian steroids on anxiety versus fear as measured by open field test and fear-potentiated startle. Behav Brain Res 166, 93-100.

Insel, T.R., Shapiro, L.E., 1992. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci USA 89, 5981-5985.

Jinks AL, McGregor IS (1997) Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. Brain Res 772: 181-90.

Johnston, A.L., File, S.E., 1991. Sex differences in animal tests of anxiety. Physiol Behav 49, 245-250.

Knobloch, H.S., Charlet, A., Hoffmann, L.C., Eliava, M., Khrulev, S., Cetin, A.H., Osten, P., Schwarz, M.K., Seeburg, P.H., Stoop, R., Grinevich, V., 2012. Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73, 553-566.

Lacroix, L., Spinelli, S., Heidbreder, C.A., Feldon, J., 2000. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci 114, 1119-1130.

Lahoud N, Maroun M (2013) Oxytocinergic manipulations in corticolimbic circuit differentially affect fear acquisition and extinction. Psychoneuroendocrinology 38: 2184-95.

Lang, P.J., Davis, M., and Ohman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *J Affect Disord* 61**,** 137-159.

Lapiz-Bluhm, M.D., Bondi, C.O., Doyen, J., Rodriguez, G.A., Bedard-Arana, T., Morilak, D.A., 2008. Behavioural assays to model cognitive and affective dimensions of depression and anxiety in rats. J Neuroendocrinol 20, 1115-1137.

Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res 1156, 152-167.

Leuner, B., and Shors, T.J. (2013). Stress, anxiety, and dendritic spines: What are the connections? *Neuroscience* 251**,** 108-119. doi: 10.1016/j.neuroscience.2012.04.021.

Lim MM, and Young LJ (2006) Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm & Behav 50: 506-17.

Liu, W., Pappas, G.D., Carter, C.S., 2005. Oxytocin receptors in brain cortical regions are reduced in haploinsufficient (+/-) reeler mice. Neurol Res 27, 339-345.

Lonstein, J.S., and Gammie, S.C. (2002). Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. *Neurosci Biobehav Rev* 26**,** 869-888.

Ludwig, M., Leng, G., 2006. Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 7, 126-136.

Lukas, M., Toth, I., Reber, S.O., Slattery, D.A., Veenema, A.H., Neumann, I.D., 2011. The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. Neuropsychopharmacology 36, 2159-2168.

Maaswinkel, H., Gispen, W.H., Spruijt, B.M., 1996. Effects of an electrolytic lesion of the prelimbic area on anxiety-related and cognitive tasks in the rat. Behav Brain Res 79, 51-59.

Macdonald, K., and Feifel, D. (2014). Oxytocins role in anxiety: A critical appraisal. *Brain Res*. doi: 10.1016/j.brainres.2014.01.025.

Mak, P., Broussard, C., Vacy, K., Broadbear, J.H., 2012. Modulation of anxiety behavior in the elevated plus maze using peptidic oxytocin and vasopressin receptor ligands in the rat. J Psychopharmacol 26, 532-542.

Manning, M., Misicka, A., Olma, A., Bankowski, K., Stoev, S., Chini, B., Durroux, T., Mouillac, B., Corbani, M., and Guillon, G. (2012). Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. *J Neuroendocrinol* 24**,** 609-628. doi: 10.1111/j.1365-2826.2012.02303.x.

Manning, M., Stoev, S., Chini, B., Durroux, T., Mouillac, B., and Guillon, G. (2008). Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V1a, V1b, V2 and OT receptors: research tools and potential therapeutic agents. *Prog Brain Res* 170**,** 473-512. doi: S0079-6123(08)00437-8 [pii] 10.1016/S0079-6123(08)00437-8.

Mantella, R.C., Vollmer, R.R., Li, X., Amico, J.A., 2003. Female oxytocin-deficient mice display enhanced anxiety-related behavior. Endocrinology 144, 2291-2296.

Marcondes, F.K., Miguel, K.J., Melo, L.L., Spadari-Bratfisch, R.C., 2001. Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav 74, 435-440.

Maroun, M. (2013). Medial prefrontal cortex: multiple roles in fear and extinction. *Neuroscientist* 19**,** 370-383. doi: 10.1177/1073858412464527.

Meyer-Lindenberg, A., Domes, G., Kirsch, P., and Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci* 12**,** 524-538. doi: 10.1038/nrn3044 nrn3044 [pii].

McCarthy, M.M., McDonald, C.H., Brooks, P.J., Goldman, D., 1996. An anxiolytic action of oxytocin is enhanced by estrogen in the mouse. Physiol Behav 60, 1209-1215.

Mora, S., Dussaubat, N., Diaz-Veliz, G., 1996. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 21, 609-620.

Myers-Schulz, B., and Koenigs, M. (2012). Functional anatomy of ventromedial prefrontal cortex: implications for mood and anxiety disorders. *Mol Psychiatry* 17**,** 132-141. doi: 10.1038/mp.2011.88 mp201188 [pii].

Nakajima, M., Gorlich, A., and Heintz, N. (2014). Oxytocin Modulates Female Sociosexual Behavior through a Specific Class of Prefrontal Cortical Interneurons. *Cell* 159**,** 295-305. doi: 10.1016/j.cell.2014.09.020.

Naseri, MH, S. Hesami-Tackallou, M. Torabi-Nami, MR Zarrindast, and M. Nasehi. Involvement of the CA1 GABAA Receptors in MK-801-induced Anxiolytic-like Effects: An Isobologram Analysis. Behavioral Pharmocology 25.3 (2014): 197-205.

Neumann, I.D., Torner, L., Wigger, A., 2000. Brain oxytocin: differential inhibition of neuroendocrine stress responses and anxiety-related behaviour in virgin, pregnant and lactating rats. Neuroscience 95, 567-575.

Neumann, I.D., 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. Prog Brain Res 139, 147-162.

Neumann, I.D., Landgraf, R., 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35, 649-659.

Ninan, I., 2011. Oxytocin suppresses basal glutamatergic transmission but facilitates activity-dependent synaptic potentiation in the medial prefrontal cortex. J Neurochem 119, 324-331.

Nyuyki, K.D., Waldherr, M., Baeuml, S., Neumann, I.D., 2011. Yes, i am ready now: differential effects of paced versus unpaced mating on anxiety and central oxytocin release in female rats. PLoS One 6, e23599.

Paxinos, G., Watson, C., 1998. The rat brain in stereotaxic coordinates. Academic Press, San Diego, CA.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14, 149-167.

Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16: 279-88.

Postina R, Kojro E, Fahrenholz F (1998) Identification of neurohypophysial hormone receptor domains involved in ligand binding and G protein coupling. Adv Exp Med Biol 449: 371-85.

Qi, J., Han, W.Y., Yang, J.Y., Wang, L.H., Dong, Y.X., Wang, F., Song, M., and Wu, C.F. (2012). Oxytocin regulates changes of extracellular glutamate and GABA levels induced by methamphetamine in the mouse brain. *Addict Biol* 17**,** 758-769. doi: 10.1111/j.1369-1600.2012.00439.x.

Qi, J., Yang, J.Y., Wang, F., Zhao, Y.N., Song, M., and Wu, C.F. (2009). Effects of oxytocin on methamphetamine-induced conditioned place preference and the possible role of glutamatergic neurotransmission in the medial prefrontal cortex of mice in reinstatement. *Neuropharmacology* 56**,** 856-865. doi: 10.1016/j.neuropharm.2009.01.010S0028-3908(09)00023-9 [pii].

Ramos, L., Hicks, C., Kevin, R., Caminer, A., Narlawar, R., Kassiou, M., McGregor, I.S., 2013. Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-methylenedioxymethamphetamine in rats: involvement of the V1A receptor. Neuropsychopharmacology 38, 2249-2259.

Resstel, L.B., Souza, R.F., Guimaraes, F.S., 2008. Anxiolytic-like effects induced by medial prefrontal cortex inhibition in rats submitted to the Vogel conflict test. Physiol Behav 93, 200-205.

Ring, R.H., Malberg, J.E., Potestio, L., Ping, J., Boikess, S., Luo, B., Schechter, L.E., Rizzo, S., Rahman, Z., Rosenzweig-Lipson, S., 2006. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology 185, 218-225.

Rotzinger, S., Lovejoy, D.A., Tan, L.A., 2010. Behavioral effects of neuropeptides in rodent models of depression and anxiety. Peptides 31, 736-756.

Sabihi, S., Dong, S.M., Durosko, N.E., and Leuner, B. (2014a). Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Front Behav Neurosci* 8**,** 258. doi: 10.3389/fnbeh.2014.00258.

Sabihi, S., Durosko, N.E., Dong, S.M., and Leuner, B. (2014b). Oxytocin in the prelimbic medial prefrontal cortex reduces anxiety-like behavior in female and male rats. *Psychoneuroendocrinology* 45**,** 31-42. doi: 10.1016/j.psyneuen.2014.03.009.

Saitoh A, Ohashi M, Suzuki S, Tsukagoshi M, Sugiyama A, Yamada M, Oka J, Inagaki M, Yamada M (2014) Activation of the prelimbic medial prefrontal cortex induces anxiety-like behaviors via N-Methyl-D-aspartate receptor-mediated glutamatergic neurotransmission in mice. Journal of neuroscience research 92: 1044-53.

Sala, M., Braida, D., Lentini, D., Busnelli, M., Bulgheroni, E., Capurro, V., Finardi, A., Donzelli, A., Pattini, L., Rubino, T., Parolaro, D., Nishimori, K., Parenti, M., and Chini, B. (2011). Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: a neurobehavioral model of autism. *Biol Psychiatry* 69**,** 875-882. doi: 10.1016/j.biopsych.2010.12.022.

Shah, A.A., Sjovold, T., Treit, D., 2004. Inactivation of the medial prefrontal cortex with the GABAA receptor agonist muscimol increases open-arm activity in the elevated plus-maze and attenuates shock-probe burying in rats. Brain Res 1028, 112-115.

Shah, A.A., Treit, D., 2003. Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. Brain Res 969, 183-194.

Sierra-Mercado, D., Padilla-Coreano, N., and Quirk, G.J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharm* 36**,** 529-538. doi: 10.1038/npp.2010.184.

Slattery, D.A., Neumann, I.D., 2010. Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. Neuropharmacology 58, 56-61.

Smeltzer, M.D., Curtis, J.T., Aragona, B.J., Wang, Z., 2006. Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. Neurosci Lett 394, 146-151.

Sofroniew, M.V., 1983. Morphology of vasopressin and oxytocin neurones and their central and vascular projections. Prog Brain Res 60, 101-114.

Solati J, Hajikhani R, Golub Y (2013) Activation of GABAA receptors in the medial prefrontal cortex produces an anxiolytic-like response. Acta neuropsychiatrica : officieel wetenschappelijk orgaan van het IGBP 25: 221-6.

Stack, A., Carrier, N., Dietz, D., Hollis, F., Sorenson, J., Kabbaj, M., 2010. Sex differences in social interaction in rats: role of the immediate-early gene zif268. Neuropsychopharmacology 35, 570-580.

Stern, C.A., Do Monte, F.H., Gazarini, L., Carobrez, A.P., Bertoglio, L.J., 2010. Activity in prelimbic cortex is required for adjusting the anxiety response level during the elevated plus-maze retest. Neuroscience 170, 214-222.

Sullivan, R.M., Gratton, A., 2002. Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. Brain Res 927, 69-79.

Sylvers, P., Lilienfeld, S.O., and Laprairie, J.L. (2011). Differences between trait fear and trait anxiety: implications for psychopathology. *Clin Psychol Rev* 31**,** 122-137. doi: 10.1016/j.cpr.2010.08.004.

Toth, I., Neumann, I.D., and Slattery, D.A. (2012). Central administration of oxytocin receptor ligands affects cued fear extinction in rats and mice in a timepoint-dependent manner. *Psychopharmacology (Berl)* 223**,** 149-158. doi: 10.1007/s00213-012-2702-4.

Uvnas-Moberg, K., Ahlenius, S., Hillegaart, V., Alster, P., 1994. High doses of oxytocin cause sedation and low doses cause an anxiolytic-like effect in male rats. Pharmacol Biochem Behav 49, 101-106.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L., and Quirk, G.J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn Mem* 13**,** 728-733. doi: 13/6/728 [pii]

Waldherr, M., Neumann, I.D., 2007. Centrally released oxytocin mediates mating-induced anxiolysis in male rats. Proc Natl Acad Sci USA 104, 16681-16684.

Walf, A.A., Frye, C.A., 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2, 322-328.

Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138, 2829-2834.

Yamada M, Saitoh A, Ohashi M, Suzuki S, Oka JI, Yamada M (2015) Induction of c-Fos immunoreactivity in the amygdala of mice expressing anxiety-like behavior after local perfusion of veratrine in the prelimbic medial prefrontal cortex. Journal of neural transmission.

Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., Nishimori, K., 2009. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. J Neurosci 29, 2259-2271.

Yosten, G.L., and Samson, W.K. (2010). The melanocortins, not oxytocin, mediate the anorexigenic and antidipsogenic effects of neuronostatin. *Peptides* 31**,** 1711-1714. doi: 10.1016/j.peptides.2010.06.017.

Zarrindast, Mohammad R., Hatam Ahmadi, and Mohammad Nasehi. "Interaction of GABA (A) System in the Nucleus Accumbens Ahell with ACPA on Anxiety-like Behaviors in Wistar Male Rat." Journal of Experimental and Applied Animal Science 1.1 (2013): 44-55. Web.