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**AN INVESTIGATION OF
OXYTOCINERGIC MODULATION OF
STRIATAL DOPAMINE
NEUROCHEMISTRY AND BEHAVIOR
IN THE MALE PRAIRIE VOLE**

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Abstract

The neuropeptide oxytocin (OXT) is notable for its diverse range of physiological and behavioral effects across mammalian taxa. Beyond OXT's well-documented role in modulating basic reproductive processes during parturition and lactation, OXT is also involved in the modulation of complex social behaviors such as pair bonding, mother-infant bonding, sexual behavior, and selective affiliation with conspecifics. Converging evidence from human and rodent studies indicate that OXT modulates a number of these behaviors via interactions with mesolimbic dopamine (DA) circuitry. Mesolimbic DA systems are strongly implicated in the attribution of salience and motivation towards cues for natural rewards and drugs of abuse, suggesting that centrally released OXT may serve to modulate the attribution of incentive salience towards social cues. Here, we used male prairie voles (*Microtus ochrogaster*) to investigate the reinforcing effects of central administration of OXT via the conditioned place preference paradigm (CPP) and DA neurotransmission in the striatum using *in vitro* fast scan cyclic voltammetry (FSCV). A centrally infused 100ng dose of OXT proved to be ineffective for inducing a CPP. Additionally, we found no significant differences in DA release dynamics in the nucleus accumbens (NAcc) shell, NAcc core, and dorsal striatum following OXT experience in the CPP training paradigm. Together, these experiments demonstrate a need for further research on OXT's dose dependent effects on the mesolimbic DA system. Given OXT's promise as a potential therapy for drug addiction, autism, and schizophrenia, future research will result in novel insights regarding how OXT might be used in a clinically relevant context.

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For A.M.M., A.A.V., C.M.E., W.G.V., T.J.C., N.M., and of course my dog, Casper.

Introduction

Among mammalian taxa, primate species are defined by their uniquely social nature. The complex social interactions that characterize primate group living are essential for proper development, survival, and reproduction. The navigation of challenging social environments requires an ability to selectively direct attention and behavior towards conspecifics that promote fitness, while avoiding individuals or social interactions that cause bodily harm or prevent mating opportunities. Assessing which social interactions will result in increases or decreases in fitness benefits necessitates the analysis of valence and the attribution of salience to discrete social interactions. Thus, the neurobiological mechanisms underlying selective social behaviors likely were the target of intense selective pressure in the evolution of modern human behaviors. However, the neural mechanisms underlying the attribution of social salience remains poorly studied in primates, and mammals more generally. Due to its role in modulating both positive and negative social behaviors, mother-infant interactions, and its unique anatomical overlap with mesolimbic dopamine circuitry, the neuropeptide oxytocin (OXT) has been recently investigated as a likely candidate molecule for influencing the attribution of social salience. OXT-like peptide hormones are remarkably evolutionarily conserved and have been identified in a diverse range of taxa including hydra, insects, and vertebrates, where they play a general role in the modulation of social and reproductive behaviors (Knobloch & Grinevich, 2014; Donaldson & Young, 2008). Recent evidence from studies in monogamous rodents suggests that interactions between OXT and DA may be necessary for the formation of a selective social attachment known as a pair bond. Only 3-5% of mammals exhibit monogamous pair bonded social structures (Kleiman, 1977), and some have posited that these long lasting forms of social bonding may have been a critical factor in the evolutionary development of large brains in primates (Dunbar & Shultz, 2007). Although qualitative differences exist between primate and rodent pair bonding

behaviors, the monogamous prairie vole (*Microtus ochrogaster*) has emerged as a powerful model organism for the neurobiological investigation of affiliative social behaviors in the laboratory (Carter et al. 1995; Young & Wang, 2004). Prairie voles are small rodents that range primarily in the grasslands of the central United States (Hoffmann and Koepl, 1985). The low annual rainfall and food resources of minimal caloric value which typify these environments are posited as likely factors contributing towards the selection of monogamy in prairie voles, as food scarcity increases female territorial behaviors and inhibits the abilities of males to monopolize multiple females (Emlen & Oring, 1977; Carter et al., 1995). Both male and female prairie voles engage in pair bonded relationships which are defined as a stable relationship between members of a breeding pair that share parental duties and common territory (Aragona & Wang, 2004). In contrast to most other rodent species, male prairie voles contribute to the construction and defense of the nest, engage in extensive social contact, and retrieve pups that wander from the nest (Getz & Carter, 1996; Gruder-Adams & Getz, 1985). The long term maintenance of a pair bond is usually also accompanied by selective aggression by the male towards non-bonded females (Aragona et al., 2006; Gobrogge et al., 2007). Importantly, prairie voles are considered to be socially monogamous and not genetically monogamous, as extra-pair copulations have been observed in both laboratory and field conditions (McGraw & Young, 2010). Socially monogamous behaviors are reliably expressed under laboratory conditions and can be assessed using the resident-intruder test and partner preference test paradigms (for a review of these techniques, see Aragona & Wang, 2009).

OXT is a nonapeptide neurohormone composed of a six amino acid ring and a three amino acid tail. Biochemically, the ability of OXT to form both temporary and long-lasting interactions with other molecules may stem from the properties of the sulfur bonds that create the amino acid

ring (Martin & Carter, 2013; Carter, 2014). OXT is synthesized primarily in magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Once synthesized, OXT molecules are packaged into vesicles that are transported down axonal projections through the infundibular stalk to the posterior pituitary for release into the peripheral circulation (Stoop, 2012; Meyer-Lindenberg et al. 2011). Peripherally, oxytocin acts on the uterine myometrial cells to induce contractions during parturition and on myoepithelial cells in the alveolar ducts to stimulate milk ejection during lactation (Dale 1906; Insel et al., 1997; Greenspan et al. 2007). Secretory vesicles containing OXT are distributed in the cell bodies, dendrites, axons, and nerve terminals of PVN and SON neurons and are capable of somatodendritic release into the extracellular space in response to rapid increases in intracellular calcium concentration. This property of OXT neurons allows the diffusion of OXT via volume transmission to distant targets in the central nervous system (Neumann & Landgraf, 2012; Stoop 2012). Additionally, a smaller subset of parvocellular neurons in the PVN that produce OXT send direct projections to diverse brain regions including the amygdala, hippocampus, nucleus accumbens (NAcc), ventral tegmental area (VTA), and brainstem (Ross et al., 2009; Stoop, 2012). Once it reaches the target site, OXT primarily binds to a seven transmembrane Class 1-type G protein coupled receptor (OXTR) and additionally has weak affinity for the three classes of vasopressin receptors (Gimple & Fahrenholtz, 2001; Gimple et al., 2008). OXTRs are densely expressed throughout the spinal cord and brain, including in the accessory olfactory bulb, CA1 of hippocampus, ventral medial hypothalamus, and NAcc (for review, see Stoop, 2012). Critically, the Insel group has demonstrated that the distribution of OXTRs in the brain is both species dependent and correlated with within species differences in social behaviors such as pair bonding (Insel & Shapiro, 1992; Insel & Young, 2001). Specifically, prairie voles have increased

expression of OXTRs in the prelimbic cortex and NAcc, while polygamous montane voles exhibit increased expression in the lateral septum (Insel & Shapiro, 1992).

The processing of sensory stimuli into motivated behavioral output occurs in the basal ganglia and is mediated by DA signaling in the mesocorticolimbic DA system. This system is primarily composed of dopaminergic neurons within the VTA and their widespread projections to the cortex and limbic regions which act in concert to promote motivated behaviors. Specifically, the VTA projects to the ventral striatum, hippocampus, amygdala, ventral pallidum, and the prefrontal cortex (Love, 2014; for review, see Ikemoto, 2007, 2010). Within the ventral striatum, the NAcc serves as a critical brain region for reward processing and motivated behaviors. The NAcc, via its dense reciprocal interconnections with regions such as the prefrontal cortex, amygdala, thalamus, and hippocampus, serves as junction for the cortico-limbic and motor system and is critical for the generation of voluntary motor movement (Kelley & Swanson, 1995; Kelley, 1999; Kelley, 2004). Moreover, the unique anatomical connectivity of the NAcc aids in the ability to translate affective or emotional cognition into voluntary action, permitting the expression of instrumental learning behaviors or the motivated pursuit of food, water, reproductive partners, or cues for drugs of abuse (Kelley, 2004; Berridge, 2007; Nicola, 2007). Based on its anatomical connectivity to the VTA, the NAcc is functionally subdivided into the NAcc core and NAcc shell regions (Ikemoto, 2007). DA transmission in the NAcc core is critical in mediating learning and the performance of motivated behaviors (Ikemoto & Panksepp, 1999; Cardinal et al., 2002; Saunders et al., 2012, 2013), while DA signaling in the NAcc shell is implicated in the modulation of appetitive and consummatory motivational behavior in rodents (Swanson et al., 1997). Importantly, pioneering work by Calipari et al. (2012) demonstrated the existence of an evolutionarily conserved dorsal-ventral gradient of DA

release and uptake in the striatum of mice, rats, and rhesus macaques. Specifically, they found a high to low gradient of stimulated DA release from the dorsal striatum, NAcc core, and the NAcc shell using fast scan cyclic voltammetry to measure sub-second fluctuations in DA release. These differences in overall dopamine signaling contribute to the functionally distinct subnuclei of the striatum.

Pioneering work over the last 20 years has established a critical role for OXT and DA microcircuitry in the expression of socially monogamous behaviors. Intracerebroventricular (i.c.v.) administration of an OXT receptor antagonist in female prairie voles blocks mating-induced partner preference formation, while administration of OXT facilitates the formation of a partner preference in the absence of mating (Williams et al., 1994). Similarly, administration of DA antagonists block, while DA agonists induce, the formation of partner preferences in female prairie voles (Wang et al. 1999; Gingrich et al., 2000). Liu and Wang (2003) demonstrated that the NAcc region of the mesocorticolimbic dopamine system of the striatum was a critical area for OXT and DA interactions and the formation of partner preferences. Specifically, antagonistic blockade of OXT receptors prevented partner preferences induced by DA D2-like receptor agonists, while administration of a DA D2-like antagonist, but not a D1-like antagonist, prevented the formation of OT-induced partner preferences. Thus, access to NAcc OXT receptors and DA D2-like receptors are necessary for the formation of a partner preference. Subsequent research building from these findings have further illuminated the importance of the NAcc in the expression monogamous behaviors. Low dose infusions of the non-selective DA receptor agonist apomorphine into the NAcc of prairie voles facilitates partner preference formation, but high dose infusions of apomorphine do not (Aragona et al., 2003). This dosage effect of apomorphine suggested that a receptor specific mechanism modulated the observed

behavioral effects. DA D1-like receptors (D1 and D5 receptors) have a lower affinity for DA binding than the DA D2-like receptors (D2, D3, and D4 receptors) (Neve et al., 2004). Aragona et al. (2006) showed that activation of D2-like receptors within the NAcc shell region, but not the NAcc core region, induced partner preferences in the absence of mating. Furthermore, the researchers determined that administration of a specific D1-like receptor agonist not only failed to produce a partner preference, but that D1-like receptor activation blocked partner preferences induced by administration of a specific D2-like receptor agonist. Thus, differential activation of the two classes of DA receptors dynamically regulates the formation of this important monogamous behavior. Further evidence for the duplicity of DA signaling on the facilitation of partner preference formation stemmed from manipulations of the intracellular signaling mechanisms of the DA D1-like and D2-like receptors which stimulate and inhibit the conversion of ATP to cAMP via adenylyl cyclase, respectively (Nesse et al., 2004; Missale et al., 1998; Aragona & Wang, 2007). Binding of cAMP to PKA releases PKA's regulatory subunits, ultimately resulting in altered gene expression in the cell nucleus (Lynch & Taylor, 2005; Self et al., 1998). In support of their previous findings, Aragona and Wang (2007) found that decreasing PKA activity induced partner preferences in the absence of mating while increasing PKA activity failed to induce partner preferences and prevented mating-induced pair bond formation.

Behaviorally, prairie voles undergo dynamic changes as they shift from sexually naïve to pair bonded (Carter et al. 1995). As expected, sexually naïve male prairie voles show affiliative behavior towards novel females. After an extended cohabitation period with mating and the induction of a pair bond, males switch their behavior and become aggressive towards novel females (Gobrogge et al., 2007; Wang et al., 1997). Aggressive behaviors were abolished via the administration of a selective D1-like, but not D2-like, receptor antagonist, showing that D1-like

receptors mediates selective aggression. Importantly, Aragona et al. (2006) showed that D1-like receptors are substantially upregulated in the NAcc of pair bonded males, providing a physiological marker of the transition from naivety to pair bonded.

The molecular mechanisms underlying the effects of OXT on pair bonding are less clear. OXT receptors are hypothesized to be post-synaptic as selective lesions of dopaminergic terminals using 6-OHDA in the NAcc did not alter levels of OXT receptor expression (Lim et al., 2004). As both OXT and D2-like receptors are G_i coupled, each of these receptors may facilitate partner preference formation by independently or additively inhibiting cAMP signaling pathways (Aragona & Wang, 2007, 2009). Direct infusion of OXT into the VTA of lactating rats increased dopamine release in the NAcc, demonstrating the direct influence of OXT on striatal neurochemistry (Shahrokh et al., 2010). Romero-Fernandez et al. (2013) recently reported evidence for the existence of a D2-OXT receptor heteromer in the ventral and dorsal striatum. OXT receptor agonist binding to the D2-OXT receptor heteromer increased the affinity of the D2 receptor for its ligand (Romero-Fernandez et al., 2013). In addition to DA and OXT interactions, coordinated NAcc OXT and serotonin transmission has recently been shown to be required for the rewarding properties of social interactions in mice (Dölen et al., 2013). Further investigations are needed to fully illuminate the molecular interactions between OXT and striatal microcircuitry.

The widespread distribution of OXT receptor expression in the mesocorticolimbic dopamine system strongly supports the hypothesis that central OXT release may modulate reward and motivated behaviors via interactions with DA. Additionally, the observation that the effects of OXT appears to be context dependent and can be both positive and negative has led some groups to conclude that the behavioral effects of OXT are a result of the strong functional

interactions between OXT and the DA system to increase the salience of social cues (Shamay-Tsoory & Abu-Akel, 2016; Love, 2014; Averbach, 2010; Bartz et al., 2011; Burkett & Young, 2012). The ability to noninvasively deliver intranasal OXT (inOXT) has served as an impetus for investigations of the positive and negative effects of OXT in humans (Born et al. 2002; for review of translational implications and current research, see Meyer-Lindenberg et al. 2011). Myriad evidence exists for the prosocial effects of OXT. Subjects given inOXT experienced improved performance on emotional recognition tasks when observing other people's facial expressions, increased cooperative trust, and increased generosity in a financial game (Lishke et al., 2012; Marsh et al. 2010; Kosfeld et al. 2005; Zak et al., 2007). inOXT increases negative social emotions in a game of chance played with another human where the participant either won more money, less money, or won/lost equal amounts of money. Specifically, intranasal OXT increased ratings of gloating (*schadenfreude*) during relative gain conditions, while ratings of envy were increased during relative loss conditions (Shamay-Tsoory et al., 2009). Despite OXT's reputation as a non-selective love hormone or cuddle chemical, intranasal OXT administration appears to increase trust and cooperation with familiar members of an in-group, increase ethnocentrism, and increase defensive aggression towards members of an out-group (De Dreu, 2012; De Dreu et al., 2010, 2011). Additionally, OXT administration increased the willingness of participants to lie when doing so benefited their social group financially, but not when lying benefited only the individual. Thus, OXT seems to shift individual's choices from self to group interests (Shalvi & De Dreu, 2014). Interestingly, in studies of wild chimpanzees, urinary oxytocin levels increase after grooming sessions with bond partners compared with non-bond partners (Crockford et al., 2013), and also after food sharing events between group

members compared to non-social feeding events (Wittig et al., 2014; for review, see Crockford et al., 2014).

Given the evidence showing that OXT dynamically modulates social behaviors, OXT has been investigated as a potential treatment for disorders characterized by severe social dysfunction as autism, schizophrenia, and drug addiction (Guastella et al., 2010; Pederson et al., 2011; Rubin et al., 2010; Kovacs et al., 1998; McGregor & Bowen, 2012; Sarnyai & Kovacs, 2014; Lee et al., 2016). Deficits in social behavior are well documented in drug and alcohol abusers, suggesting that drugs of abuse may modify the endogenous oxytocin system as well as reward and motivational circuitry (Strathearn & Mayes 2010; Young et al. 2011). Interestingly, having strong social bonds in the form of parent-infant relationships, family structure, and peer groups, appear to have protective effects against vulnerability for substance abuse and associated psychological disorders (Kendler et al. 2000; Bell et al. 2000; Ellickson et al. 1999). Although the biological mechanisms are unknown, repeated administration of psychostimulants, opiates, and alcohol each appear to reduce central OXT synthesis, suggesting strong reciprocal interactions exist between OXT and motivational circuitry (Kovacs., 1998; Sarnyai & Kovacs, 2014). OXT limits the psychoactive behavioral effects of both methamphetamine and cocaine (Carson et al. 2010a; Carson et al. 2010b; Sarnyai et al. 1990; Sarnyai et al. 1992), and pre-clinical human studies in schizophrenics have shown oxytocin administration reduces psychotic symptoms and improves social cognition (Pederson et al. 2011; Rubin et al. 2010). However, little is known about OXT's effects on striatal dopamine neurotransmission, which have been strongly implicated in the processing of drug reward, motivation, and the attribution of incentive salience (Hyman et al. 2006; Kelley & Berridge, 2002; Berridge & Robinson 1993).

Based on the research reviewed here, OXT is a primary candidate for the modulation of the reinforcing properties of positive and negative social interactions within the NAcc. Herein, we assessed the reinforcing properties of centrally administered OXT using the CPP paradigm. Second, we tested how experience with OXT conditioning in the CPP paradigm modulated DA release dynamics in the NAcc shell, NAcc core, and dorsal striatum using fast scan cyclic voltammetry to measure real-time dopamine transmission in the socially monogamous male prairie vole.

Materials and methods

Subjects

Subjects were sexually naïve adult male prairie voles from a laboratory breeding colony housed at the University of Michigan. Subjects were weaned at 21 days of age and housed in same-sex sibling pairs inside plastic cages (12 x 28 x 16 cm) containing pine chip bedding, with food and water provided *ad libitum*. All cages were maintained at approximately 20° C with a 14:10 h light:dark cycle (lights on at 0600 h) and all experiments were conducted between 0800h and 1800h. All subjects were between 90 and 150 days of age when tested, weighed between 35 and 50 g, and were randomly assigned to a drug condition. Experimental procedures were conducted in accordance with the animal care guidelines of the University of Michigan and approved by the University of Michigan Institutional Animal Care and Use Committee.

Stereotaxic intracerebroventricular cannulation surgery

For intracerebroventricular (i.c.v) cannula placement, subject animals were anesthetized and underwent stereotaxic surgery. A 26-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA) was aimed at the right lateral ventricle (+0.6 mm anterior-posterior, -1.0 mm medial-lateral, and -2.65 mm dorsal-ventral from bregma). Three sterile stainless-steel jeweler's

screws (Fine Science Tools) and dental cement were used to secure the guide cannula and to create a head cap. All subjects were given a 10mg/kg ketoprofen injection immediately after surgery and triple antibiotic ointment was applied around the edges of the head cap to prevent infection. Animals were placed on a heating pad until they regained consciousness and then they were transferred back to their home cage. Animals were allowed 3-5 days of recovery in their home cage with their cage mate prior to the first day of CPP testing.

Microinfusion

During the conditioning trials (CPP Days 2-4), subjects received a single infusion in both the morning and evening conditioning session of either OT (oxytocin acetate salt hydrate, Sigma) in artificial cerebrospinal fluid (aCSF) or aCSF as a control (refrigerated aCSF solution consists of: 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 2.4 mM CaCl₂, 1.2 mM MgCl₂, and 25 mM NaHCO₃; pH 7.38-7.4). Briefly, animals were removed from their home cages and gently restrained on a flat surface while an infusion cannula (33 gauge, Plastics One, Roanoke, VA) attached to polyethylene tubing was lowered into the guide cannula. Using an electronic infusion pump (Harvard Apparatus??), a total volume of 1 μ L of OT (100 ng/ μ L in aCSF) or aCSF was injected via a Hamilton 10 μ L microsyringe through the infusion cannula over a 1-minute period (0.5 μ L/minute flow rate). Infusion cannulas were left in place for an additional 1-minute after the injection to ensure full delivery of vehicle and drug. Subjects were then placed in the appropriate conditioning chambers as described below.

Conditioned Place Preference Testing

The conditioned place preference (CPP) paradigm has been extensively used to study the motivational or reinforcing properties of various pharmacological agents (Bardo et al., 1995). CPP procedures were performed as previously described (Aragona et al., 2007; Liu et al., 2010;

(Aragona, Detwiler, & Wang, 2007; Yan Liu et al., 2010; Yan Liu, Young, Curtis, Aragona, & Wang, 2011). Briefly, the CPP testing apparatus consisted of two opaque plastic cages (12 X 28 X 16 cm), one black with a metal top and one white with a mesh top, connected by a clear Plexiglas hollow tube (7.5 X 16 cm). The metal lid allowed less light into the black cage thereby creating a darker environment compared to the white cage. Although previous work suggests prairie voles tend to prefer the white cage over the black cage (Aragona et al., 2007), we observed a great deal of individual differences in this preference. Therefore, subjects were tested in the CPP apparatus for their initial cage preference on the morning of Day 1 in a 30-minute pretest. At the start of the pretest, one half of the subjects were initially placed in the black cage and the other half were initially placed in the white cage. All subjects were allowed to roam freely between the two cages during the pretest, and behavior was videotaped for later analysis. On Days 2 through 4, subjects underwent two 60-minute conditioning sessions 6 hours apart during the light cycle. All experimental subjects were counter-balanced in regard to whether they received 100 ng OT in aCSF during the morning session or evening session. Control subjects received 1 μ L aCSF infusions for both the morning and evening conditioning sessions. Experimental subjects were given OT in the cage in which they had spent less time during the pre-test (conditioned cage) in an attempt to reverse the initial preference; i.e. a biased test. During the conditioning sessions, wire mesh was placed over the hole left by the removed Plexiglas tube for both the black and white cages. 30-minute post-tests were conducted on the morning of Day 5 to test for the presence of a CPP. A CPP was defined as a significant change in the duration of time spent inside the OT-paired cage before and after conditioning. Data is presented as the change in time from the pretest for both the OT and aCSF (control) groups: total time spent in the OT (or aCSF) cage during the post-test divided by the total time spent in the OT

(or aCSF) cage during the pre-test, multiplied by 100 (Aragona et al. 2007). Subjects were videotaped during all test and conditioning sessions and the videos were later scored for the amount of time each individual spent in each cage using BehaviorTracker video analysis software. Immediately after the post-test, subjects were rapidly decapitated and their brains halved along the midline. The right hemisphere was frozen on dry ice for future tissue microdissection experiments and the left hemisphere was processed for analysis using fast scan cyclic voltammetry as described below.

in vitro fast scan cyclic voltammetry

In order to assess the functional effects of oxytocin administration on real time dopamine transmission, fast scan cyclic voltammetry (FSCV) was performed according to established protocols (Maina et al., 2012; Singer et al., 2016). Approximately 20 minutes after the post-test, subjects were euthanized by rapid decapitation and brains were immediately extracted. The left hemisphere was used for FSCV experiments while the right hemisphere was immediately frozen on dry ice and stored at -80°C for cannula placement verification and future autoradiography and protein analysis studies to be completed Summer 2016 (Resendez et al., 2012). Brains were sectioned into 400 μm coronal slices containing the striatum with a vibratome and submerged in oxygenated (95% O_2 /5% CO_2) aCSF maintained at 32°C (aCSF prepared the day of the experiment; aCSF solution consists of: 126 mM NaCl, 2.5 mM KCl, 2.4 CaCl_2 , 1.2 mM MgCl_2 , 25 NaHCO_3 , 1.2 mM NaH_2PO_4 , 11 D-glucose, 0.4 ascorbic acid; pH 7.38-7.4). A glass-encased carbon fiber recording electrode was targeted to the NAc shell, NAc core, and dorsal striatum. Pre-calibration of the recording electrode allows for current measurements to be converted to dopamine concentration (Singer et al., 2016). A bipolar stimulating electrode was positioned nearby the recording electrode to evoke optimal DA release. Following stabilization of the

recording electrode, three rounds of alternating single-pulse (300 μ A, 4 ms), five-pulse (300 μ A, 20 Hz), and twenty-pulse (300 μ A, 20 Hz) electrical stimulations were applied once every 5 minutes to the NAc shell, NAc core, and dorsal striatum. All stimulations and recordings were performed using TarHeel Voltammetry software.

Results

i.c.v. administration of 100 ng of OXT does not reliably induce a CPP

To our knowledge, this study represents the first investigation of the reinforcing properties of centrally infused OXT in male prairie voles. As previously described, a conditioned place preference was defined as a significant increase in the time spent in the OXT-paired cage during the posttest relative to the pretest after 3 days of OXT conditioning. OXT has been shown to induce CPP at high doses (6 mg/kg) when injected subcutaneously in male Sprague-Dawley rats (Liberzon et al. 1997). Relatively high doses of oxytocin are required when administered systemically as OXT has poor penetration of the blood-brain barrier (Ermisch et al., 1985). Therefore, we hypothesized that a 100ng dose of OXT in a non-social CPP paradigm would not have reinforcing effects. Statistical analyses were performed using data from a total of 15 male prairie voles (vehicle: $n = 6$; 100ng of OXT: $n = 9$). Overall, there were no significant differences in the time spent in the un-preferred cage during the posttest relative to the pretest between the vehicle (aCSF) and vehicle + 100 ng of OXT subjects (Mann-Whitney two-tailed t test; $U = 18.00$, $p = 0.3265$) (Fig. 1). Therefore, centrally infused OXT (100 ng) does not reliably produce a CPP in male prairie voles. However, these data should be considered preliminary until cannula placement analyses have been performed (analyses scheduled for Summer 2016). Additionally, we noted high levels of variation in the amount of time spent manipulating bedding materials

during the test (data analysis to be completed Summer 2016), which may explain the high standard error measurements obtained from the analysis.

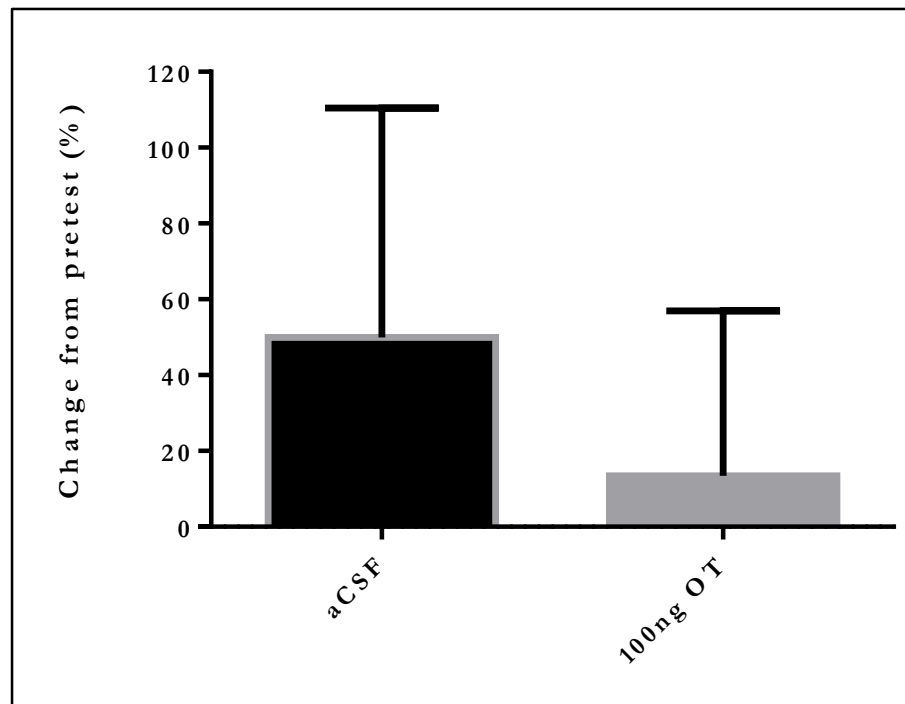


Figure 1. Reinforcing effects of 100ng of OXT. Experience with OXT during the CPP paradigm did not significantly alter the percent change in time spent in the unpreferred cage during the posttest relative to the pretest.

No differences in striatal dopamine release following experience in the CPP paradigm

We first compared stimulated DA release in the NAcc shell, NAcc core, and dorsal striatum of sexually naïve male prairie voles following three consecutive days of experience with either aCSF or OXT in the CPP paradigm (Fig. 2). We found within-subject regional differences in stimulated DA release (Two-way ANOVA with multiple comparisons; $F_{(2,8)} = 9.583$, $p = 0.008$), but no between-subject differences in stimulated DA release ($F_{(1,4)} = 1.581$, $p = 0.277$). Within-subject region X condition interactions were not significant ($F_{(2,8)} = 1.774$, $p = 0.230$). Stimulation effects ($F_{(2,8)} = 2.686$, $p = 0.128$) and stimulation X condition ($F_{(2,8)} = 0.240$, $p = 0.792$) effects were not significant. Region X stimulation effects ($F_{(4,16)} = 0.854$, $p = 0.512$) and

region X stimulation X condition effects ($F_{(4,16)} = 0.578$, $p = 0.682$) were also not significant (Fig. 3 and 4). Therefore, experience with administration of 100ng of OXT in the CPP paradigm did not robustly alter stimulated DA release across brain areas and across different stimulation regimens (1 pulse, 5 pulse, and 20 pulse stimulations).

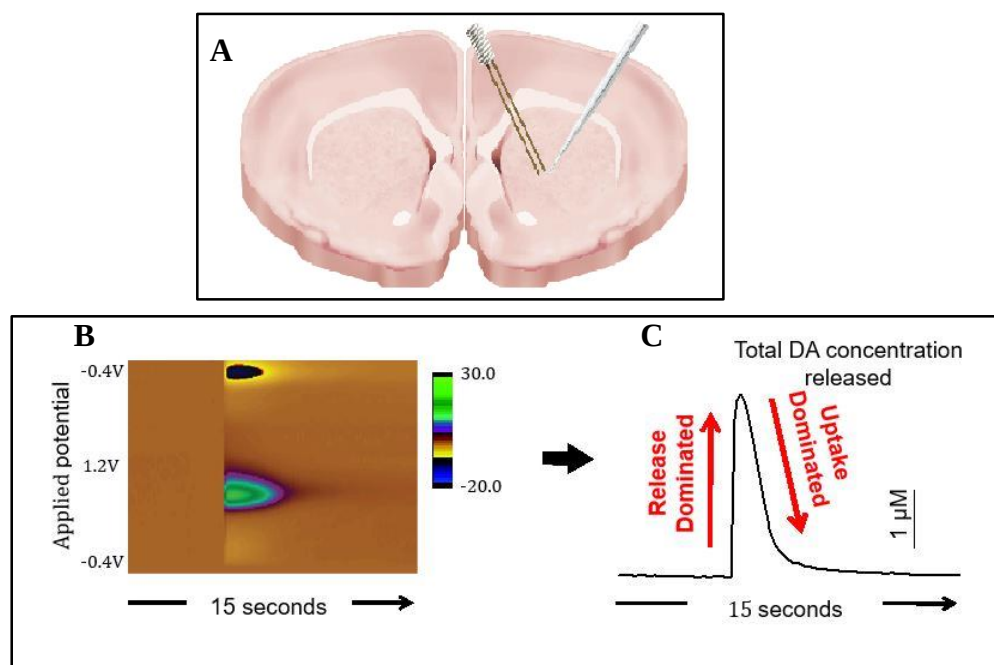


Figure 2. Striatal *in vitro* FSCV methods. A) A bipolar stimulating electrode and a carbon fiber recording electrode are placed into the target brain region (NAcc shell, NAcc core, or dorsal striatum). A depolarizing electrical stimulation evokes DA release, which is measured by the carbon fiber electrode. B) A representative color plot shows changes in current during the 15s recording period. 1 pulse, 5, pulse, and 20 pulse stimulations are applied 5 seconds into the recording (20 Hz). C) Following calibration of the electrode, current is converted into DA concentration.

Although many of the above results were not significant, we believe that these results warrant replication and further investigation using higher doses of OXT, as systemically administered OXT has been shown to dose-dependently attenuate tolerance and self-administration behavior to heroin and alcohol (see Kovacs et al., 1998 for a thorough review). As these data are preliminary, the addition of more animals to control condition will yield more

significant results. Furthermore, in future investigations the injections should be given to freely moving, unrestrained animals to limit the possibly confounding effects of stress.

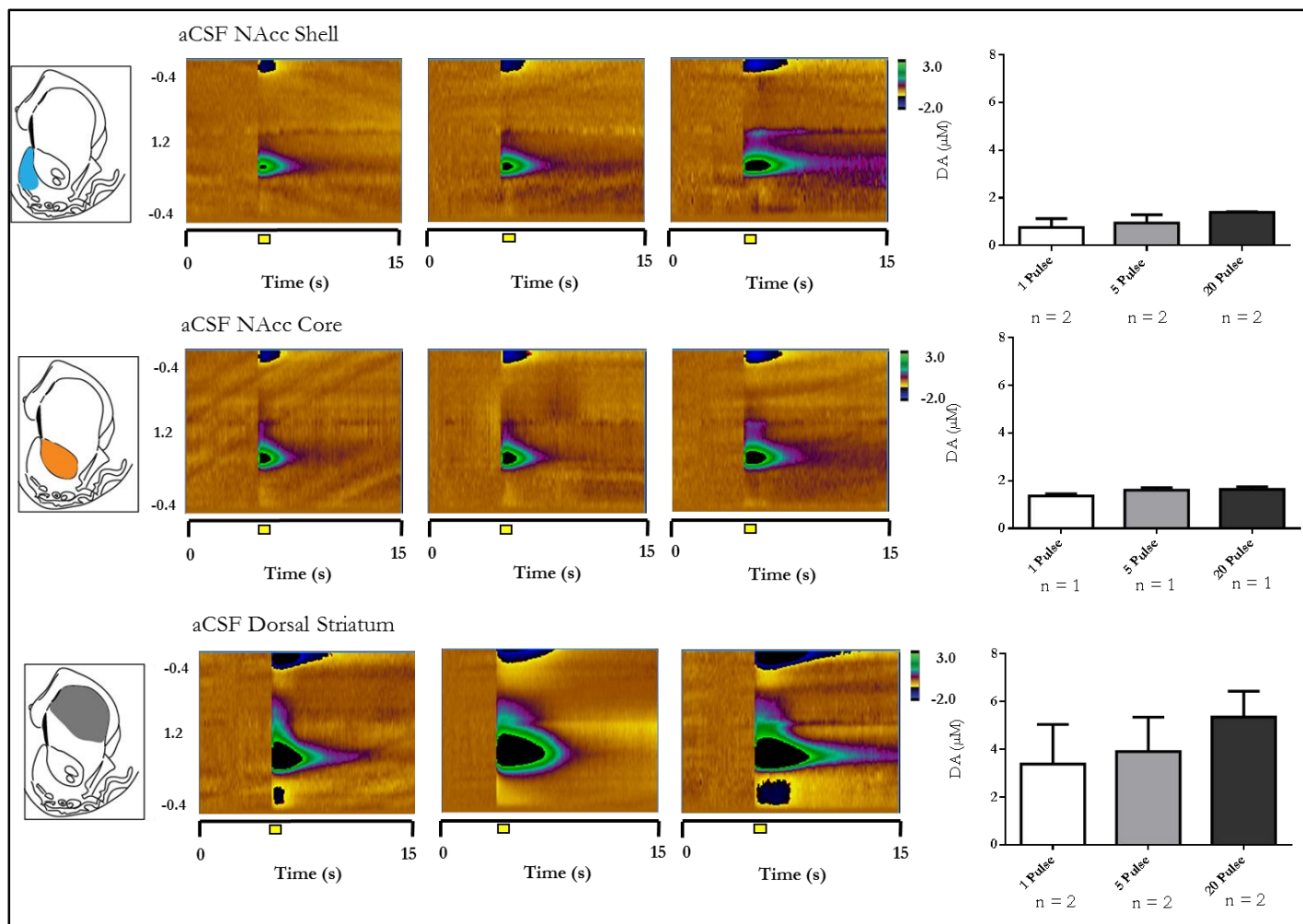


Figure 3. Effect of aCSF exposure in CPP paradigm on striatal DA release. There were no significant differences between stimulations within the NAcc shell, NAcc core, or dorsal striatum after three days of conditioning with aCSF in the CPP paradigm. Similarly, there were no significant effects of the pulse stimulation (1 pulse, 5 pulse, and 20 pulse) on evoked dopamine release within each brain region.

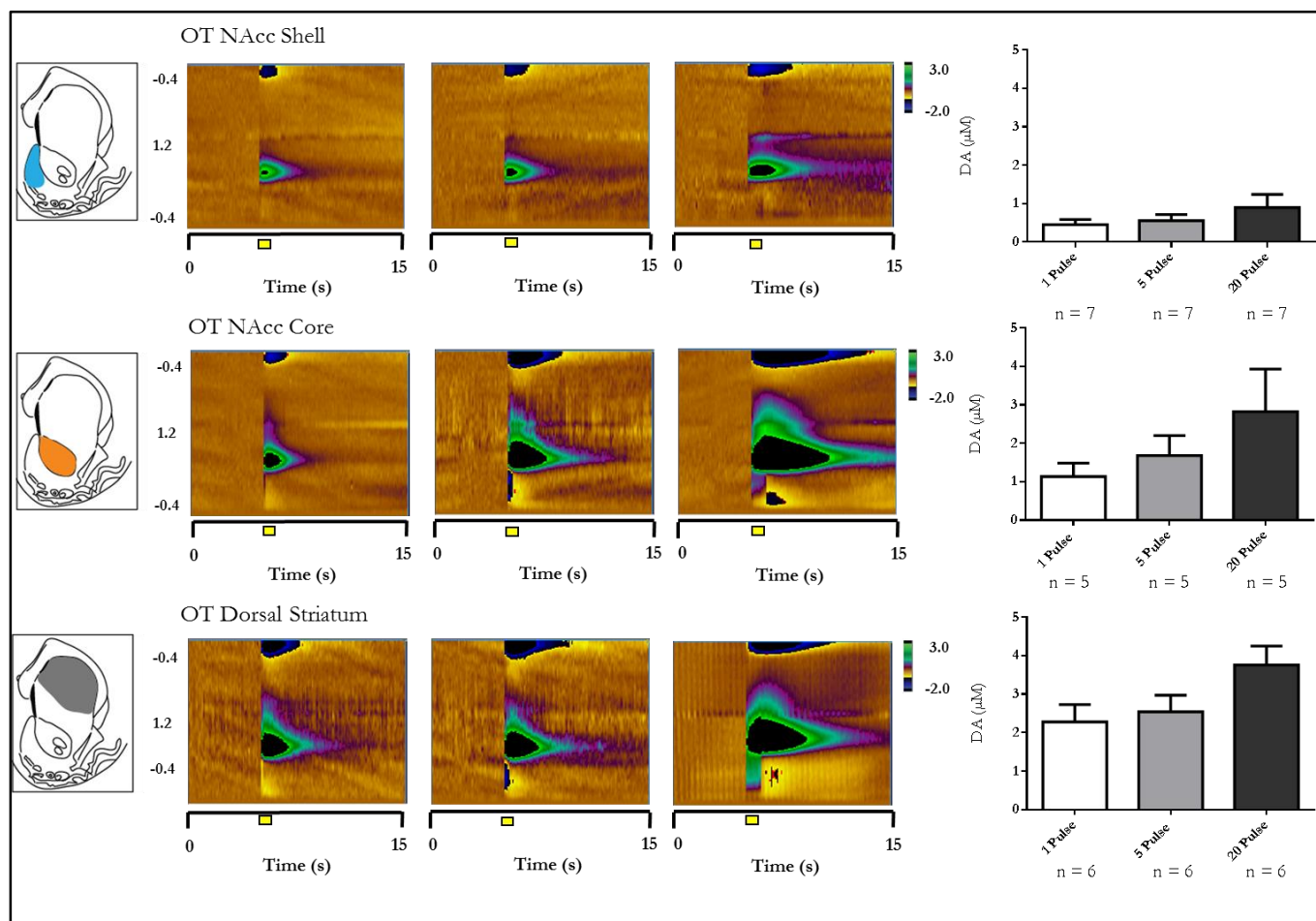


Figure 4. Effect of aCSF exposure in CPP paradigm on striatal DA release. There were no between-subject differences between stimulations within the NAcc shell, NAcc core, or dorsal striatum after three days of conditioning with 100ng of OXT. Similarly, we found no significant effect of the pulse stimulation (1 pulse, 5 pulse, and 20 pulse) on evoked dopamine release within each brain region.

Discussion

The formation of selective social bonds is a critical attribute of group living mammalian species, yet the neurobiological underpinnings of these behaviors remain poorly understood. Elucidating the cellular and molecular mechanisms underlying the development of social behaviors remains a key goal for understanding the evolution of modern human behavior, and will likely result in the development of novel therapies for ailments associated with dysregulated social behaviors including autism, schizophrenia, and drug abuse. To our knowledge, this study

represents the first examination of the reinforcing properties of centrally administered OXT in the male prairie vole. Our data show that an i.c.v. administration of 100 ng dose of OT is not reinforcing as assessed by the CPP paradigm. However, our data showed extreme variability within groups in the amount of time spent in the unpreferred cage during the posttest. The observed variability may have been the result of the animals chewing and manipulating the bedding material on the bottom of the CPP chambers. Video scoring of these behaviors are scheduled for Summer 2016, and therefore these results should be considered preliminary. Moreover, at the start of the CPP test, all subjects had relatively few experiences being handled by experimenters. Therefore, handling during the microinjection procedure, which involved physical restraint on a flat surface over the course of three minutes, may have served as an acute stressor. Given the well documented effects of stress on dopamine release, social behavior, and locomotor activity (Abercrombie et al., 1989; Kumsta & Heinrichs, 2013; Gentsch et al., 1981), subject handling may have influenced our results.

In conclusion, we found that i.c.v. administration of a 100ng dose of OXT was not reinforcing as assessed by the CPP paradigm, and that three days of conditioning experience with OXT had no effect on dopamine neurotransmission in the striatum of the socially monogamous male prairie voles. Despite these results, evidence from prior studies as summarized above strongly suggest the existence of DA and OXT systems interacting at the level of the NAcc. Further investigations into the effects of dose, stress, and individual variation in animal behavior will prove beneficial in developing clinically relevant therapies using OXT for the treatment of disorders such as social anxiety, schizophrenia, and drug addiction.

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