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Systemic Oxytocin Administration Alters Mesolimbic Dopamine Relase

Mary Kathlyn Estes

SYSTEMIC OXYTOCIN ADMINISTRATION ALTERS MESOLIMBIC DOPAMINE RELEASE

by

Mary Kathlyn Estes

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Abstract

Growing research indicates oxytocin may be involved in relieving anxiety and attenuating the rewarding effects of psychostimulants. The present study investigated the effects of subchronic oxytocin treatments on mesolimbic dopamine transmission in areas associated with anxiety (amygdala) and addiction (nucleus accumbens, NAc). Using in vivo fixed potential amperometry, stimulation-evoked dopamine release was recorded either in the amygdala or NAc in oxytocin pretreated mice. During dopamine recordings, mice received a drug challenge of either oxytocin, the dopamine reuptake blocker nomifensine (psychostimulant), or saline. In the amygdala, dopamine release was decreased following the oxytocin challenge but only in oxytocin pretreated mice. In the NAc, baseline dopamine release was decreased and the dopaminergic response to nomifensine was decreased but only in oxytocin pretreated mice. Together these results provide neurochemical support for previous behavioral studies suggesting oxytocin may be useful at treating aspects of anxiety and drug abuse.

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Oxytocin is a neuropeptide synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. Oxytocin is released into the bloodstream by the pituitary gland thus functioning as a hormone, but this neuropeptide is also released into the central nervous system thus additionally functioning as a neuromodulator. Oxytocin has been classically credited with initiating contractions and assisting in lactation, and has been used for these purposes clinically for decades (Magon & Kalra, 2011). However, recent studies have shown that oxytocin plays a variety of roles in physiological and psychological behaviors. For example, oxytocin also has been shown to influence behaviors related to maternal bonding, stress, memory and learning, and social bonding (Magon, & Kalra, 2011). Therefore, it is not surprising that oxytocin is being increasingly applied in clinical settings. Clinically, oxytocin is usually delivered intranasally (Veening & Oliver, 2013), and as of September 2018, a search for "intranasal oxytocin" on ClinicalTrials.gov returned 198 ongoing, planned, or completed studies, of which 26 are related to treating autism, 19 to anxiety or posttraumatic stress disorder, 25 to drug abuse, and 24 to schizophrenia. Interestingly, most of the therapeutic applications of oxytocin are aimed at disorders associated with dopamine transmission (Baskerville & Douglas, 2010); however, the effect of oxytocin administration on dopamine transmission is not yet understood. Both oxytocin and dopamine receptors and fibers have been shown to exist in corresponding brain regions, often close in proximity to each other (Ross et al., 2009; Smeltzer, Curtis, Aragona, & Wang, 2006; Love, 2014). Many animal studies have shown that oxytocin and dopamine interact to affect aspects of social behaviors, stress, and attention (for review see Baskerville & Douglas, 2010; Love, 2014). Furthermore, stimulations of oxytocin and dopamine pathways are known to

have comparable results on social behaviors such as sexual behaviors and pair bonding (Baskerville & Douglas, 2010).

Neuronal dopamine cell bodies predominately originate in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc), and have 3 main projection pathways. The nigrostriatal dopamine pathway has projections from the SNc to the dorsal striatum within the basal ganglia and is important for healthy motor functioning (Lester, Rogers, & Blaha, 2010). The mesocortical dopamine pathway projects from the VTA to the prefrontal cortex and is associated with emotional behavior and cognition (Goldin, McRae, Ramel, & Gross, 2008; Bechara, Damasio, Damasio, & Lee, 1999). The mesolimbic dopamine pathway consists of projections from the VTA to areas within the limbic system, specifically the nucleus accumbens (NAc) and amygdala. Dopamine transmission in the NAc is associated with attention and reward-motivated behaviors (O'brien & Gardner, 2005; Salgado & Kaplitt, 2015; Volkow et al., 2006), while the amygdala is involved in regulation of fear, anxiety, and emotional intelligence (Anderson & Phelps, 2000; LeDoux, 2007; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Abnormalities of dopamine systems are considered to be contributors to Parkinson's disease, schizophrenia, major depressive disorders, attention-deficit hyperactivity disorder, anxiety, addiction, binge eating, and compulsive disorders (Baskerville & Douglas, 2010).

Anxiety

Anxiety is a behavioral state with physiological and psychological changes due to environmental threats, either perceived or real. The threat causes involuntary responses that include an increased state of arousal and reflexive responses from the autonomic nervous system and the endocrine system. The autonomic nervous system is constantly at work maintaining a

balance of internal functions; however, when one encounters or perceives a danger, this system reacts. The initial response prepares our bodies for action, generally by increasing heart rate, blood pressure, and muscle tension while slowing immune responses and digestion (Steimer, 2002). When danger is evaded, or deemed unrealistic, homeostasis resumes, and the body relaxes. Anxiety is a necessary, normal reaction to the environment and assists in survival. Anxiety-related behaviors become maladaptive, however, when they are displayed at an excessive degree relative to the threat level or in response to uncertain and unlikely threat (Brosschot, Verkuil, & Thayer, 2016; Grupe & Nitschke, 2013). Unfortunately, the occurrence of faulty perceptions regarding threat assessment and consequently increased unwarranted anxiety are commonplace in our species.

Imaging studies have repeatedly shown structural damage and/or functional alterations in the amygdala of patients with chronic anxiety disorder, post-traumatic stress disorder, social anxiety, and depressive disorders (Holzel et al., 2009; Pine, 2007). The neural connections of the amygdala highlight its role in regulating emotional behaviors. The amygdala receives sensory input from thalamic and cortical regions, which is then integrated and potentially sent as a distress signal to the hypothalamus. This begins the activation of the autonomic nervous system via excitation of the hypothalamus-pituitary-adrenal (HPA) axis, resulting in release of cortisol throughout the body (McEwen, 2007). The amygdala also projects to the prefrontal cortex, hippocampus, and striatum to allow an evaluation of the situation, planning motor responses, and an opportunity to code information into memory (Pine, 2007). This reciprocal connection between the prefrontal cortex and the amygdala ultimately keeps amygdala activity in check. The prefrontal cortex has inhibitory projections that can reduce amygdala activation when the environmental situation does not require a fear/anxiety response (Ochsner et al., 2004; Urry et

al., 2006; Ohira et al., 2006). Thus, the balance between excitatory and inhibitory activity in the amygdala influences the anxiety state of animal.

Dopamine plays a role in regulating activity levels of the amygdala and has been associated with anxiety (Zarrindast & Khakpai, 2015). Under stress, dopamine neurons are activated in the VTA leading to increased release of dopamine in the amygdala and increased occurrence of anxiety-related behaviors (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Borowski & Kokkindis, 1998; Forster, Novick, Scholl, & Watt, 2012; de la Mora, Gallegos-Cari, Arizmendi-Garcia, Marcellino, & Fuxe, 2010). Dopamine release in the amygdala is thought to potentiate anxiety by dampening the inhibition of amygdala activity maintained by the prefrontal cortex (Diaz, Chappell, Christian, Anderson, & McCool, 2011). Lesions of the VTA were shown to attenuate conditioned fear behavior and responses (de la Mora et al, 2010), and research suggests that the administration of some anxiolytic drugs reduces amygdalar dopamine release evoked by exposure to aversive stimuli (Coco, Kuhn, Ely, & Kilts, 1992). The general mechanism of most anxiolytic medications includes reducing activity of the amygdala.

The most commonly used anxiolytics, a class of drugs called benzodiazepines (such as Valium and Xanax), reduce neurotransmission in the amygdala by agonizing the inhibitory neurotransmitter GABA (Davis, Rainnie, & Cassell, 1994; Sanders & Shekhar, 1995). Unfortunately, patients using these as chronic treatments are at risk for developing benzodiazepine dependence (Farach et al., 2012). In 2016, over 10,000 adults died from overdose involving a benzodiazepine; this accounts for nearly 15% of all overdose-related deaths [National Institute on Drug Abuse (NIDA), 2018]. Such problems caution the use of benzodiazepines for treating chronic anxiety and have physicians seeking alternate pharmaceuticals. Some patients are prescribed antidepressants for chronic treatment of anxiety;

however, these medications have substantial non-response rates (e.g. 44%) and can lead to increased severity of anxiety symptoms initially (Gollan et al., 2012; Montgomery, Sheehan, Meoni, Haudiquet, & Hackett, 2002). Thus, the field is in search of alternative anxiolytic pharmaceuticals. Oxytocin administration is one promising option.

Oxytocin is endogenously released in response to stressful stimuli and reduces activity in the amygdala and HPA axis, thus serving as a natural anxiolytic (Sobota, Mihara, Forrest, Featherstone, & Siegel, 2015; Viero et al., 2010). Preclinical studies have produced promising findings that suggest oxytocin administration may provide anxiety relief (see Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). For example, mice administered oxytocin over a 5 day period displayed significant decreases in blood pressure and cortisol levels (Petersson, Alster, Lundeberg, & Uvnäs-Moberg, 1996). Subchronic oxytocin administration in mice has also shown to reduce anxiety-related behaviors (using open field, four-plate, and elevated plus maze tasks) and reduce amygdala activity (using EEG recordings) (Mantella, Vollmer, Li, & Amico, 2003; Neumann & Landgraf, 2012; Ring et al., 2006; Sobota et al., 2015). Anxiogenic stimuli have shown to stimulate CNS and peripheral oxytocin systems, leading some researchers to suggest endogenous oxytocin may serve as a natural anxiolytic and that an imbalance in the oxytocin system may play a role in the etiology of anxiety disorders (Engelmann, Landgraf, & Wotjak, 2004; Neumann & Slattery, 2016). Although clinical trials administering oxytocin have shown promise, such as a reduction in HPA axis activity, lower cortisol, lowered ACTH, and reduced amygdala activity (Dodhia et al., 2014; Parker, Buckmaster, Schatzberg, & Lyons, 2005; Windle, Shanks, Lightman, & Ingram, 1997) many questions, mostly surrounding the mechanism of action and required dosing parameters, need to be addressed before this treatment becomes mainstream (see Guastella & Hickie, 2016; Veening & Oliver, 2013).

Addiction

The DSM 5 (2013) explains addiction, or substance abuse disorder specifically, as a dysfunctional habit that causes affliction or distress as indicated by maladaptive social skills, impaired restraint, tolerance and withdrawal symptoms from substance. NIDA (2015) reports that addiction costs over $700 billion dollars annually due to lost work productivity, health care, and crime associated with addiction. The anterior cingulate, orbital cortex, and temporal lobe (includes amygdala) has been shown through functional imaging studies to be interactive during drug craving (Everitt & Robbins, 2005). Everitt and Robbins (2005) suggest that drug seeking may be influenced by mechanisms in the medial PFC, amygdala, and the hippocampus through the connections to the nucleus accumbens.

The mesolimbic dopaminergic pathway has long been identified as playing a key role in addiction-related behaviors (Koob & Le Moal, 1997; Volkow, Fowler, Wang, & Swanson, 2004). While different drugs of abuse affect different parts of the brain, the common trait is increased dopamine release in limbic brain regions. The NAc is responsible for the primary reinforcing effects of drugs, while the amygdala and hippocampus facilitate conditioned learning (Feltenstein & See, 2008; Salgado & Kaplitt, 2015). Animal studies have shown an attenuation of cocaine and heroin self-administration after lesions of the VTA or NAc (Roberts & Koob, 1982; Hubner & Koob, 1990). Furthermore, dopamine antagonist or synthesis inhibition also decreased self-administration (Ikemoto, Yang, & Tan, 2015; Feltenstein & See, 2008). Animal studies will self-administrate opioids into the VTA and NAc reliably but not consistently in other brain regions. Rat and monkey studies have demonstrated that self-administration of amphetamines is consistent in the orbitofrontal cortex (Phillips, Mora, & Rolls, 1981), the amygdala and nucleus accumbens (Chevrette, Stellar, Hesse, & Markou, 2002).

Oxytocin has recently been identified as a potential modulator implicated in the biology of drug addiction (Buisman-Pijlman, Sumracki, Gordon, Hull, Carter, & Tops, 2014; Leong, Zhou, Ghee, See, & Reichel, 2016; Kovács, Sarnyai, & Szabó, 1998; Sarnyai & Kovács, 2014). Many animal studies have found that oxytocin administration can specifically reduce behavioral responses to psychostimulants (for review see Carson, Guastella, Taylor, & McGregor, 2013). Despite widespread abuse and detrimental health effects of psychostimulants (such as cocaine and amphetamines) in our culture, no effective pharmacological treatments are currently available (Kaye, Darke, Duflou, & McKetin, 2008; Lee & Rawson, 2008; Rose & Grant, 2008), which emphasizes the excitement surrounding these preclinical studies of oxytocin and addiction. Oxytocin impaired methamphetamine-induced conditioned place preference and stress-induced reinstatement (Qi et al., 2009), and oxytocin has been shown to decrease lever pressing in both methamphetamine and cocaine self-administration studies as well as reduce drug seeking after extinction (Leong et al., 2016; Cox, Young, See, & Reichel, 2013; Zhou et al., 2014). Locomotor hyperactivity and constant sniffing behavior observed following cocaine administration were both attenuated by injections of oxytocin (Leong et al., 2016; Sarnyai and Kovacs, 1994). Given this support for the use of oxytocin to combat the effects of drugs of abuse, many clinical trials have begun testing this, and although most results are pending, some have already shown promise (see Lee & Weerts, 2016).

Current Study

The proposed study aims to determine the effects of systemic oxytocin administration on mesolimbic dopamine transmission in brain areas associated with anxiety (amygdala) and addiction (NAc). In vivo fixed potential amperometry with carbon fiber recording electrodes

will be used to measure real-time stimulation-evoked dopamine release in anesthetized mice in response to subchronic and acute oxytocin administration. With the mice anesthetized and their dopamine systems not being naturally activated, we will mimic environmentally activated patterns via electrical stimulation (Lester et al., 2010). Given that both the amygdala and NAc receive dopaminergic input from cell bodies in the VTA, the VTA will be our electrical stimulation site. Anxiolytic drugs have shown to reduce dopamine release in the amygdala (Coco et al., 1992), and oxytocin has shown to attenuate anxiety-related behaviors (Ring et al., 2006; Sobota et al., 2015). Thus, we expect that systemic oxytocin administration will reduce VTA stimulation-evoked dopamine release in the amygdala. As mentioned above, oxytocin is also being evaluated as a potential treatment for drug addiction, and our recordings in the NAc will shed light on potential mechanisms for this therapeutic use. In order to determine the effect of oxytocin treatment on psychostimulant addiction, some mice will receive a drug challenge of nomifensine, which is a dopamine reuptake blocker with a similar neuropharmacological mechanism as cocaine. Our hypothesis is that oxytocin pretreatment will attenuate the psychostimulant-induced increase in NAc dopamine release. To our knowledge, no studies have measured in vivo real-time dopamine release following oxytocin administration; therefore, the results of the present study will be extremely interesting and beneficial to basic scientists aiming to understand neurochemical interactions but also to clinicians interested in using oxytocin as a treatment option.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Memphis and were also aligned with those outlined in The Public

Health Service Policy on Humane Care and Use of Laboratory Animals (National Institutes of Health 2012) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

Subjects

Fifty-two male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA), between 3-5 months, were housed 3-5 per cage. Cages were kept in a temperature (21± 1° C) controlled room with a 12 h light/ 12h dark cycle. Food and water will be available ad libitum.

Apparatus and Materials

Stimulation-evoked dopamine release was measured using in vivo fixed potential amperometry with carbon fiber microelectrodes. Amperometry is an electrochemical technique in which a constant potential is delivered to an electrode. We chose 0.8V since our molecule of interest, dopamine, oxidizes at 0.6V. Thus, the recorded current was a representation of the amount of extracellular dopamine efflux following electrical stimulation (Carter & Shieh, 2015). Pharmacological studies using the same electrical stimulation sites have confirmed these recorded current changes to be dopamine based (Lee et al., 2006; Mittleman, Goldowitz, Heck, & Blaha, 2008). Fixed potential amperometry is an established technique for real-time (10K samples/sec) recording of stimulation-evoked dopamine release in cortical and subcortical brain regions (Forster & Blaha, 2003; Lester et al., 2010).

Our lab consists of 3 functional amperometry stations, each equipped with a stereotaxic frame and electrode carriers (David Kopf Instruments, CA, USA), a programmable stimulator (Iso-Flex/Master 8, AMPI, Israel), and an electrometer (e-corder 401 and Picostat, Edaq Inc.,

CO, USA) connected to a computer. Each amperometry station was surrounded by a faraday cage to block interfering electrical noise. Approval was given by the Environmental Health and Safety department at the University of Memphis for the standard operating procedures concerning urethane (U2500 Sigma-Aldrich), oxytocin (03251 Sigma-Aldrich), nomifensine (N1530 Sigma-Aldrich), and dopamine (H8502 Sigma-Aldrich).

Procedure

The subchronic oxytocin pretreatment regimen of this proposed study was the same as that used by previous behavioral studies showing that oxytocin is capable of improving social interactions, reducing anxiety-related behaviors, and reducing amygdala EEG activity in adolescent and adult mice (Sobota et al., 2015; Teng et al., 2016). The pretreatment consisted of each mouse receiving 4 i.p. injections of either saline (vehicle) or oxytocin (1.0 mg/kg) across 8 days with a minimum of 48 hours between each injection. The injection was given at the same time on sequential weekdays. Forty-eight hours after the last injection, dopamine release was measured using fixed potential amperometry.

The amperometry procedure began with each mouse being anesthetized by urethane (1.5 g/kg, i.p.). The mouse was evaluated 10 minutes after injection by three different reflex checks (eye-blink, mild tail and foot-pinch) to ensure induction of anesthesia. If necessary, a supplemental dose of urethane (0.5 g/kg, i.p.) was given to mouse. All records of anesthetized animals were kept in a designated lab notebook. The mouse was then placed in a stereotaxic frame with a mouse head-holder adapter for electrode placement. Body temperature was kept at 37 ± 0.5 °C as well as monitored and maintained throughout procedure with a temperature-regulated heating pad (TC-1000; CWE, NY).

A longitudinal incision was made to expose the surface of the skull. Three trephine holes (~ 1-1.5 mm o.d.) were drilled through the skull. A stimulating electrode (SNE-100; Rhodes Medical Co., CA, USA) was inserted into the VTA (coordinates in mm from bregma: AP + 3.3, ML + 0.3, and DV – 4.0 from dura). A stainless-steel auxiliary and Ag/AgCl reference electrode combination was positioned in contact with contralateral cortical tissue approximately 2.0 mm posterior to bregma. A carbon fiber recording electrode was either implanted in the left amygdala or nucleus accumbens (amygdala coordinates in mm from bregma: AP +1.0, ML +2.5, and DV -5.0 from dura; nucleus accumbens coordinates in mm from bregma: AP +1.5, ML +1.0, and DV -4.0 from dura; Paxinos & Franklin, 2001).

A series of cathodal monophasic current pulses (20 pulses at 50 Hz applied every 30 secs over a 1 hr recording period) were delivered to the stimulating electrode. Following a 10-min baseline recording of stimulation-evoked dopamine release, mice received an i.p. injection of either saline (0.9%), oxytocin (1.0 mg/kg), or the selective dopamine transporter (DAT) inhibitor nomifensine (10 mg/kg). See Table 1 for a list of experimental groups. Recordings proceeded for 1 hour following the drug challenge. At the end of each experiment, a direct anodic current (100μAmps for 10 sec) was applied to the stimulating electrode to mark electrode placement. Mice were then euthanized (intracardial urethane 0.345g/ml). The brain was removed and prepared in 30%/10% sucrose/formalin plus 0.1% potassium ferricyanide for cryostat sectioning. Electrode placement was confirmed with a light microscope and recorded on coronal diagrams (Paxinos & Franklin, 2001). The mean change in dopamine oxidation current (nAmp), corresponding to stimulation-evoked dopamine efflux, was converted to a mean dopamine concentration (μM) by post-experiment *in vitro* calibration of the carbon fiber electrode in

solutions of dopamine (0.5-2.0 μM) using a flow injection system (Michael and Wightman, 1999).

Data analysis

In order to quantify the recorded dopamine efflux, data points occurring at 0.25 sec pre- and 10 sec post-stimulation were extracted at 5 min intervals. From baseline recordings we compared the effects of the subchronic pretreatment (oxytocin or saline) on dopamine release (the magnitude of the response peak) and dopamine half-life (i.e. the time for 50% decrease from the maximum evoked increase to the prestimulus baseline level). Independent samples t-tests were used to determine differences between pretreatment groups in baseline dopamine release and half-life. In order to determine the effect of the i.p. drug challenge (oxytocin, nomifensine, or saline) during amperometric recordings, changes in stimulation-evoked dopamine release and half-life were converted to mean percent change with respect to baseline (Lester, Miller, Pate, & Blaha, 2008). A repeated measures ANOVA was used to determine the effect of pretreatment on percent change in dopamine release and half-life over time following the drug challenge. When appropriate a one way ANOVA with Tukey's HSD posthoc tests was used to determine group differences at certain time points. All statistical analyses were conducted using IBM SPSS Statistics 23 (IBM Corp).

Results

Histology

The tips of the stimulating electrodes were positioned within the anatomical boundaries of the VTA. The placements of the electrochemical recording electrode surfaces were confined

to the core of the basolateral amygdala (BLA) or NAc. Figure 1A-C is a depiction of the placement ranges and coordinates from bregma (Paxinos & Franklin, 2001).

Dopamine efflux in the amygdala

Stimulation-evoked dopamine efflux in the amygdala was compared between the two pretreatment groups, subchronic oxytocin (n = 14) or saline (n = 12) (Fig 2A). Two specific components of these responses were quantified: dopamine release (the magnitude of the response peak) and dopamine half-life (the time dopamine remained in the synapse). Dopamine release in the amygdala did not differ between oxytocin and saline pretreated mice; t (24) = 0.092, p = .927 (Fig 2B). Likewise, there was no significant difference in dopamine half-life between the pretreatment groups; t (24) = 1.162, p = .257 (Fig 2C).

Dopamine efflux in the amygdala following oxytocin administration

During amperometric recordings in the amygdala, a subset of mice received an i.p. drug challenge of either oxytocin (1 mg/kg; n = 4 from each pretreatment group) or saline (n = 4 from each pretreatment group). Percent change in dopamine release and dopamine half-life were analyzed at 20, 40, and 60 min post drug challenge (oxytocin or saline). A two-way repeated measures ANOVA revealed a nearly significant pretreatment x drug challenge interaction on percent change in dopamine release over time [F(3, 36) = 2.85, p = .051, n_p^2 = 0.19], indicating the effect of the oxytocin injection on dopamine release differed based on whether the animal had been pretreated with oxytocin or saline. Further investigations using one-way ANOVAs revealed that at 20 min post oxytocin/saline injection, no alterations in dopamine release were observed in either pretreatment group [F (3,12) = 0.68, p = .579]. However, at 40 and 60 min

post oxytocin/saline differences in dopamine release were observed between pretreatment groups [40 min: F (3,12) = 6.08, p = .009, n_p^2 = 0.60; 60 min: F (3,12) = 9.06, p = .002, n_p^2 = 0.69]. Specifically, oxytocin administration decreased dopamine release at these time points in oxytocin pretreated mice relative to saline pretreated mice (sal-oxy vs. oxy-oxy: p = .017 and .008 at 40 and 60 min) (Fig 3A). Regarding the effect of the oxytocin drug challenge on dopamine half-life, a two-way repeated measures ANOVA revealed no interaction between pretreatment and drug challenge on percent change in dopamine half-life over time [F(3, 36) = 1.68, p = .188] (Fig 3B).

Dopamine efflux in the amygdala following nomifensine administration

During amperometric recordings in the amygdala, a subset of mice received an i.p. drug challenge of the dopamine reuptake blocker nomifensine (10 mg/kg, n = 4-5 per pretreatment group). Percent change in stimulation-evoked dopamine release and half-life following nomifensine were compared with mice that received an i.p. saline injection (n = 4 from each pretreatment group) 20, 40, and 60 min post injection. A two-way repeated measures ANOVA revealed no interaction between pretreatment and nomifensine drug challenge on percent change in dopamine release over time [F(3, 39) = 0.72, p = .55] (Fig 3C). Similarly, regarding the effect of the nomifensine drug challenge on dopamine half-life, a two-way repeated measures ANOVA revealed no interaction between pretreatment and drug challenge on percent change in dopamine half-life over time [F(3, 39) = 0.50, p = .69]. However, there was a significant main effect of drug challenge on percent change in dopamine half-life over time [F(3,39) = 10.11, p < .001, n_p^2 = 0.438]. Specifically, relative to saline injections, nomifensine significantly increased the dopamine half-life at the 20 and 40 min time points in saline pretreated mice (sal-sal vs. sal-

nomi: p = .017 at 20 min; sal-sal vs. sal-nomi: p = .029 at 40 min). Interestingly though, in oxytocin pretreated mice, nomifensine did not significantly alter dopamine half-life at these time points (oxy-sal vs. oxy-nomi: p = .213 at 20 min; oxy-sal vs. oxy-nomi: p = .130 at 40 min) (Fig 3D).

Dopamine efflux in the NAc

Stimulation-evoked dopamine efflux in the NAc was compared between the two pretreatment groups, subchronic oxytocin (n = 15) or saline (n = 12) (Fig 4A). Oxytocin pretreated mice had a significantly lower baseline dopamine release compared to the saline pretreated mice; t (25) = 2.80, p = 0.01 (Fig 4B). The half-lives of baseline dopamine responses did not differ between the mice pretreated with oxytocin and saline; t (25) = 1.17, p = 0.25 (Fig 4C).

Dopamine efflux in the NAc following oxytocin administration

During amperometric recordings in the NAc, a subset of mice received an i.p. drug challenge of either oxytocin (1 mg/kg; n = 4-5 per pretreatment group) or saline (n = 4-5 per pretreatment group). Percent change in dopamine release and dopamine half-life were analyzed at 20, 40, and 60 min post drug challenge (oxytocin or saline). A two-way repeated measures ANOVA revealed no interaction between pretreatment and oxytocin drug challenge on percent change in NAc dopamine release over time [F(3, 42) = 0.55, p = .649] and no main effect of oxytocin drug challenge over time on dopamine release [F(3, 42) = 0.34, p = .799] (Fig 5A). Likewise, no interaction was observed between pretreatment and oxytocin drug challenge on percent change in dopamine half-life over time [F(3, 42) = 1.41, p = .254] with no main effect of oxytocin drug challenge over time on dopamine half-life [F(3, 42) = 0.24, p = .867] (Fig 5B).

These results indicate that the oxytocin drug challenge during amperometric recordings did not alter NAc dopamine release or half-life in either pretreatment group (oxytocin or saline).

Dopamine efflux in the NAc following nomifensine administration

During amperometric recordings in the NAc, a subset of mice received an i.p. drug challenge of the dopamine reuptake blocker nomifensine (10 mg/kg, n = 4-5 per pretreatment group). Percent change in stimulation-evoked dopamine release and half-life following nomifensine were compared with mice that received an i.p. saline injection (n = 4-5 per pretreatment group) 20, 40, and 60 min post injection. A two-way repeated measures ANOVA revealed no interaction between pretreatment and nomifensine drug challenge on percent change in dopamine release over time [$F(3, 42) = 0.96$, $p = .420$] (Fig 5C), indicating nomifensine had a similar effect on NAc dopamine release in both pretreatment groups (saline and oxytocin). However, regarding the effect of the nomifensine drug challenge on dopamine half-life in the NAc, a two-way repeated measures ANOVA revealed a trending interaction of pretreatment x drug challenge on percent change in dopamine half-life over time [$F(3, 42) = 2.48$, $p = .074$, $n_p^2 = 0.15$], indicating the effect of nomifensine on dopamine half-life differed based on whether the animal had been pretreated with oxytocin or saline. One way ANOVAs showed significant differences in percent change in dopamine half-life following the nomifensine/saline drug challenge between pretreatment groups at all time points [20 min: $F(3,14) = 15.86$, $p < .001$, $n_p^2 = 0.77$; 40 min: $F(3,14) = 16.83$, $p < .001$, $n_p^2 = 0.78$; 60 min: $F(3,14) = 11.80$, $p < .001$, $n_p^2 = 0.72$]. Post hoc analyses revealed that nomifensine significantly increased dopamine half-life at all time points in both pretreatment groups ($p < 0.05$), but nomifensine administration increased

dopamine half-life to a lesser extent in oxytocin pretreated mice compared to saline pretreated mice at 20 min (sal-nomi vs. oxy-nomi: $p = 0.04$) (Fig 5D).

Discussion

Studies at both the preclinical and clinical level have demonstrated that oxytocin may play a role in multiple behavioral disorders including anxiety (see Meyer-Lindenberg et al., 2011) and addiction (see Kovács et al., 1998). Dopamine has also been associated with anxiety (see Zarrindast & Khakpai, 2015) and addiction (see Volkow et al., 2004); however, the impact of oxytocin administration on dopamine transmission is not clear. The current study investigated the effects of subchronic oxytocin administration on the release of mesolimbic dopamine in the amygdala and NAc. Subchronic pretreatment dosages were chosen based on previous behavioral studies (Sobota et al., 2015; Teng et al., 2016) and consisted of 4 i.p. injections of oxytocin (1 mg/kg) or saline, with each injection scheduled 48 hours apart. Dopamine release was quantified using *in vivo* fixed potential amperometry 48 hours after the final pretreatment injection. Baseline levels of stimulation-evoked dopamine release were established and followed by an i.p. drug challenge of acute oxytocin (1 mg/kg), the dopamine reuptake blocker nomifensine (10 mg/kg), or saline (vehicle control).

To our knowledge, the present study is the first to show that systemic oxytocin administration can lower baseline dopamine release. Oxytocin, a nonapeptide characterized by a six amino acid ring structure with a three amino acid tail, should be too large to cross the blood brain barrier (Ermisch, Rühle, Landgraf, & Hess, 1985; Meisenberg & Simmons, 1983). However, our results indicate that peripheral administration of oxytocin has the ability to influence neurotransmission. Several studies have shown elevated oxytocin levels in in cerebral

spinal fluid following peripheral administration (Dal Monte, Noble, Turchi, Cummins, & Averbeck, 2014; Freeman et al., 2016; Lee et al., 2018). Neumann et al. (2013) used microdialysis to specifically confirm increased concentrations of oxytocin in the hippocampus, amygdala, and plasma of rats and mice following both intranasal and intraperitoneal routes. Some have suggested peripheral administration of oxytocin stimulates release of endogenous central oxytocin via activation of hypothalamic nuclei (Carson, Cornish, Guastella, Taylor, & McGregor, 2010; Maejima et al., 2015). However, recently Lee et al. (2018) utilized a sensitive and specific quantitative mass spectrometry assay, capable of distinguishing endogenous and administered oxytocin, to confirm oxytocin crossed the blood brain barrier in rhesus macaques following both intranasal and intravenous administration. Although our experimental protocol does not allow conclusions to be drawn regarding the routes of transport and pharmacodynamics of administered oxytocin, our data supports the growing literature showing peripherally administered oxytocin has CNS impact. Our data supplements the field by revealing an influential role of oxytocin on functional mesolimbic dopamine transmission.

Subchronic oxytocin administration altered dopamine transmission in the amygdala

Typical anxiolytic drugs, which generally directly agonize GABA, have been shown to indirectly reduce mesolimbic dopamine activity evoked by both electrical stimulation (Gomez-A et al., 2017) and stressful stimuli (Coco et al., 1992). Therefore, we expected oxytocin administration, which has shown to have anxiolytic effects behaviorally (Kim et al., 2011; Sobota et al., 2015), would reduce dopamine release in the amygdala. Amperometric recordings took place 48 hours following the last pretreatment injection. In the amygdala, no differences in baseline dopamine release or half-life were observed between pretreatment groups. Thus,

subchronic oxytocin administration did not have a lasting effect (at least not spanning 48 hours) on stimulation-evoked dopamine release or reuptake kinetics. However, subchronic oxytocin administration did alter the functioning of this brain region, priming it to respond differently to future oxytocin administrations. Oxytocin administered during dopamine recordings resulted in attenuated dopamine release but only in the oxytocin pretreated group (meaning this was the 5th oxytocin injection for this group of mice). Likewise, using the same subchronic oxytocin administration and EEG recordings in the same mouse strain, Sobota et al. (2015) observed a reduction in amygdala activity following oxytocin administration. Given that dopamine transmission in the saline pretreated mice was not affected in the current study, an acute injection of oxytocin (first exposure) at this dose was not sufficient to decrease amygdalar dopamine release.

The response to the dopamine reuptake blocker nomifensine was also influenced by subchronic oxytocin pretreatment. Saline pretreated mice showed an increase in dopamine half-life following nomifensine administration compared to saline administration, as expected; however, nomifensine did not have the same impact in oxytocin pretreated mice. Dopamine reuptake blockers, similar to nomifensine used in the present study, are regularly prescribed, such as Ritalin and Adderall for ADHD, and abused, such as cocaine and amphetamines. Anxiety is a common side effect of such drugs (Coughlin, Cohen, Mulqueen, Ferracioli-Oda, Stuckelman, & Bloch, 2015; Koob, 2009). The present results suggest repeated oxytocin treatment may lessen the amygdalar effect of these dopaminergic drugs to some degree. Correspondingly, Morales-Rivera et al. (2014) showed that oxytocin treatment mediated anxiety behavioral responses triggered by cues previously paired with cocaine intake. The present

findings can help provide a neurochemical mechanism to studies relating oxytocin with amygdala-associated behavioral functions.

We examined the influence of oxytocin administration on dopamine in the amygdala due to the well-known role of this brain region in anxiety and input from midbrain dopamine neurons (de la Mora, 2010). In the amygdala, increased dopamine concentrations have been observed following stressful conditions such as handling, being restrained, or conditioned fear paradigms (Diaz et al., 2011; Forster et al., 2012; Torres, Gamaro, Vasconcellos, Silveira, & Dalmaz, 2002), and dopamine antagonists elicited anxiolytic affects when microinjected into the basolateral amygdala (Linsambarth, Moraga-Amaro, Quintana-Donoso, Rojas, & Stehberg, 2017). Oxytocin seems to play a role in the excitability of the amygdala as well. In elevated plus maze tests, oxytocin deficient mice displayed anxious behaviors (less time spent in open arms and less rearing), which were mitigated by oxytocin administration (Mantella et al., 2003; Neumann & Landgraf, 2012; Ring et al., 2006). These studies, combined with previously mentioned studies showing endogenous oxytocin levels rise in stressful conditions, suggest oxytocin plays a natural role in relieving anxiety (see Engelmann, Landgraf, & Wotjak, 2004; Neumann & Slattery, 2016). Clinical studies support this notion in that patients with social anxiety present increased amygdala activation (Ziegler, Dannlowski, Brauer, Stevens, Laeger, & Wittman, 2015) and oxytocin administration decreases amygdala activity associated with generalized social anxiety disorder (Dodhia et al., 2014). Given that dopamine is excitatory in the amygdala (Diaz et al., 2011; Rosenkranz & Grace, 1999), the present results indicate oxytocin administration may have anxiolytic properties due to its regulatory role over dopamine transmission in the amygdala, at least in part. Thus, an interesting follow-up to the present study would be to repeat these procedures in mice having been exposed to a stress-inducing paradigm.

Subchronic oxytocin administration altered dopamine transmission in the nucleus accumbens

At the time of amperometric recordings, 48 hours after the last subchronic oxytocin pretreatment, oxytocin pretreated mice displayed reduced accumbal dopamine release compared to saline pretreated mice. Interestingly, however, oxytocin administered during amperometric recordings did not alter dopamine release or half-life, indicating no acute/immediate effect of oxytocin in dopamine neurotransmission in this brain region. These findings suggest oxytocin lacks reinforcing properties, which mirrors behavioral findings in which oxytocin did not produce conditioned place preference in mice when administered via intracutaneous, intraperitoneal (Baracz et al., 2012) or intranasal routes (Kosaki & Watanabe, 2016). These findings may be sex and dose-dependent given female rodents have been shown to self-administer oxytocin and develop conditioned place preference for oxytocin delivered at higher doses through intracerebroventricular cannula (Donhoffner, Goings, Atabaki, & Wood, 2016; Kent et al., 2016) or intraperitoneal injection 8 mg/kg (Liberzon, Trujillo, Akil, & Young, 1997).

Oxytocin has been shown to attenuate self-administration, conditioned place preference, and locomotor hyperactivity related to use psychostimulant and other dopamine agonists in animal models (Leong et al., 2016; Carson et al., 2010; Qi et al., 2009; Zhou et al., 2014), and as expected, in the present study, mice pretreated with subchronic oxytocin had a reduced dopaminergic response to the drug challenge of nomifensine, a dopamine reuptake blocker with a similar neuromechanism as cocaine. Specifically, 20 min post nomifensine injection, dopamine half-life in the NAc of oxytocin pretreated mice was increased relative to baseline (pre-nomifensine levels), but to a significantly reduced extent (nearly half as much) as saline

pretreated mice. Dopamine half-life is commonly used as an indication of the influence of a DAT inhibitor (Mittleman et al., 2008; Siciliano, Calipari, Ferris, and Jones 2014). Attenuated drug-induced increases in dopamine half-life (a measure of time in the synapse) may result in a diminished salience for the psychostimulant. At 40 and 60 min post injection, the nomifensine-induced increases in dopamine half-life were the same in both pretreatment groups, but the observed reduced dopaminergic response at the 20 min mark may have implications for the rewarding effects of dopamine agonists. The sooner an increase in dopamine efflux occurs following a drug administration, the more reinforcing ability that drug has (Volkow and Morales, 2015); thus, our data indicates nomifensine may be less rewarding following subchronic administration of oxytocin. These findings can help provide a neurochemical mechanism for studies relating oxytocin with NAc-associated behavioral functions.

Clinical studies have begun trying to determine how these effects can be put to use in the human population to combat maladaptive drug use. Recent clinical studies have shown that oxytocin reduces the withdrawal symptoms of alcohol (Pedersen et al., 2013), attenuated cocaine craving in cocaine-dependent users receiving methadone (Stauffer et al., 2016), and decreased the amount of cannabis consumed daily by habitual cannabis smokers (Sherman, Baker, & McRae-Clark, 2017; McRae-Clark, Baker, Moran-Santa Maria, & Brady, 2013). Given these clinical findings, an interesting follow-up to the present study would be to repeat these procedures in mice previously exposed to and dependent on a psychostimulant.

Conclusion

The present study is the first to our knowledge to show that systemic oxytocin administration can alter dopamine release in limbic structures. The effect of systemic oxytocin,

as least at the present dose in this mouse strain, seems to be cumulative given that acute injections in oxytocin-naïve mice had no dopaminergic effect in either limbic brain region. In the amygdala, mice pretreated with subchronic oxytocin displayed decreased dopamine release following oxytocin administration relative to saline pretreated mice. In the NAc, mice pretreated with subchronic oxytocin displayed decreased baseline dopamine release and a reduced dopaminergic response to a dopamine agonist. Together these results provide neurochemical support for preclinical and clinical studies suggesting oxytocin may be useful in treating aspects of anxiety and drug addiction. The prospect of an anxiolytic without abuse liability is particularly attractive given the current issues surrounding benzodiazepines. Reciprocally, a potential pharmaceutical that decreases the rewarding effects of psychostimulants while also reducing anxiety, a common withdrawal symptom, is enticing and warrants further study. The present study provides a starting point for deciphering the relationship between systemic oxytocin administration and dopamine transmission. More studies are necessary to determine the relative effects of oxytocin administration in both sexes, as oxytocin expression levels (de Vries, 2008) and oxytocin-mediated behaviors (see Dumais & Veenema, 2016) are known to differ between sexes. Overall, the present study adds to our understanding of the neural effects of oxytocin, which is critical given the potential of therapeutic uses for this drug.

References

Abercrombie, E. D., Keefe, K. A., DiFrischia, D. S., & Zigmond, M. J. (1989). Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *Journal of neurochemistry*, *52*(5), 1655-1658.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing

Anderson, A. K., & Phelps, E. A. (2000). Expression without recognition: contributions of the human amygdala to emotional communication. *Psychological Science*, *11*(2), 106-111.

Baracz, S. J., Rourke, P. I., Pardey, M. C., Hunt, G. E., McGregor, I. S., & Cornish, J. L. (2012). Oxytocin directly administered into the nucleus accumbens core or subthalamic nucleus attenuates methamphetamine-induced conditioned place preference. *Behavioural brain research*, *228*(1), 185-193.

Baskerville, Tracey, & Douglas, A. J. (2010). Dopamine and oxytocin interactions underlying behaviors: potential contributions to behavioral disorders. *CNS neuroscience & therapeutics*, *16*(3), e92-e123.

Bechara, A., Damasio, H., Damasio, A. R., & Lee, G. P. (1999). Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. *Journal of Neuroscience*, *19*(13), 5473-5481.

Borowski, T. B., & Kokkindis, L. (1998). The effects of cocaine, amphetamine, and the d1 receptor agonist skf 3893 on fear extinction as measured with potentiated startle: Implications for psychomotor stimulant psychosis. *Behavioral Neuroscience*, *112*, 952 – 965. doi: 10.1037/0735-7044.112.4.952

Brosschot, J. F., Verkuil, B., & Thayer, J. F. (2016). The default response to uncertainty and the importance of perceived safety in anxiety and stress: An evolution-theoretical perspective. *Journal of Anxiety Disorders, 41,* 22-34.

Buisman-Pijlman, F. T., Sumracki, N. M., Gordon, J. J., Hull, P. R., Carter, C. S., & Tops, M. (2014). Individual differences underlying susceptibility to addiction: role for the endogenous oxytocin system. *Pharmacology Biochemistry and Behavior*, *119*, 22-38.

Carson, D. S., Cornish, J. L., Guastella, A. J., Hunt, G. E., & McGregor, I. S. (2010). Oxytocin decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats. *Neuropharmacology*, *58*(1), 38-43.

Carson, D. S., Guastella, A. J., Taylor, E. R., & McGregor, I. S. (2013). A brief history of oxytocin and its role in modulating psychostimulant effects. *Journal of Psychopharmacology*, 0269881112473788.

Carter, M., & Shieh, J. C. (2015). *Guide to research techniques in neuroscience*. Academic Press.

Chevrette, J., Stellar, J. R., Hesse, G. W., & Markou, A. (2002). Both the shell of the nucleus accumbens and the central nucleus of the amygdala support amphetamine self-administration in rats. *Pharmacology Biochemistry and Behavior*, *71*(3), 501-507.

Coco, M. L., Kuhn, C. M., Ely, T. D., & Kilts, C. D. (1992). Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. *Brain research*, *590*(1), 39-47.

Coughlin, C. G., Cohen, S. C., Mulqueen, J. M., Ferracioli-Oda, E., Stuckelman, Z. D., & Bloch, M. H. (2015). Meta-analysis: reduced risk of anxiety with psychostimulant treatment in children with attention-deficit/hyperactivity disorder. *Journal of child and adolescent psychopharmacology*, *25*(8), 611-617.

Cox, B. M., Young, A. B., See, R. E., & Reichel, C. M. (2013). Sex differences in methamphetamine seeking in rats: Impact of oxytocin. *Psychoneuroendocrinology*, *38*(10), 2343-2353.

Dal Monte, O., Noble, P. L., Turchi, J., Cummins, A., & Averbeck, B. B. (2014). CSF and blood oxytocin concentration changes following intranasal delivery in macaque. *PloS one*, *9*(8), e103677.

Davis, M., Rainnie, D., & Cassell, M. (1994). Neurotransmission in the rat amygdala related to fear and anxiety. *Trends in neurosciences*, *17*(5), 208-214.

de la Mora, M. P., Gallegos-Cari, A., Arizmendi-García, Y., Marcellino, D., & Fuxe, K. (2010). Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. *Progress in neurobiology*, *90*(2), 198-216.

de Vries, G. J. (2008). Sex differences in vasopressin and oxytocin innervation of the brain. *Progress in brain research*, *170*, 17-27.

Diaz, M. R., Chappell, A. M., Christian, D. T., Anderson, N. J., & McCool, B. A. (2011). Dopamine d3-like receptors modulate anxiety-like behavior and regulate gabaergic transmission in the rat lateral/basolateral amygdala. *Neuropsychopharmacology*, *36*, 1090 – 1103. doi: 10.1038/npp.2010.246

Dodhia, S., Hosanagar, A., Fitzgerald, D. A., Labuschagne, I., Wood, A. G., Nathan, P. J., & Phan, K. L. (2014). Modulation of resting-state amygdala-frontal functional connectivity by oxytocin in generalized social anxiety disorder. *Neuropsychopharmacology*, *39*(9), 2061.

Donhoffner, M. E., Goings, S. P., Atabaki, K., & Wood, R. I. (2016). Intracerebroventricular

Oxytocin Self-Administration in Female Rats. *Journal of neuroendocrinology*, *28*(10).

Dumais, K. M., & Veenema, A. H. (2016). Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior. *Frontiers in neuroendocrinology*, *40*, 1-23.

Engelmann, M., Landgraf, R., & Wotjak, C. T. (2004). The hypothalamic–neurohypophysial system regulates the hypothalamic–pituitary–adrenal axis under stress: an old concept revisited. *Frontiers in neuroendocrinology*, *25*(3-4), 132-149.

Ermisch, A., Rühle, H. J., Landgraf, R., & Hess, J. (1985). Blood—brain barrier and peptides. *Journal of Cerebral Blood Flow & Metabolism*, *5*(3), 350-357.

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature neuroscience*, *8*(11), 1481-1489.

Farach, F. J., Pruitt, L. D., Jun, J. J., Jerud, A. B., Zoellner, L. A., & Roy-Byrne, P. P. (2012). Pharmacological treatment of anxiety disorders: Current treatments and future directions. *Journal of anxiety disorders*, *26*(8), 833-843.

Feltenstein, M. W., & See, R. E. (2008). The neurocircuitry of addiction: an overview. *British journal of pharmacology*, *154*(2), 261-274.

Forster, G.L., & Blaha, C.D. (2003). Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and the pons of the rat. *European Journal of Neuroscience, 17* (4), 751-762.

Forster, G. L., Novick, A. M., Scholl, J. L., & Watt, M. J. (2012). The role of the amygdala in anxiety disorders. In The Amygdala-A Discrete Multitasking Manager. InTech.

Freeman, S. M., Samineni, S., Allen, P. C., Stockinger, D., Bales, K. L., Hwa, G. G., & Roberts, J. A. (2016). Plasma and CSF oxytocin levels after intranasal and intravenous oxytocin in awake macaques. *Psychoneuroendocrinology*, *66*, 185-194.

Goldin, P. R., McRae, K., Ramel, W., & Gross, J. J. (2008). The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biological psychiatry*, *63*(6), 577-586.

Gollan, J. K., Fava, M., Kurian, B., Wisniewski, S. R., Rush, A. J., Daly, E. ... & Trivedi, M. H. (2012). What are the clinical implications of new onset or worsening anxiety during the first two weeks of SSRI treatment for depression?. *Depression and anxiety*, *29*(2), 94-101.

Gomez-A, A., Fiorenza, A. M., Boschen, S. L., Sugi, A. H., Beckman, D., Ferreira, S. T., ... &

Da Cunha, C. (2017). Diazepam inhibits electrically evoked and tonic dopamine release in the nucleus accumbens and reverses the effect of amphetamine. *ACS chemical neuroscience*, *8*(2), 300-309.

Grupe, D. W., & Nitschke, J. B. (2013). Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. *Nature Reviews Neuroscience*, *14*(7), 488-501.

Guastella, A. J., & Hickie, I. B. (2016). Oxytocin treatment, circuitry, and autism: A critical review of the literature placing oxytocin into the autism context. *Biological psychiatry*, *79*(3), 234-242.

Hölzel, B. K., Carmody, J., Evans, K. C., Hoge, E. A., Dusek, J. A., Morgan, L., ... & Lazar, S. W. (2009). Stress reduction correlates with structural changes in the amygdala. *Social cognitive and affective neuroscience*, nsp034.

Hubner, C. B., & Koob, G. F. (1990). The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. *Brain research*, *508*(1), 20-29.

Ikemoto, S., Yang, C., & Tan, A. (2015). Basal ganglia circuit loops, dopamine and motivation: a review and enquiry. *Behavioural brain research*, *290*, 17-31.

Kaye, S., Darke, S., Duflou, J., & McKetin, R. (2008). Methamphetamine-related fatalities in Australia: demographics, circumstances, toxicology and major organ pathology. *Addiction*, *103*(8), 1353-1360.

Kent, P., Awadia, A., Zhao, L., Ensan, D., Silva, D., Cayer, C., ... & Merali, Z. (2016). Effects of intranasal and peripheral oxytocin or gastrin-releasing peptide administration on social interaction and corticosterone levels in rats. *Psychoneuroendocrinology*, *64*, 123-130.

Kim, M. J., Loucks, R. A., Palmer, A. L., Brown, A. C., Solomon, K. M., Marchante, A. N., & Whalen, P. J. (2011). The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioural brain research*, *223*(2), 403-410

Kosaki, Y., & Watanabe, S. (2016). Conditioned social preference, but not place preference, produced by intranasal oxytocin in female mice. *Behavioral neuroscience*, *130*(2), 182.

Koob, G. F. (2009). Neurobiological substrates for the dark side of compulsivity in addiction. *Neuropharmacology*, *56*, 18-31.

Koob, G. F., & Le Moal, M. (1997). Drug abuse: hedonic homeostatic dysregulation. *Science*, *278*(5335), 52-58.

Kovács, G. L., Sarnyai, Z., & Szabó, G. (1998). Oxytocin and addiction: a review. *Psychoneuroendocrinology*, *23*(8), 945-962.

LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron*, *20*(5), 937-945.

LeDoux, J. (2007). The amygdala. *Current biology*, *17*(20), R868-R874.

Lee, K. H., Blaha, C. D., Harris, B. T., Cooper, S., Hitti, F. L., Leiter, J. C., … & Kim, U. (2006). Dopamine efflux in the rat striatum evoked by electrical stimulation of the subthalamic nucleus: potential mechanism of action in Parkinson's disease. *European Journal of Neuroscience*, *23*(4), 1005-1014.

Lee, N. K., & Rawson, R. A. (2008). A systematic review of cognitive and behavioural therapies for methamphetamine dependence. *Drug and alcohol review*, *27*(3), 309-317.

Lee, M. R., Scheidweiler, K. B., Diao, X. X., Akhlaghi, F., Cummins, A., Huestis, M. A., … & Averbeck, B. B. (2018). Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay. *Molecular psychiatry*, *23*(1), 115.

Lee, M. R., & Weerts, E. M. (2016). Oxytocin for the treatment of drug and alcohol use disorders. *Behavioural Pharmacology*, *27*(8), 640-648.

Leong, K. C., Zhou, L., Ghee, S. M., See, R. E., & Reichel, C. M. (2016). Oxytocin decreases cocaine taking, cocaine seeking, and locomotor activity in female rats. *Experimental and clinical psychopharmacology*, *24*(1), 55.

Lester, D. B., Miller, A. D., Pate, T. D., & Blaha, C. D. (2008). Midbrain acetylcholine and glutamate receptors modulate accumbal dopamine release. *Neuroreport*, *19*(9), 991-995.

Lester, D. B., Rogers, T. D., & Blaha, C. D. (2010). Acetylcholine–dopamine interactions in the pathophysiology and treatment of CNS disorders. *CNS neuroscience & therapeutics*, *16*(3), 137-162.

Liberzon, I., Trujillo, K. A., Akil, H., & Young, E. A. (1997). Motivational properties of oxytocin in the conditioned place preference paradigm. *Neuropsychopharmacology*, *17*(6), 353.

Linsambarth, S., Moraga-Amaro, R., Quintana-Donoso, D., Rojas, S., & Stehberg, J. (2017). The Amygdala and Anxiety. In *The Amygdala-Where Emotions Shape Perception, Learning and Memories*. InTech.

Love, T. M. (2014). Oxytocin, motivation and the role of dopamine. *Pharmacology Biochemistry and Behavior*, *119*, 49-60.

Maejima, Y., Rita, R. S., Santoso, P., Aoyama, M., Hiraoka, Y., Nishimori, K., ... & Yada, T.

(2015). Nasal oxytocin administration reduces food intake without affecting locomotor activity and glycemia with c-Fos induction in limited brain areas. *Neuroendocrinology*, *101*(1), 35-44.

Magon, N., & Kalra, S. (2011). The orgasmic history of oxytocin: Love, lust, and labor. *Indian journal of endocrinology and metabolism*, *15*(7), 156.

Mantella, R. C., Vollmer, R. R., Li, X., & Amico, J. A. (2003). Female oxytocin-deficient mice display enhanced anxiety-related behavior. Endocrinology, 144(6), 2291-2296.

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiological reviews*, *87*(3), 873-904.

McRae-Clark, A. L., Baker, N. L., Moran-Santa Maria, M., & Brady, K. T. (2013). Effect of oxytocin on craving and stress response in marijuana-dependent individuals: a pilot study. *Psychopharmacology*, *228*(4), 623-631.

Meisenberg, G., & Simmons, W. H. (1983). Peptides and the blood-brain barrier. *Life sciences*, *32*(23), 2611-2623.

Meyer-Lindenberg, A., Domes, G., Kirsch, P., & Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nature Reviews Neuroscience*, *12*(9), 524.

Michael, D. J., & Wightman, R. M. (1999). Electrochemical monitoring of biogenic amine neurotransmission in real time. *Journal of pharmaceutical and biomedical analysis*, *19*(1), 33-46.

Mittleman, G., Goldowitz, D., Heck, D. H., & Blaha, C. D. (2008). Cerebellar modulation of frontal cortex dopamine efflux in mice: relevance to autism and schizophrenia. *Synapse*, *62*(7), 544-550.

Montgomery, S. A., Sheehan, D. V., Meoni, P., Haudiquet, V., & Hackett, D. (2002). Characterization of the longitudinal course of improvement in generalized anxiety disorder during long-term treatment with venlafaxine XR. *Journal of psychiatric research*, *36*(4), 209-217.

Morales-Rivera, A., Hernández-Burgos, M. M., Martínez-Rivera, A., Pérez-Colón, J., Rivera, R., Montalvo, J., ... & Maldonado-Vlaar, C. S. (2014). Anxiolytic effects of oxytocin in cue-induced cocaine seeking behavior in rats. *Psychopharmacology*, *231*(21), 4145-4155.

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends in neurosciences*, *35*(11), 649-659.

Neumann, I. D., Maloumby, R., Beiderbeck, D. I., Lukas, M., & Landgraf, R. (2013). Increased

brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology*, *38*(10), 1985-1993.

Neumann, I. D., & Slattery, D. A. (2016). Oxytocin in general anxiety and social fear: a translational approach. *Biological psychiatry*, *79*(3), 213-221.

NIDA (2015). Trends & Statistics. Retrieved March 1, 2017, from https://www.drugabuse.gov/related-topics/trends-statistics

NIDA (2018). Trends & Statistics. Retrieved September 18, 2018, from https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates

O'brien, C. P., & Gardner, E. L. (2005). Critical assessment of how to study addiction and its treatment: human and non-human animal models. *Pharmacology & therapeutics*, *108*(1), 18-58.

Ochsner, K. N., Ray, R. D., Cooper, J. C., Robertson, E. R., Chopra, S., Gabrieli, J. D., & Gross, J. J. (2004). For better or for worse: neural systems supporting the cognitive down-and up-regulation of negative emotion. *Neuroimage*, *23*(2), 483-499.

Ohira, H., Nomura, M., Ichikawa, N., Isowa, T., Iidaka, T., Sato, A., …& Yamada, J. (2006). Association of neural and physiological responses during voluntary emotion suppression. *Neuroimage*, *29*(3), 721-733.

Parker, K. J., Buckmaster, C. L., Schatzberg, A. F., & Lyons, D. M. (2005). Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. Psychoneuroendocrinology, 30(9), 924-929.

Paxinos G, and KB Franklin. (2001) The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2001.

Pedersen, C. A., Smedley, K. L., Leserman, J., Jarskog, L. F., Rau, S. W., Kampov-Polevoi, A., ... & Garbutt, J. C. (2013). Intranasal oxytocin blocks alcohol withdrawal in human subjects. *Alcoholism: Clinical and Experimental Research*, *37*(3), 484-489.

Petersson, M., Alster, P., Lundeberg, T., & Uvnäs-Moberg, K. (1996). Oxytocin causes a long-term decrease of blood pressure in female and male rats. *Physiology & behavior*, *60*(5), 1311-1315.

Phillips, A. G., Mora, F., & Rolls, E. T. (1981). Intracerebral self-administration of amphetamine by rhesus monkeys. *Neuroscience Letters*, *24*(1), 81-86.

Pine, D. S. (2007). Research review: a neuroscience framework for pediatric anxiety disorders. *Journal of Child Psychology and Psychiatry*, *48*(7), 631-648.

Qi, J., Yang, J. Y., Wang, F., Zhao, Y. N., Song, M., & 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*(5), 856-865.

Ring, R. H., Malberg, J. E., Potestio, L., Ping, J., Boikess, S., Luo, B., … & Rosenzweig-Lipson, S. (2006). Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology*, *185*(2), 218-225.

Roberts, D. C., & Koob, G. F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacology Biochemistry and Behavior*, *17*(5), 901-904.

Rose, M. E., & Grant, J. E. (2008). Pharmacotherapy for methamphetamine dependence: a review of the pathophysiology of methamphetamine addiction and the theoretical basis and efficacy of pharmacotherapeutic interventions. *Annals of Clinical Psychiatry*, *20*(3), 145-155.

Rosenkranz, J. A., & Grace, A. A. (1999). Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo. *Journal of Neuroscience*, *19*(24), 11027-11039.

Ross, H. E., Freeman, S. M., Spiegel, L. L., Ren, X., Terwilliger, E. F., & Young, L. J. (2009). Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. *Journal of Neuroscience*, *29*(5), 1312-1318.

Salgado, S., & Kaplitt, M. G. (2015). The nucleus accumbens: a comprehensive review. *Stereotactic and functional neurosurgery*, *93*(2), 75-93.

Sanders, S. K., & Shekhar, A. (1995). Anxiolytic effects of chlordiazepoxide blocked by injection of GABA A and benzodiazepine receptor antagonists in the region of the anterior basolateral amygdala of rats. *Biological psychiatry*, *37*(7), 473-476.

Sarnyai, Z., & Kovács, G. L. (1994). Role of oxytocin in the neuroadaptation to drugs of abuse. *Psychoneuroendocrinology*, *19*(1), 85-117.

Sarnyai Z, & Kovács G.L. (2014) Oxytocin in learning and addiction: from early discoveries to the present. Pharmacology Biochemistry and Behavior. 2014; 119:3–9.

Sherman, B. J., Baker, N. L., & McRae-Clark, A. L. (2017). Effect of oxytocin pretreatment on cannabis outcomes in a brief motivational intervention. *Psychiatry research*, *249*, 318-320.

Siciliano, C. A., Calipari, E. S., Ferris, M. J., & Jones, S. R. (2014). Biphasic mechanisms of amphetamine action at the dopamine terminal. *Journal of Neuroscience*, *34*(16), 5575-5582.

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. *Neuroscience letters*, *394*(2), 146-151.

Sobota, R., Mihara, T., Forrest, A., Featherstone, R.E., & Siegel, S.J. (2015). Oxytocin reduces amygdala activity, increases social interactions, and reduces anxiety-like behavior irrespective of NMDAR antagonism. *Behavioral Neuroscience, 129 (4)*, 389-398.

Stauffer, C. S., Musinipally, V., Suen, A., Lynch, K. L., Shapiro, B., & Woolley, J. D. (2016). A two-week pilot study of intranasal oxytocin for cocaine-dependent individuals receiving methadone maintenance treatment for opioid use disorder. *Addiction research & theory*, *24*(6), 490-498.

Steimer, T. (2002). The biology of fear-and anxiety-related behaviors. *Dialogues in clinical neuroscience*, *4*, 231-250.

Teng, B.L., Nikolova, V.D., Riddick, N.V., Agster, K.L., Crowley, J.J., Baker, L.K., …& Moy, S.S. (2016). Reversal of social deficits by subchronic oxytocin in two autism mouse models. *Neuropharmacology, 105*, 61-71.

Torres, I. L. S., Gamaro, G. D., Vasconcellos, A. P., Silveira, R., & Dalmaz, C. (2002). Effects of chronic restraint stress on feeding behavior and on monoamine levels in different brain structures in rats. *Neurochemical research, 27*(6), 519-525.

Urry, H. L., Van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., … & Davidson, R. J. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *Journal of Neuroscience*, *26*(16), 4415-4425.

Veening, J.G., & Olivier, B. (2013). Intranasal administration of oxytocin: behavioral and clinical effects, a review. *Neuroscience and biobehavioral Reviews, 37 (8)*, 1445-1465.

Viero, C., Shibuya, I., Kitamura, N., Verkhratsky, A., Fujihara, H., Katoh, A., ... & Dayanithi, G. (2010). REVIEW: Oxytocin: Crossing the bridge between basic science and pharmacotherapy. *CNS neuroscience & therapeutics*, *16*(5), e138-e156.

Volkow, N. D., Fowler, J. S., Wang, G. J., & Swanson, J. M. (2004). Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Molecular psychiatry*, *9*(6), 557.

Volkow, N., & Morales, M. (2015). The brain on drugs: from reward to addiction. *Cell*, *162*(4), 712-725.

Volkow, N. D., Wang, G. J., Telang, F., Fowler, J. S., Logan, J., Childress, A. R., … & Wong, C. (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of

craving in cocaine addiction. *Journal of Neuroscience*, *26*(24), 6583-6588.

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(7), 2829-2834.

Zarrindast, M. R., & Khakpai, F. (2015). The Modulatory Role of Dopamine in Anxiety-like Behavior. *Archives of Iranian Medicine (AIM)*, *18*(9).

Zhou, L., Sun, W. L., Young, A. B., Lee, K., McGinty, J. F., & See, R. E. (2014). Oxytocin reduces cocaine seeking and reverses chronic cocaine-induced changes in glutamate receptor function. *International Journal of Neuropsychopharmacology*, pyu009.

Ziegler, C., Dannlowski, U., Bräuer, D., Stevens, S., Laeger, I., Wittmann, H., ... & Lesch, K. P. (2015). Oxytocin receptor gene methylation: converging multilevel evidence for a role in social anxiety. *Neuropsychopharmacology*, *40*(6), 1528.

Appendix

Table 1

Experimental groups

Pre-treatment	Recording Site	Systemic drug during recording	N
Subchronic Oxytocin	Amygdala	Oxytocin	4
		Nomifensine	5
		Saline	4
	Nucleus accumbens	Oxytocin	5
		Nomifensine	5
		Saline	5
Subchronic Saline	Amygdala	Oxytocin	4
		Nomifensine	4
		Saline	4
	Nucleus Accumbens	Oxytocin	4
		Nomifensine	4
		Saline	4
--	--	--	52

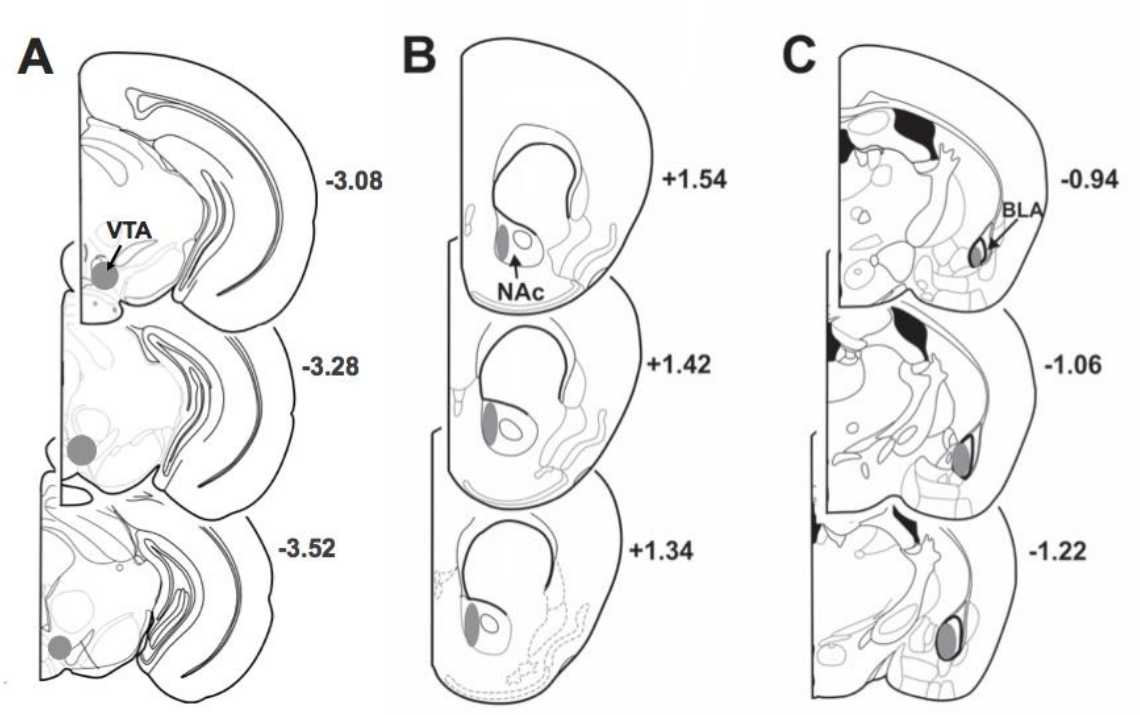

Figure 1. Representative coronal sections of the mouse brain (adapted from the atlas of Paxinos and Franklin, 2001), with grey shaded areas indicating the placements of (A) stimulating electrodes in the ventral tegmental area (VTA) and amperometric recording electrodes in the (B) nucleus accumbens (NAc) or (C) basolateral amygdala (BLA). Numbers correspond to mm from bregma.

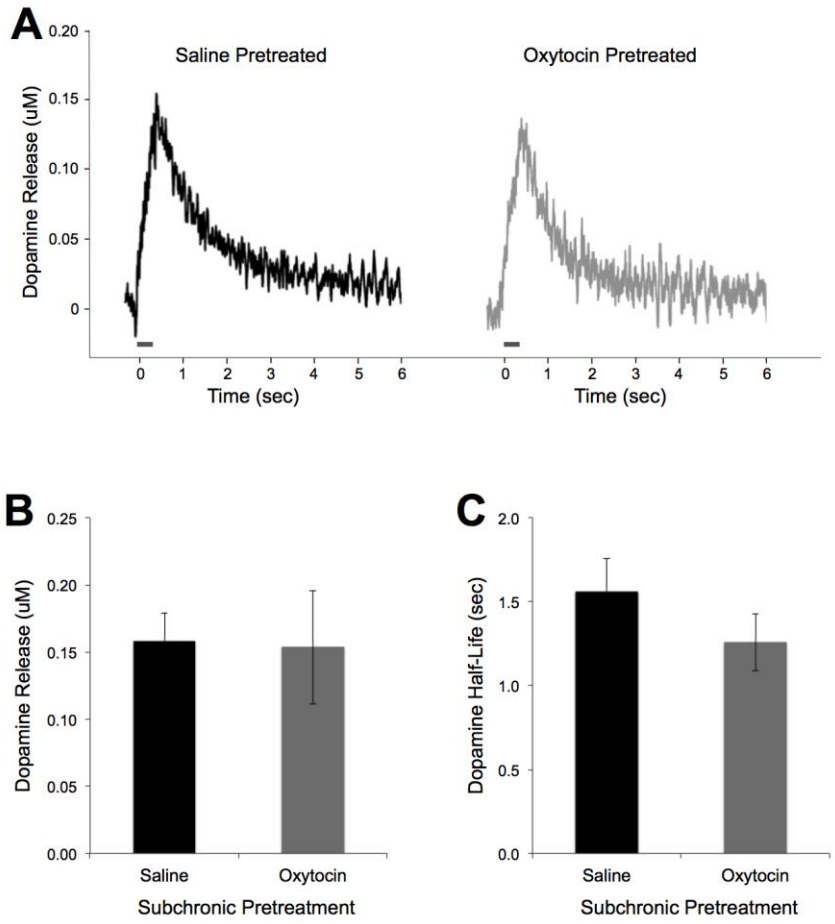

Figure 2. Amperometric recordings of stimulation-evoked dopamine release in the amygdala. (A) Profiles illustrate example responses from each pretreatment group. Time zero indicates the start of the stimulation train of 20 pulses at 50 Hz. Neither means (± SEM) in (B) dopamine release or (C) dopamine half-life, i.e. the time required for 50% decrease from the maximum evoked release to the prestimulus baseline level, were different between pretreatment groups.

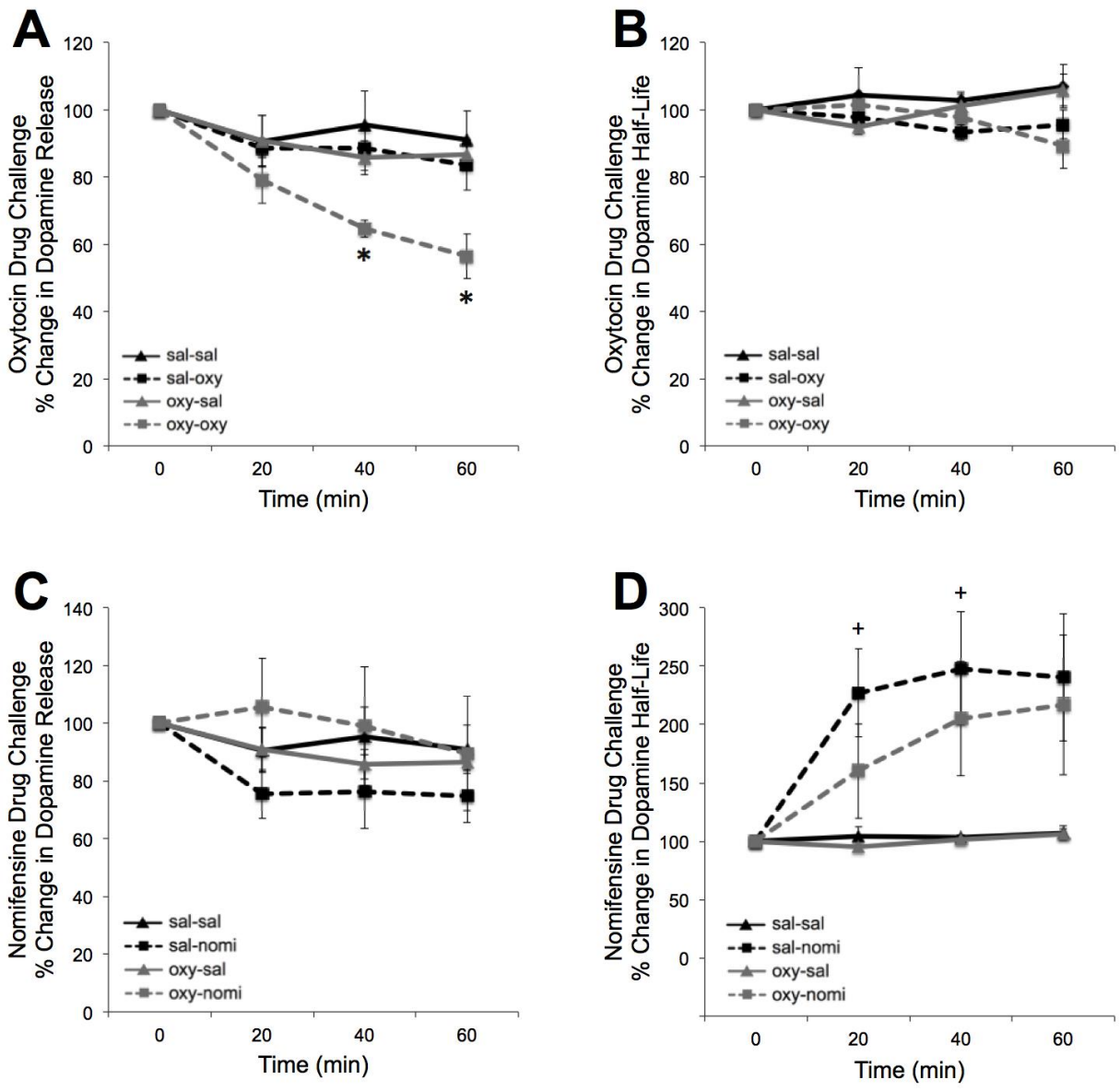

Figure 3. Amperometric recordings of stimulation-evoked dopamine release in the amygdala following an intraperitoneal drug challenge of either saline (control), oxytocin, or nomifensine (dopamine reuptake blocker). (A) Oxytocin administration significantly decreased dopamine release at 40 and 60 min post injection but only in the mice subchronically pretreated with oxytocin. * indicates significant difference between pretreatment groups. (B) Oxytocin administration did not alter dopamine half-life in any pretreatment group. (C) Nomifensine did not alter dopamine release in any pretreatment group. (D) Nomifensine significantly increased the dopamine half-life of mice pretreated with saline but not oxytocin. + indicates significant difference between drug challenges (nomifensine and saline). Legend signifies "pretreatment - drug challenge"; for example, "oxy-nomi" denotes the group of mice pretreated with subchronic oxytocin and administered nomifensine as the drug challenge during amperometric recordings.

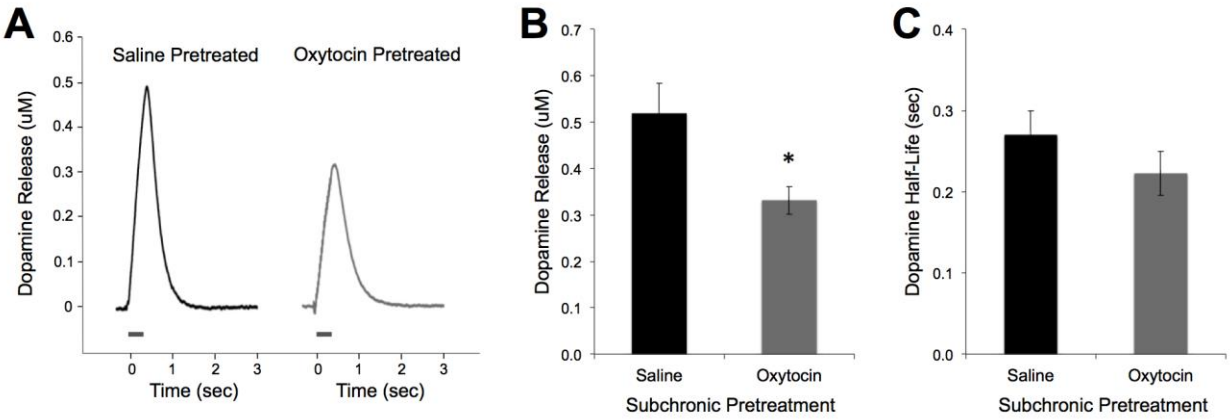

Figure 4. Amperometric recordings of stimulation-evoked dopamine release in the nucleus accumbens (NAc). (A) Profiles illustrate example responses from each pretreatment group. Time zero indicates the start of the stimulation train of 20 pulses at 50 Hz. (B) Mean (± SEM) differences in dopamine release were observed between treatment groups. * indicates $p < 0.05$. (C) No differences in mean (± SEM) dopamine half-life, i.e. the time required for 50% decrease from the maximum evoked release to the prestimulus baseline level, were observed between pretreatment groups.

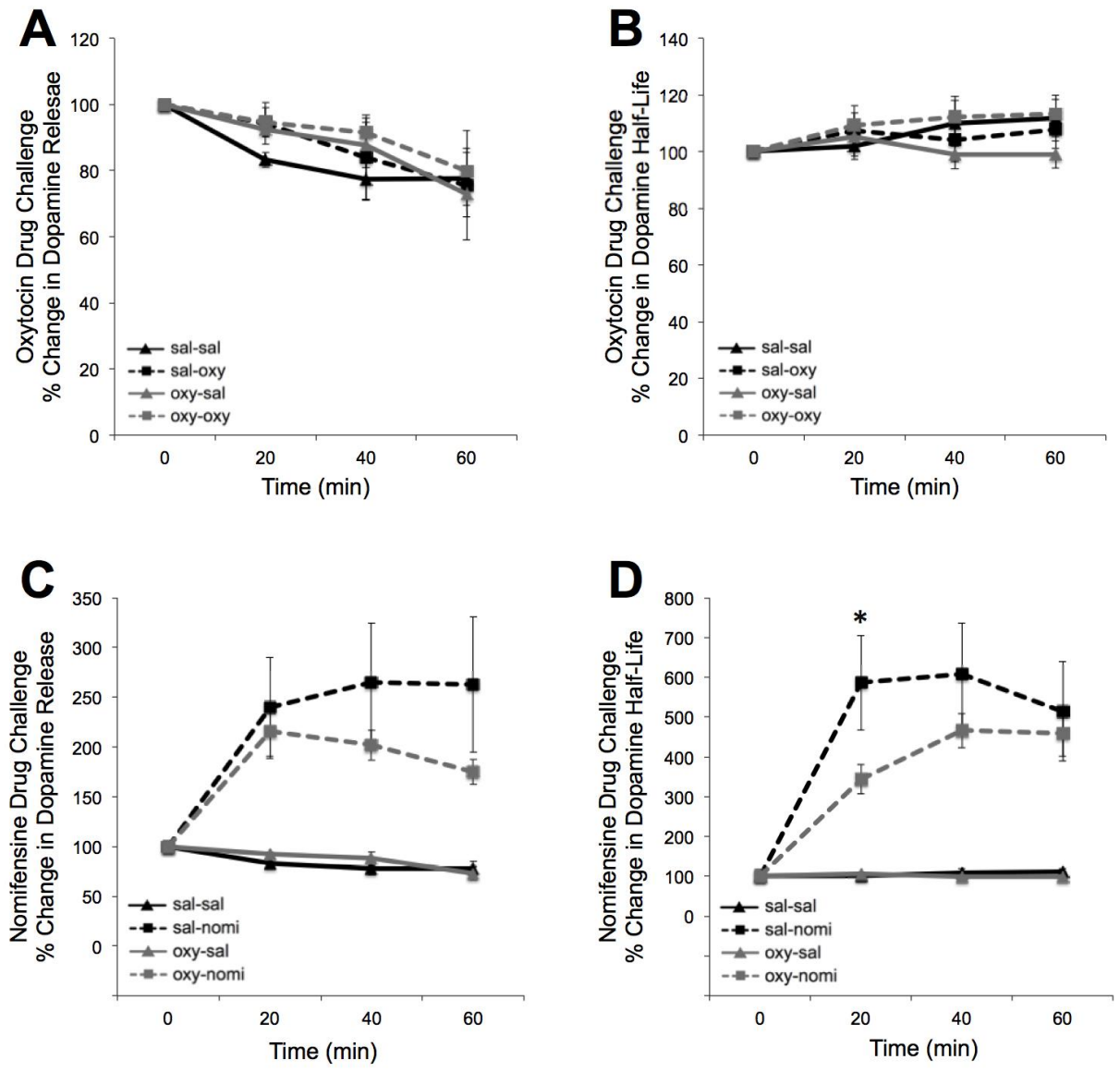

Figure 5. Amperometric recordings of stimulation-evoked dopamine release in the nucleus accumbens (NAc) following an intraperitoneal drug challenge of either saline (control), oxytocin, or nomifensine (dopamine reuptake blocker). Oxytocin administration did not alter (A) dopamine release or (B) dopamine half-life in any pretreatment group relative to saline. (C) Nomifensine acted similarly, increasing NAc dopamine release, in both pretreatment groups. (D) Nomifensine also significantly increased the dopamine half-life of mice in both pretreatment groups, but to a greater degree at 20 min in the mice pretreated with saline. * indicates significant difference between pretreatment groups. Legend signifies "pretreatment - drug challenge"; for example, "oxy-nomi" denotes the group of mice pretreated with subchronic oxytocin and administered nomifensine as the drug challenge during amperometric recordings.