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Examining Oxytocin as a Potential Pharmacotherapy for Methamphetamine Addiction

Brittney M. Cox
Medical University of South Carolina

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by

Brittney M. Cox

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Approved by:

Gary Aston-Jones
Advisory Committee Chairman

Ronald E See
Co-Chairman

Carmela M Reichel

Judson Chandler

Patrick J Mulholland

Stan B Floresco

Abstract

Human and animal studies suggest that females differ in their motivation to use methamphetamine (meth), and have increased propensity to relapse. However, addiction pharmacotherapies have primarily only been tested in males, which may not accurately predict treatment outcomes in females. Evidence suggests that oxytocin, an endogenous peptide well known for its role in social behaviors and childbirth, is a promising addiction pharmacotherapy. This dissertation first examines sex differences in the effect of oxytocin to decrease meth and sucrose seeking. Our data indicate that systemic oxytocin decreased responding for meth differentially in males and females on a progressive ratio schedule of reinforcement (a measure of motivation) in females but not males, although it reduced reinstatement of meth seeking similarly in both sexes. To further examine these sex differences, we next employed a translational within-session behavioral-economic (BE) model designed specifically for meth self-administration. The BE paradigm allows for measurement of drug demand at high effort (motivation; α), normalized based on intake at low effort (baseline consumption; Q_0) within the same session. This approach also allowed us to assess individual variability in meth demand in relation to relapse behaviors, and in response to oxytocin administration. Our rodent BE paradigm was modeled after BE procedures commonly used to assess motivation for reward in humans and non-human primates. Importantly, the same BE variables (α, Q_0) are

assessed across species, and these variables have been shown to predict later relapse behavior. Therefore, the translational potential of preclinical BE studies is particularly strong. We showed that this BE model can predict relapse-like behaviors, and that systemic oxytocin acts similarly in both males and females to decrease demand (i.e., motivation) for meth and attenuated reinstatement to meth seeking. We also demonstrated that oxytocin is most effective at decreasing meth seeking in rats with the strongest addiction phenotype. Finally, we showed that systemic oxytocin acts via a central mechanism, and more specifically through actions in the NAc core. Together these results demonstrate that oxytocin modifies multiple meth-seeking behaviors, show the efficacy of oxytocin as a pharmacotherapy for addiction in both sexes, and characterize the effects of oxytocin on mesolimbic brain circuitry implicated in addiction. Overall, these data indicate that oxytocin-based therapies would be a promising treatment approach for meth addiction in humans.

I dedicate this dissertation to my father, Galen Cox.
Although you were unable to see me finish this work,
I will forever know how proud you are of me.

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CHAPTER 1: Introduction

History of Methamphetamine Abuse

Methamphetamine (meth), a derivative of amphetamine, was first synthesized in 1893 (Suwaki, Fukui et al. 1997). However, the first widespread use of the drug did not occur until World War II (WWII), when soldiers in the United States, Germany, United Kingdom, and Japan were all documented to have used amphetamine or methamphetamine for its stimulant and performance enhancing properties (Meredith, Jaffe et al. 2005, Rasmussen 2011). In fact, Adolf Hitler was known to use intravenous meth whenever he needed energy, and later in his life he exhibited signs of Parkinson's disease thought to possibly be due to his excessive meth use (Doyle 2005). After WWII, Japan suffered from the first meth epidemic (Lineberry and Bostwick 2006); however, prescription regulations slowed the onset of the epidemic in the United States. Regardless, by the 1950's prevalence of amphetamine abuse was on the rise and prescription forms were being promoted for treatment of obesity, narcolepsy, and depression (Wolkoff 1997). The Controlled Substance Act of 1970 and the classification of

amphetamines as a Schedule II drug in 1971 helped to reduce some of the abuse problems (Beebe and WALLE 1995, McGuinness 2006). However, due to production by clandestine labs, meth use began rising again in the 1980's (Cunningham and Thielemeir 1996). In response, the United States government began to regulate the precursor chemicals used to make meth (Cunningham and Liu 2003). This approach caused the market to transition to acquire meth primarily from foreign producers (Cunningham and Liu 2008), namely Mexico, and attempting to control this production has been challenging (Shukla, Crump et al. 2012). Overall, meth addiction has been a problem since the early twentieth century and remains a significant problem today.

Methamphetamine Addiction

Meth is a highly addictive psychostimulant that is most commonly smoked or snorted, but can also be taken orally or injected (Brecht, O'Brien et al. 2004, SAMHSA 2005). However, compared to amphetamine, meth is a much more potent stimulant that can more easily cross the blood brain barrier and has a longer half-life (~10 h in humans) (Cruickshank and Dyer 2009). Meth addiction is a particularly detrimental health problem in the United States and worldwide, with over 12 million people (4.7% of the US population) estimated to have tried meth, with the number of new users significantly increasing from the previous year (SAMHSA 2010). When occasional abuse of meth transitions into compulsive use and addiction, various long-term health consequences result including: significant weight loss, severe dental problems, damage to the cardiovascular

system, psychotic behavior, increased impulsivity, deficits in memory and attention, increased aggression and violence, and alterations in affect and mood (Paulus, Hozack et al. 2003, Klasser and Epstein 2005, Hoffman, Moore et al. 2006, Ikeda, Iwata et al. 2006, McKetin, McLaren et al. 2006, Sekine, Ouchi et al. 2006, Looby and Earleywine 2007, Salo, Nordahl et al. 2007, Salo, Leamon et al. 2008, Salo, Nordahl et al. 2008). While the detrimental effects to the addict are clear, meth abuse is estimated to cost the United States $23.4 billion a year through its effects on crime, unemployment, child abuse or neglect, and other social issues (Nicosia, Pacula et al. 2009).

Meth addiction is a chronically relapsing disorder and addicts who try to quit often face severe withdrawal symptoms including craving, disturbed sleep, anxiety, depression, and cognitive deficits (Kalechstein, Newton et al. 2003, London, Berman et al. 2005, McGregor, Srisurapanont et al. 2005). While most of these symptoms can resolve in days to weeks, cognitive deficits have been shown to persist months to years after abstinence (Volkow, Chang et al. 2001, Volkow, Chang et al. 2001, Nordahl, Salo et al. 2003, Johanson, Frey et al. 2006, Salo, Nordahl et al. 2007). These cognitive deficits early in abstinence can compromise executive and psychomotor function, and likely contribute to the high rates of relapse (Kalechstein, Newton et al. 2003, Paulus, Tapert et al. 2005). Thus, despite the great need for effective behavioral and pharmacotherapeutic intervention, no satisfactorily effective treatments for meth addiction currently exist.

Pharmacology of Methamphetamine

Similar to other psychostimulants like cocaine, meth produces much of its effects by increasing monoamine release. However, as compared to cocaine, meth has a significantly longer duration of action and increases monoamine release to a much greater degree. Meth has an average half-life of 10 hours (in humans), depending on many factors, including the route of administration and inter-individual variability (Cruickshank and Dyer 2009). Meth acts as an indirect agonist at the dopamine, norepinephrine, and serotonin receptors, and also acts as a substitute for these neurotransmitters at their transporters (dopamine (DAT), norepinephrine (NET), serotonin (SERT), and the vesicular monoamine transporter-2 (VMAT-2) (Sulzer, Sonders et al. 2005). By acting at VMAT-2, meth reverses transporter function and causes the monoamines to leave the vesicles and enter the cytosol at the synaptic terminal. In turn, this alters the pH gradient, which reverses the endogenous function of DAT, NET, and SERT, releasing the neurotransmitters into the synapse (Cruickshank and Dyer 2009). In addition, meth inhibits monoamine metabolism by inhibiting monoamine oxidase (Sulzer, Sonders et al. 2005). *In vitro* studies examining these mechanisms have shown that meth is twice as effective as releasing norepinephrine than dopamine, and 60x greater than serotonin (Rothman, Baumann et al. 2001).

Neurotoxicity

Studies in animals and humans have examined the neurotoxic effects of meth, and the mechanisms by which meth produces these effects. Repeated

exposure to amphetamines damages dopaminergic and serotonergic neurons, potentially via oxidization of these transmitters in the cytosol, which can damage nearby proteins and lipids (Cruickshank and Dyer 2009). Additionally, increased body temperature is also thought to heavily contribute to these effects (Riddle, Hanson et al. 2007). Primate studies show that repeated exposure to meth (in doses consistent with human abuse) causes neurotoxicity that can persist for more than a year (Villemagne, Yuan et al. 1998, Harvey, Lacan et al. 2000). Human PET studies in various brain regions have shown many indicators of neurotoxicity including reduced density of DAT (Volkow, Chang et al. 2001, Sekine, Minabe et al. 2003) VMAT-2 (Johanson, Frey et al. 2006), SERT (Sekine, Ouchi et al. 2006), and D2 receptors (Volkow, Chang et al. 2001). These reductions in receptor and transporter density have also been shown to persist months to years after cessation of prolonged meth use (Volkow, Chang et al. 2001, Wang, Volkow et al. 2004, Chang, Alicata et al. 2007).

Animal Models of Addiction

The most common animal model used to assess relapse-like behaviors is the self-administration and reinstatement (relapse) model of addiction, where animals respond to receive an i.v. drug infusion accompanied by stimuli that become associated with the rewarding effects of the drug via Pavlovian conditioning. Following a period of drug self-administration, subjects undergo extinction training. Reinstatement of drug seeking can then be elicited through presentation of meth-associated stimuli (cue-induced reinstatement), meth itself

(primed reinstatement), or stress (e.g., shock-induced reinstatement). This model of relapse has face validity largely due to the response contingent drug delivery, and the similarity of factors that elicit reinstatement to those that cause drug craving and relapse in abstinent individuals (Jaffe, Cascella et al. 1989, Sinha, Fuse et al. 2000). However, while animal models of addiction and relapse have identified numerous drugs that attenuate drug seeking in animals, most have not been effective when tested in humans (Pierce, O'Brien et al. 2012). Thus, in Chapters 3 & 4, we will build on the current model and incorporate an economic framework in order to mathematically compare animal and human studies and potentially increase predictive validity for preclinical testing of potential addiction pharmacotherapies.

Using a behavioral economic framework for valuation of drugs has been well characterized across species with the design of many different paradigms that allow for a mathematically identical method for quantifying behavior (Hursh 1980, Hursh and Silberberg 2008). Human studies indicate that behavioral economic measures can predict various measures of addiction related behaviors. For example, economic demand predicted alcohol dependence, and overall lifetime use of heroin, cocaine, and marijuana (Petry 2001, Murphy, MacKillop et al. 2009, Gray and MacKillop 2014). The behavioral economic framework and the specific paradigm used will be described in Chapter 3.

Gender and sex differences in meth addiction

Meth addiction is a substantial problem that affects both men and women, although clinical research has shown numerous sex differences in terms of use patterns and responses to treatment (Dluzen and Liu 2008). Clinical studies have shown that females have a greater vulnerability to meth addiction. Specifically, females initiate meth use at a younger age (Lin, Ball et al. 2004, Hser, Evans et al. 2005), transition faster from recreational use to dependence, exhibit greater dependence on meth (Kim and Fendrich 2002, Rawson, Gonzales et al. 2005), and have greater comorbidity to other neuropsychiatric disorders (Hser, Evans et al. 2005, Yen and Chong 2006). Importantly, these differences suggest that females may respond differently to treatment strategies for meth addiction.

Animal models of meth addiction have shown similar sex differences as to those seen clinically in human addicts. Compared to males, females acquire self-administration faster (Roth and Carroll 2004, Kucerova, Vrskova et al. 2009), have higher meth intake (Reichel, Chan et al. 2012), and show greater reinstatement of meth seeking (Holtz, Lozama et al. 2012, Reichel, Chan et al. 2012, Cox, Young et al. 2013). Females also show greater motivation for meth on a progressive ratio (PR) schedule of reinforcement (Roth and Carroll 2004, Cox, Young et al. 2013). Overall, these studies indicate clear sex differences in the motivation to seek meth in humans and animal models; however, almost all preclinical studies assessing potential pharmacotherapies for addiction have been exclusively tested in males.

The Nucleus Accumbens and Addiction

The critical role of the nucleus accumbens (NAc) in mediating the addiction process has long been a central focus of addiction research. The NAc is a critical interface between the dopaminergic input from the mesocorticolimbic projection from the ventral tegmental area and glutamatergic inputs from several regions, including the prefrontal cortex and amygdala (Wise 1996, Kalivas and O'Brien 2008, Torregrossa, Tang et al. 2008, Carlezon and Thomas 2009). Increasing dopamine levels in the NAc is characteristic of all addictive drugs (Sacchettini, Frazier et al. 1988, Wise 1996, Phillips, Robinson et al. 2003, Phillips, Stuber et al. 2003, Luscher and Ungless 2006, Sulzer 2011) and self-administration of psychostimulants in particular depends upon effects on dopamine signaling in the NAc (Roberts, Koob et al. 1980, Pulvirenti, Maldonado-Lopez et al. 1992). Further, dopamine and glutamate in the NAc critically mediate drug seeking, reinstatement, and other motivated behaviors (Cornish and Kalivas 2000, Kalivas and Volkow 2005, Kalivas 2009, Floresco 2015). The NAc is divided into two major regions: the NAc core (the area surrounding the anterior commissure) which has been associated with initiation of motivated behaviors (Cornish and Kalivas 2000, McFarland, Lapish et al. 2003) and the NAc shell (medial and ventral portions) (Zahm and Brog 1992) which mediates the immediate reinforcing actions of psychostimulants (Rodd-Henricks, McKinzie et al. 2002, Sellings and Clarke 2003). The NAc contains over 95% GABAergic medium spiny neurons (MSN’s), along with both cholinergic and GABAergic

interneurons (Pennartz, Groenewegen et al. 1994, Tepper and Bolam 2004). Work in Chapter 4 of this dissertation focuses specifically on the NAc core. The next section will elaborate on the NAc, specifically the effects of oxytocin in the NAc.

The Oxytocin System

The endogenous peptide oxytocin is best known for its peripheral actions in female parturition and childbirth (e.g., inducing uterine contractions and lactation) (Dale 1906, Ott and Scott 1910, Mackenzie 1911). Oxytocin is synthesized in magnocellular neurons of the supraoptic and paraventricular nuclei (PVN) of the hypothalamus, and secreted via the posterior pituitary for peripheral circulation (Renaud and Bourque 1991, Gimpl and Fahrenholz 2001, Baskerville and Douglas 2010). Oxytocin is also a centrally active neuropeptide that influences many behaviors, including reward-related behavior (Baskerville and Douglas 2010). Parvocellular neurons in the PVN send oxytocin efferents to multiple brain regions, modulating neuronal activity (Sawchenko and Swanson 1982, Renaud and Bourque 1991, Baskerville and Douglas 2010, Knobloch, Charlet et al. 2012). Oxytocin binds to the Gq class of G protein-coupled receptors (GPCRs) activating phopholipase C (PLC) and subsequently increasing neuronal firing and neurotransmitter release through this second messenger cascade (Strakova and Soloff 1997, Gimpl and Fahrenholz 2001). Oxytocin receptors are located throughout the CNS (Gimpl and Fahrenholz 2001) and in both males and females, central oxytocin regulates social and sexual

bonding, as well as other behaviors such as aggression, stress, and anxiety (Milesi-Halle, McMillan et al. 2007, Baskerville and Douglas 2010).

Oxytocin mediates social and sexual behaviors in part through actions in the mesocorticolimbic dopamine system (Baskerville and Douglas 2010). PVN oxytocin neurons project to multiple regions of the mesocorticolimbic dopamine system (Knobloch, Charlet et al. 2012), including the NAc, ventral tegmental area (VTA), and prefrontal cortex (PFC) (figure 1-1).

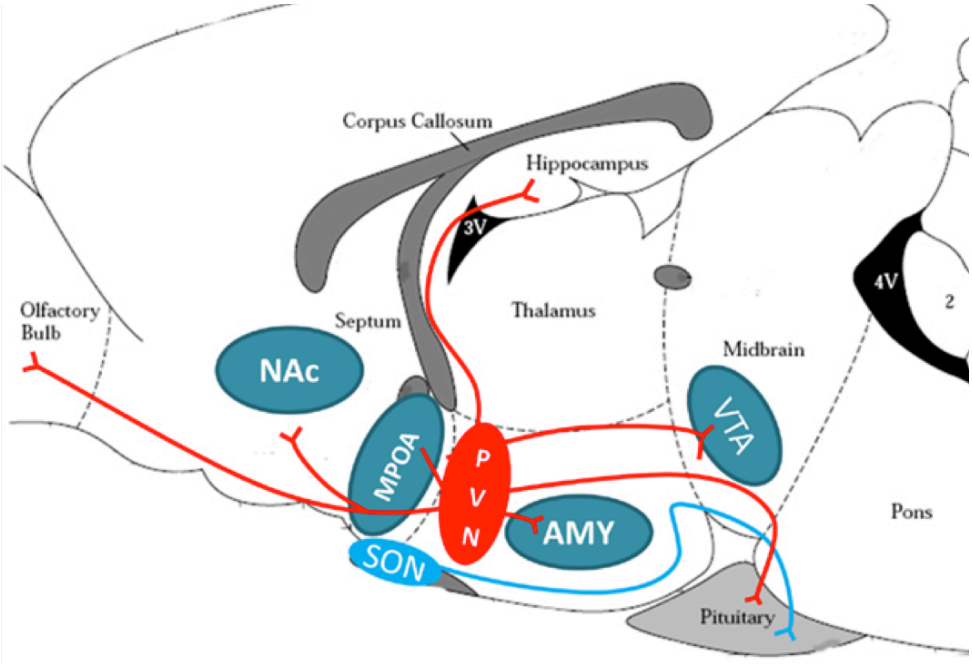

Figure 1-1 Oxytocinergic projections in the rodent brain.
The magnocellular neurons of the paraventricular nucleus (PVN, red oval) and supraoptic nucleus (SON, blue oval) project to the posterior pituitary to release oxytocin peripherally. Parvocellular neurons of the PVN have major projections to the nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus, amygdala (AMY), medial preoptic area (MPOA), and olfactory bulb (OB). This schematic was adapted from (Rutherford, Williams et al. 2011).

The majority of the studies examining how oxytocin interacts with the dopamine system have focused on social behaviors. For example, oxytocin projections from the PVN to the VTA innervate dopamine neurons in the NAc to promote sexual behavior (Melis, Melis et al. 2007) and maternal behavior (Shahrokh, Zhang et al. 2010), and oxytocin injected into the VTA increases dopamine release in NAc (Melis, Melis et al. 2007). Hypothalamic oxytocin cells also express dopamine receptors (Baskerville, Allard et al. 2009), suggesting that

dopamine may also mediate oxytocin release. In addition, pair bonding in monogamous prairie voles is dependent on dopamine interactions in the NAc; specifically, D2 receptors promote bonding and D1 receptors inhibit bonding (Liu and Wang 2003). The next section will further elaborate on the oxytocin system as it pertains to addiction behaviors.

Oxytocin as a Treatment for Addiction

More recently, oxytocin has been examined as a potential pharmacotherapy for addiction (Sarnyai and Kovacs 1994, Baskerville and Douglas 2010, Carson, Cornish et al. 2010, McGregor and Bowen 2012, Carson, Guastella et al. 2013, Sarnyai and Kovacs 2014). It has been suggested that overlap exists between how oxytocin interacts within the mesocorticolimbic dopamine system to mediate social behaviors and addiction-like behaviors (Burkett and Young 2012), as this system is known to be critical for motivation to seek drugs during relapse (Wise 1988, Koob, Sanna et al. 1998, Shaham, Shalev et al. 2003, Everitt and Robbins 2005, Kalivas and Volkow 2005, Volkow, Fowler et al. 2007, Koob and Le Moal 2008). For example, dopamine in the NAc is required for the formation of pair bonds and initial learning of psychostimulant self-administration (Burkett and Young 2012). Additionally, D1 agonists block pair bond formation and also block some drug seeking behaviors (Burkett and Young 2012).

In clinical studies, oxytocin decreased alcohol withdrawal symptoms and craving (Pedersen, Smedley et al. 2013) and craving for marijuana in dependent

individuals (McRae-Clark, Baker et al. 2013). In animal studies, oxytocin has been shown to decrease numerous drug-related behaviors for a variety of addictive drugs including opiates and alcohol (Kovacs, Sarnyai et al. 1998). For the purposes of the dissertation, only those studies that have examined the effects of oxytocin on psychostimulants will be discussed.

Peripheral and centrally-administered oxytocin (intracerebroventricular, (ICV)) inhibits cocaine-induced locomotor and stereotyped behavior (Sarnyai and Kovacs 1994), as well as meth-induced locomotor activity (Qi, Yang et al. 2008). Oxytocin administered systemically and ICV also blocks conditioned place preference for meth and reinstatement of the meth-induced preference. (Qi, Yang et al. 2009, Baracz, Rourke et al. 2012, Carson, Bosanquet et al. 2012, Subiah, Mabandla et al. 2012). In self-administration studies, oxytocin decreased cocaine intake during self-administration (Sarnyai and Kovacs 1994, Zhou, Sun et al. 2014), reinstatement of cocaine-seeking (Morales-Rivera, Hernandez-Burgos et al. 2014, Zhou, Sun et al. 2014), and reinstatement of meth-seeking (Carson, Cornish et al. 2010, Cox, Young et al. 2013).

To examine the central site of action for oxytocin on decreasing drug-reward behaviors, Carson et al. (2010) examined brain regions in which systemically-administered oxytocin decreased acute meth-induced neuronal activation (Fos activation). These regions included the NAc core, subthalamic nucleus (STN), and prelimbic region of the PFC (Carson, Hunt et al. 2010). Further, microinjections of oxytocin in the STN blocked meth-induced CPP and microinjections into the NAc core blocked meth-preference (Baracz, Rourke et al.

2012) and meth-primed reinstatement (Baracz, Rourke et al. 2012). Additionally, systemic oxytocin administration blocked cocaine-induced dopamine release in the NAc (Kovacs, Sarnyai et al. 1990). In conclusion, oxytocin appears to be interacting with the mesocortical dopamine system to attenuate meth seeking; however, specific mechanisms have not been determined.

To date, essentially all preclinical studies examining oxytocin effects on drug reward-related behaviors have only used male subjects; thus, it is unclear whether these effects extend to females. However, this is an important question given that gonadal hormones including testosterone, estrogen, and progesterone have been shown to affect oxytocin binding affinity and receptor density in various brain regions (Schumacher, Coirini et al. 1990, Coirini, Schumacher et al. 1991, Patchev, Schlosser et al. 1993). As progesterone and estrogen fluctuate during the various estrous cycle phases in female rats, it is important to consider how oxytocin may differentially affect females, especially when assessing oxytocin as a pharmacotherapy for addiction. Further studies are needed to examine whether the effects of oxytocin on drug seeking behaviors differ in females.

The work presented in this dissertation examined the efficacy of oxytocin as a pharmacotherapy for meth addiction. First we assessed the effects of systemic oxytocin on meth seeking behaviors in males and females. Next we developed a within-session behavioral economic model for meth self-administration to further explore sex differences in the effects of systemic oxytocin on meth seeking behaviors. Using this model we then examined if

systemic oxytocin is attenuating meth seeking behaviors through a peripheral or central mechanism. Finally, we examined whether the NAc core is the region where oxytocin is acting to attinuate meth seeking behaviors.

CHAPTER 2: Sex Differences in the Effects of Oxytocin on Methamphetamine Seeking

Introduction

Oxytocin may have a regulatory role in attenuating drug tolerance, dependence, and withdrawal via actions in the mesolimbic dopamine reward pathways (Baskerville and Douglas 2010). Systemic oxytocin blocked cocaine-induced dopamine release in the nucleus accumbens (Kovacs, Sarnyai et al. 1990) and decreased cocaine intake during self-administration (Sarnyai and Kovacs 1994). Additionally, recent preclinical evidence suggests that oxytocin may have therapeutic benefits in preventing relapse to methamphetamine (meth) use (Carson, Cornish et al. 2010). Of particular note, oxytocin decreased meth seeking in an animal model of addiction (Carson, Cornish et al. 2010) and reduced meth-conditioned reward (Qi, Yang et al. 2009, Baracz, Rourke et al. 2012, Subiah, Mabandla et al. 2012) in males. Oxytocin may be a critical regulator in drug addiction via modulation of dopaminergic transmission in corticolimbic structures (Sarnyai and Kovacs 1994, Baskerville and Douglas

2010). For example, oxytocin decreased dopamine release and receptor binding in mesolimbic brain structures (Sarnyai and Kovacs 1994). Support for the use of oxytocin as an addiction treatment has gained momentum, due in part to the prosocial effects associated with some drugs of abuse (Dumont, Sweep et al. 2009). Further, neural circuits mediating social bonding and drug reward may overlap (Burkett and Young 2012).

To date, the ability of oxytocin to ameliorate meth seeking or conditioned reward in female rats is unknown. However, this is an important question given that gonadal hormones regulate oxytocin's binding affinity and receptor density in various brain regions (Schumacher, Coirini et al. 1990, Coirini, Schumacher et al. 1991, Patchev, Schlosser et al. 1993). Further, progesterone and estrogen fluctuate throughout the estrous cycle in female rats, rendering studies with females a necessity to determine oxytocin's full potential as a pharmacotherapeutic treatment for meth addiction. Clinical research in meth addiction indicates numerous sex differences in terms of meth use patterns and response to treatment (reviewed in (Dluzen and Liu 2008). For example, women tend to initiate meth use at a younger age (Dluzen and Liu 2008), transition faster to dependence, exhibit greater dependence (Kim and Fendrich 2002, Rawson, Gonzales et al. 2005), and have greater comorbidity with other neuropsychiatric disorders (Hser, Evans et al. 2005, Yen and Chong 2006). While clinical evidence has shown gender differences, preclinical research investigating meth addiction and potential pharmacotheraputic treatments has primarily focused only on male subjects. This pattern is problematic, as females may respond

differentially to treatments when compared to males, suggesting a need for gender/sex specific therapies. Consistent with clinical populations, animal models indicate that female rodents have an increased sensitivity to meth's psychomotor stimulating effects (Schindler, Bross et al. 2002, Milesi-Halle, McMillan et al. 2007).

Animal models of drug self-administration incorporate various aspects of the addiction cycle, including motivation to consume a drug and/or drug seeking behavior in the absence of reinforcement. Self-administration models require animals to learn to press an operandum (typically a lever) for an intravenous drug infusion. In these models, more female rats acquire meth self-administration relative to males (Roth and Carroll 2004), exhibit higher meth intake (Reichel, Chan et al. 2012), and exert more effort for meth as a primary reward (Roth and Carroll 2004). Meth seeking can be inferred by the reinstatement of lever responding following a period of non-reinforced responding. These reinstatement tests incorporate various trigger factors (e.g., cues, drug-priming, or stress) for the testing of pharmacotherapies that may block reinstatement (Yahyavi-Firouz-Abadi and See 2009).

Here, we assessed whether systemic oxytocin may be a potential treatment for meth addiction in females and males. To this end, we first determined the effects of oxytocin on meth-primed reinstatement of meth seeking throughout the various stages of the estrous cycle in female rats. Second, we determined whether oxytocin would impact motivation for meth in both males and females during meth self-administration and in response to meth associated cues,

meth priming, or pharmacological stress (yohimbine) induced reinstatement. Finally, we determined whether oxytocin effects would extend to natural reinforcement (i.e., sucrose pellets) in males and females.

Methods

Subjects

A total of 22 male and 39 female Long-Evans rats (Charles River, Raleigh, NC) weighing 250-300 g and 180-200 g, respectively, at the time of arrival, were used. All rats were individually housed on a reversed 12:12 light-dark cycle in a temperature and humidity controlled vivarium. Water was available *ad libitum* throughout the study and rat chow (Harlan, Indianapolis, IN, USA) was provided daily until SA stabilized, after which time food was provided *ad libitum.* All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina and were in accordance with the "Guide for the Care and Use of Laboratory Rats" of the Institute of Laboratory Animal Resources on Life Sciences, National Research Council.

Surgery and drugs

Rats were anesthetized with IP injections of ketamine (66 mg/kg; Vedco Inc, St. Joseph, MO, USA), xylazine (1.3 mg/kg; Lloyd Laboratories, Shenandoah, IA, USA), and Equithesin (0.5 ml/kg; sodium pentobarbital 4 mg/kg, chloral hydrate 17 mg/kg, 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol, 10% ethanol solution). Ketorolac (2.0 mg/kg, IP; Sigma Chemical, St. Louis, MO) was given before surgery as an analgesic. Catheters (constructed with Silastic tubing, Dow Corning Corporation, Midland, MI) were

inserted 33 mm into the right jugular vein and secured with silk sutures. During recovery from surgery, catheters were flushed once daily for 5 days with 0.1 ml of Timentin (24 mg/0.1; GlaxoSmithKline, Research Triangle Park, NC) and 10 U/ml of heparinized saline (Elkins-Sinn, Cherry Hill, NJ). During meth self-administration (methamphetamine hydrochloride; Sigma Chemical, St. Louis, MO), catheters were flushed with 0.1 ml of 10 U/ml heparinized saline before and after every self-administration session. Catheter patency was periodically verified with methohexital sodium (10 mg/ml dissolved in 0.9% saline; Sigma Chemical, St. Louis, MO), a short acting barbiturate that produces a rapid loss in muscle tone when administered intravenously. Oxytocin (1 mg/ml dissolved in ddH_20; Cell Sciences, Canton, MA) was administered prior to PR and reinstatement testing, and yohimbine hydrochloride (2.5 mg/ml dissolved in ddH_20; Sigma Chemical, St. Louis, MO) was used for stress-induced reinstatement (Shepard, Bossert et al. 2004, See and Waters 2010).

Self-administration

Self-administration procedures were based on recent studies of meth self-administration in males (Reichel and See 2010, Reichel, Chan et al. 2012, Mahler, Moorman et al. 2013). All self-administration experiments were conducted during the rats' dark cycle in standard Plexiglas self-administration chambers (30 X 20 X 20 cm) that were enclosed in sound attenuating cubicles with a ventilation fan (Med Associates, St. Albans, Vermont) and linked to a computerized data collection program (MED PC, Med Associates). Each

chamber was equipped with two retractable levers with a white stimulus light above each lever, house light, and tone generator. For meth self-administration, infusion tubing enclosed in steel spring leashes (Plastics One Inc., Roanoke, VA) was connected to the infusion harness and a weighted swivel apparatus (Instech, Plymouth Meeting, PA) was suspended above the box to allow for free movement within the chamber.

Self-administration sessions were conducted 6 days/week to criterion (14 sessions > 10 infusions). The house light remained on throughout the sessions and a response on the active lever resulted in activation of the pump and delivery of a 2-sec meth infusion (17.5 µg/50 µl bolus for females and 20 µg/50 µl bolus for males) and a 5-sec presentation of a stimulus complex (illumination of the white stimulus light over the active lever and activation of tone generator; 78 dB, 4.5 kHz), followed by a 20-sec time-out. During the time-out period, responses on the active and inactive levers were recorded, but had no scheduled consequences.

Progressive ratio schedule of reinforcement

PR tests were conducted when stable self-administration was evident based on the criteria of 14 days with more than 10 reinforcers earned. Before tests, rats were injected with oxytocin (1 mg/kg) or vehicle. Test order was counterbalanced, and between tests, rats received a minimum of 2 self-administration sessions. During these tests, reinforcement was contingent upon an increasing number of responses which incrementally increased through the

following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603 (Richardson and Roberts 1996). The session terminated if a rat failed to receive an infusion or sucrose pellet for 1 h or after a total of 5 h.

Extinction and reinstatement

Following self-administration, rats underwent 2 h daily extinction sessions for a minimum of 10 days, where responses on both the active and inactive levers were recorded, but had no scheduled consequences. Extinction criterion consisted of <25% responding on the active lever relative to the last 5 days of self-administration. Upon reaching criteria, rats underwent cue-, meth-, and yohimbine-induced reinstatement testing. Prior to all reinstatement tests, rats received either an injection of oxytocin (0.3 or 1 mg/kg) or vehicle (IP) 30 min before testing (see specific experimental methods detailed below). For cue-induced reinstatement, responding on the active lever resulted in presentation of the light+tone stimulus complex along an FR5 schedule of reinforcement. For drug-primed reinstatement tests an injection of meth (1 mg/kg dissolved in 0.9% physiological saline, IP) was given immediately prior to testing (Schwendt, Rocha et al. 2009, Reichel, Chan et al. 2012). Prior to stress-induced reinstatement tests, an injection of yohimbine hydrochloride (2.5 mg/kg, IP) was given after oxytocin or vehicle, 30 min prior to testing. During both meth-primed and yohimbine-induced reinstatement tests, responses on either lever were recorded, but did not elicit any programmed consequences. Between all reinstatement

tests, rats experienced a minimum of 2 extinction sessions, or until extinction criterion was met.

Estrous cycle monitoring

Female rats were habituated to vaginal cytology procedures during self-administration. Vaginal lumen samples were collected by gently flushing 30 µl of ddH_20 with a sterile saline-dipped pipette tip and extracting the sample using a micropipette before rats were placed in the self-administration chambers. Collected samples were smeared on to a glass slide, stained with Quik-Dip Hematology Stain (Mercedes Medical, FL), and examined for classification of cycle phase (estrus, proestrus, and diestrus I/II) based on previously published criteria (Marcondes, Bianchi et al. 2002, Feltenstein, Henderson et al. 2011) with a light microscope set at 10x magnification.

Experiment 1: Oxytocin effects on meth-primed reinstatement across the estrous cycle

Female rats (n=10) self-administered meth along a FR 1 schedule of reinforcement throughout the self-administration phase. Following self-administration and extinction, rats underwent nine meth-primed reinstatement tests with pretreatment of 0, 0.3, and 1 mg/kg oxytocin. When possible, rats were tested during each of the phases of their estrous cycle (estrus, proestrus, diestrus I/II). Reinstatement tests were conducted in randomized order with a

minimum of 2 extinction sessions occurring between tests, or until subjects returned to criterion.

Experiment 2: Oxytocin effects on meth self-administration and reinstatement

Male (n=15) and female (n=17) rats initially self-administered meth along a FR1 schedule of reinforcement until they reached criterion of a minimum of 5 days with >10 infusions (with <25% change in the number of infusions for 2 consecutive days). Rats then moved to a FR3 schedule for a minimum of 3 days, followed by a FR5 schedule for the remainder of the self-administration sessions. Testing on the PR reinforcement schedule followed stable responding on the FR5. Following these tests, extinction criterion was met and all rats underwent 3 types of reinstatement tests in the following order: cue-induced, meth-primed, and yohimbine-induced, each counterbalanced for oxytocin (1 mg/kg) or vehicle pretreatment for a total of 6 reinstatement tests. Test order did not interact with reinstatement responding.

Experiment 3: Oxytocin effects on sucrose self-administration and reinstatement

Males (n=7) and females (n=12) underwent identical procedures as rats in Experiment 2, with the following exceptions. Instead of meth, sucrose pellets (45 mg, Noyes pellets, Fisher Scientific) served as the primary reinforcer. Sucrose rats did not undergo surgery. Additionally, during sucrose primed reinstatement

tests, rats received one non-contingent pellet every 2 min for the first 10 min of the session and one pellet every 30 min thereafter, modified from previous procedures (Kumaresan, Yuan et al. 2009).

Data analysis

The number of lever responses, breakpoint, and infusions were the primary dependent variables and were analyzed using analysis of variance (ANOVA). For reinstatement testing, lever responses were analyzed using a two-way ANOVA with sex and oxytocin pretreatment as between subject factors. Post hoc analyses were conducted using Bonferroni's when applicable, with the alpha set at 0.05. All data are expressed as the mean ± SEM.

Results

Experiment 1: Estrous cycle effects on meth-primed reinstatement with oxytocin pretreatment

Figure 2-1 depicts meth-primed reinstatement at three doses of oxytocin (0, 0.3, and 1 mg/kg) during the three phases of the estrous cycle (proestrus, diestrus I/II, estrus). Overall, oxytocin dose did not interact with estrous cycle, nor was there a main effect of estrous cycle. However, there was a main effect of oxytocin dose [Figure 2b, $F(2,66)=4.63$, $p<0.05$]. Specifically, 1 mg/kg oxytocin decreased responding relative to vehicle [$p<0.05$].

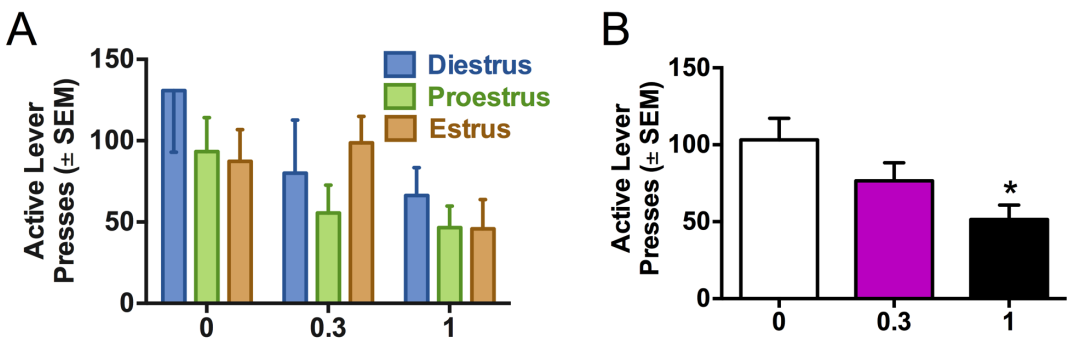

Figure 2-1 Meth-primed reinstatement to meth seeking in females following oxytocin pretreatment.

A) Active lever responding during reinstatement by estrous cycle phase and dose of oxytocin. No effect of cycle phase was seen during meth-primed reinstatement with or without oxytocin. B) Active lever responding during reinstatement by dose of oxytocin. Oxytocin (1 mg/kg) significantly decreased active lever responding as compared to vehicle (*p<0.05).

Experiment 2: Oxytocin effects on meth self-administration and reinstatement

Figure 2-2 depicts the impact of oxytocin on PR performance (lever presses, break points, and infusions) for males and females during meth self-administration. Overall, females had more active [Figure 2A, sex main effect, $F(1,28)=6.26$, $p<0.05$] and inactive (Table 1, sex main effect, $F(7,28)=4.48$, $p<0.05$] lever responses as compared to males. Oxytocin decreased active lever responding [treatment main effect, $F(1,28)=10.8$, $p<0.001$], an effect that was driven primarily by females [$p<0.05$]. Consistently, females had a higher breakpoint than males [Figure 2B, sex main effect, $F(1,28)=5.68$, $p<0.05$]. Oxytocin decreased breakpoints [treatment main effect, $F(1,28)=10.31$, $p<0.001$] to a greater extent in females [$p<0.05$]. Males and females earned similar meth

infusions (Figure 2-2C), and oxytocin pretreatment decreased infusions earned [treatment main effect, $F(1,28)=14.08$, $p<0.001$] more in females than in males [$p<0.05$].

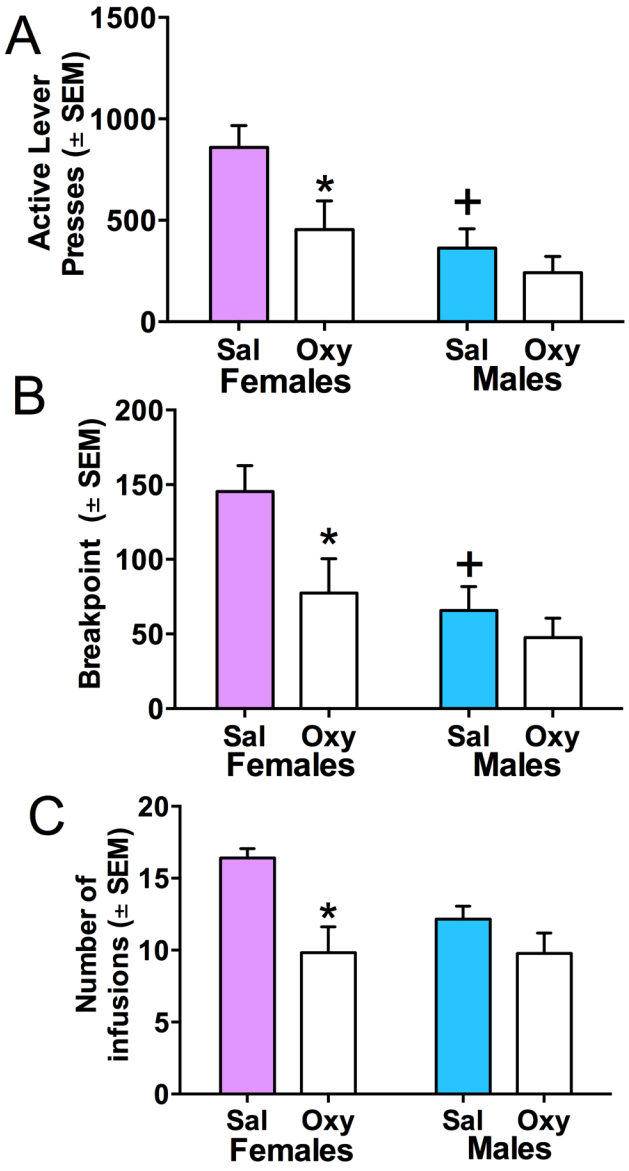

Figure 2-2. Progressive ratio (PR) responding in males and females during meth self-administration.

A) Active lever responding in males and females after oxytocin or vehicle. Females responded more on the active lever during the PR test relative to males.

Oxytocin decreased active lever responding for females, but not males. B) Breakpoint during PR tests. Females had a higher breakpoint relative to males and oxytocin (1 mg/kg) reduced the breakpoint in females, but not males. C) Number of infusions during PR tests. Oxytocin decreased the number of infusions for females, but not males. Significant differences from same sex control (*p<0.05) or between males and females treated with vehicle (†p<0.05) are indicated.

Figure 2-3 depicts self-administration, extinction, and reinstatement data for meth self-administration in males and females. Figure 2-3A shows the average active lever responding over the last 5 days of self-administration and the last 2 days of extinction. No differences emerged between males and females in active or inactive lever presses (see Table 1 for inactive lever data). However, females (14.50±1.19) took more days to meet extinction criterion than males (10.93±0.62).

After extinction, the rats were first tested on cue-induced reinstatement tests (Figure 2-3B). Active lever presses for males and females varied according to treatment. Overall, females responded more during cue-induced reinstatement than males and oxytocin attenuated active lever responding only in females [sex x treatment interaction, $F(1,24)=7.35$, $p<0.05$, and post hoc, $p<0.05$]. Oxytocin also decreased inactive lever responding [Table 1, treatment main effect, $F(1,24)=1.56$, $p<0.05$]. During meth-primed reinstatement (Figure 2-3C) females had more active lever responses [sex main effect, $F(1,24)=4.52$, $p<0.05$] and oxytocin decreased active [treatment main effect, [$F(1,24)=51.14$, $p<0.0001$] and inactive [Table 1, treatment main effect, [$F(1,24)=8.6$, $p<0.01$] lever

responding in both females and males. Rats were then tested on a yohimbine stress induced reinstatement test (Figure 2-3D). During yohimbine-induced reinstatement, females reinstated to a greater extent than males [sex main effect, $F(1,20)=5.73$, $p<0.05$] and oxytocin attenuated active [treatment main effect, $F(1,20)=16.87$, $p<0.001$] and inactive [Table 1, treatment main effect, [$F(1,22)=4.35$, $p<0.05$] lever responding in both sexes.

Table 1 Mean inactive lever responding during sucrose self-administration, progressive ratio, and reinstatement tests.

Table 1 Mean (±SEM) inactive lever responding during meth self-administration, progressive ratio, and reinstatement tests.

	Females		Males	
Self-administration	50.8 (±18.3)		29.3 (±6.5)	
Extinction	21.4 (±10.4)		14.7 (±3.5)	
	Females		Males	
	Vehicle	Oxytocin	Vehicle	Oxytocin
Progressive ratio*	130.8 (±36.7)	98.2 (±30.5)	43.7 (±12.9)	31.0 (±10.1)
Reinstatement test				
Cue*	31.7 (±10.7)	10.4 (±3.3)	15.4 (±3.8)	10.9 (±2.4)
Prime*	57.1 (±21.3)	24.3 (±12.5)	19.8 (±4.2)	12.5 (±3.9)
Yohimbine*	22.6 (±8.9)	14.3 (±4.1)	16.5 (±3.3)	10.0 (±2.0)

* Significant difference between oxytocin and vehicle.
+ Significant difference between males and females.

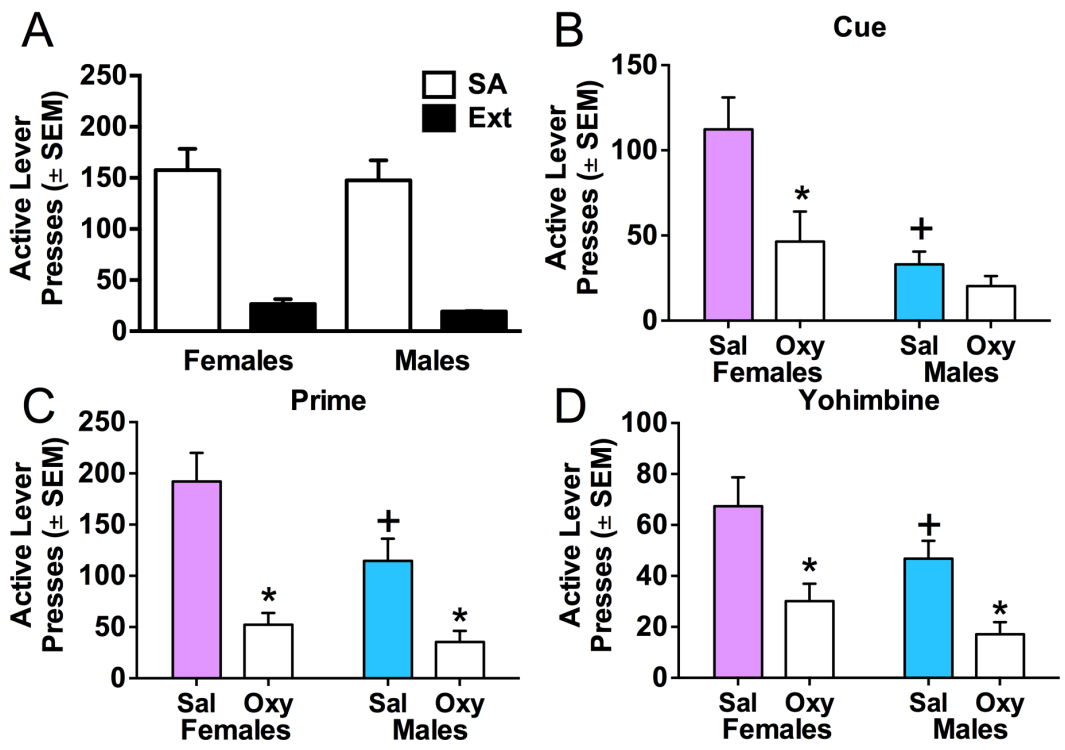

Figure 2-3 Active lever responding during meth self-administration and subsequent reinstatement tests.

A) Active lever responding during that last 5 days of self-administration and the last 2 days of extinction in males and females. B) Active lever responding during cue-induced reinstatement in males and females after oxytocin or vehicle. Females reinstated to conditioned cues to a greater extent than males. Oxytocin decreased cue-induced reinstatement in females, but not males. C) Active lever responding during meth-primed reinstatement. Females responded more during the meth prime test than males. Oxytocin decreased meth-primed reinstatement in both males and females. D) Active lever responding during stress-induced reinstatement by yohimbine. Oxytocin decreased yohimbine-induced reinstatement in males and females. Significant differences from same sex control (*$p<0.05$) or between males and females treated with vehicle (†$p<0.05$) are indicated.

Experiment 3: Oxytocin effects on sucrose self-administration and reinstatement

Figure 4 depicts the impact of oxytocin on lever presses, infusions, and break points during the PR tests for sucrose self-administration in females and males. There were no sex differences or oxytocin effects on the number of active (Figure 2-4A) or inactive (Table 2) lever presses, break point (Figure 4B), or pellets earned (Figure 2-4C).

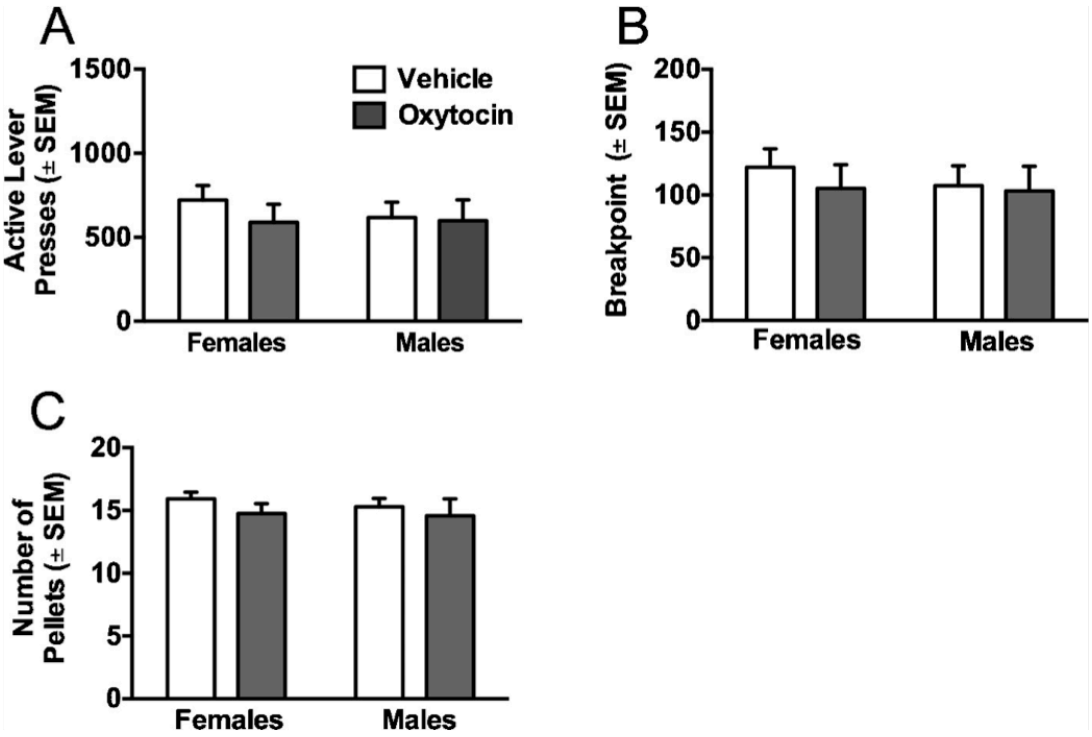

Figure 2-4 Progressive ratio (PR) responding for males and females during sucrose self-administration.

There were no differences in A) active lever responding, B) breakpoints, or C) pellets earned during PR tests in males and females after oxytocin or vehicle.

Figure 2-5A shows the average active lever responding over the last 5 days of sucrose self-administration (when self-administration behavior had

stabilized), and no differences were seen between males and females. Extinction responding did not differ during the last 2 days between sexes. Following extinction, the rats were first tested on a cue-induced reinstatement test (Figure 2-5B). Females responded more on the active lever than males [sex main effect, $F(1,13)=6.97$ $p<0.05$]. Oxytocin did not affect responding in either sex. On the sucrose prime test (Figure 2-5C), oxytocin decreased active lever responding in both females and males [treatment main effect, [$F(1,13)=8.61$, $p<0.05$]. In addition, during the yohimbine-induced reinstatement test, oxytocin attenuated active lever responding in both sexes [Figure 2-5D, $F(1,13)=8.01$, $p<0.005$]. Responding on the inactive lever did not differ on any measure for sucrose-trained rats (Table 2.)

Table 2 Mean inactive lever responding during sucrose self-administration, progressive ratio, and reinstatement tests.

Table 2 Mean (±SEM) inactive lever responding during sucrose self-administration, progressive ratio, and reinstatement tests.

	Females		Males	
Self-administration	34.5 (±5.9)		28.4 (±2.5)	
Extinction	17.1 (±2.7)		22.1 (±0.6)	
	Females		Males	
	Vehicle	Oxytocin	Vehicle	Oxytocin
Progressive ratio	70.3 (±28.2)	37.4 (±24.2)	36.6 (±8.8)	28.9 (±10.4)
Reinstatement test				
Cue	16.4 (±7.4)	9.3 (±5.0)	26.0 (±7.6)	15.1 (±5.4)
Prime	11.5 (±5.2)	8.8 (±3.0)	14.7 (±5.0)	13.4 (±3.7)
Yohimbine	77.4 (±37.1)	19.4 (±16.3)	79.7 (±36.4)	27.9 (±13.2)

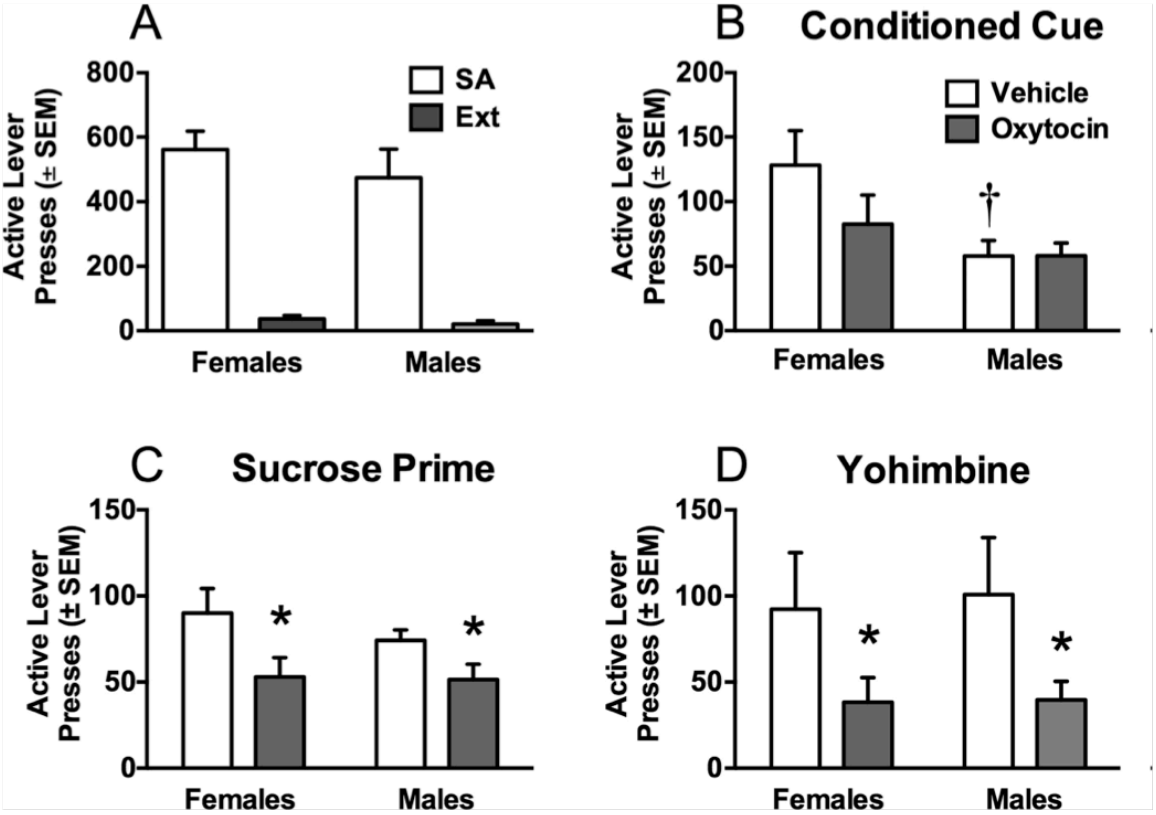

Figure 2-5 Active lever responding during sucrose self-administration and subsequent reinstatement tests in males and females treated with oxytocin or vehicle.

A) Active lever responding during that last 5 days of self-administration and the last 2 days of extinction in males and females. B) Active lever responding during cue-induced reinstatement in males and females after oxytocin or vehicle. Females reinstated to conditioned cues to a greater extent than males. Oxytocin decreased cue-induced reinstatement in females, but not males. C) Active lever responding during sucrose-primed reinstatement. Females responded more during the sucrose prime test than males. Oxytocin decreased sucrose-primed reinstatement in both males and females. D) Active lever responding during stress-induced reinstatement by yohimbine. Oxytocin decreased yohimbine-induced reinstatement in males and females. Significant differences from same sex control (*$p<0.05$) or between males and females treated with vehicle (†$p<0.05$) are indicated.

Discussion

Here, we have established that the ability of systemic oxytocin to reduce reinstatement of meth seeking may be dependent on sex specific relapse triggers.

Also, we found sex specific effects of oxytocin in reducing motivation to take meth, as well as reward seeking in response to conditioned reinforcers. The similarities between sexes were evident by the ability of oxytocin to decrease meth seeking in both males and females in response to a drug prime and a pharmacological stressor. To date, only one study exists (Holtz, Lozama et al. 2012) that directly compared the ability of a treatment compound (allopregnanolone or modafinil) to block reinstatement to meth seeking in both males and females. However, prior studies with rats have clearly demonstrated pronounced differences between male and female meth intake (Reichel, Chan et al. 2012), motivation to self-administer meth (current report, (Roth and Carroll 2004), and reinstatement of meth seeking (Holtz, Lozama et al. 2012, Reichel, Chan et al. 2012).

In the first experiment, estrous cycle did not interact with oxytocin during meth-primed reinstatement testing and females reinstated to a meth prime regardless of cycle phase. As such, neither meth-primed reinstatement nor attenuation with oxytocin appeared to be influenced by circulating ovarian hormones. However, PR responding and reinstatement tests were conducted based on response criteria, rather than estrous cycle for the females. Therefore, the possibility remains that the reported sex differences may be due, in part, to differential regulation of oxytocin receptor affinity, surface expression, and/or coupling mechanisms by estrogen and progesterone. For example, estrogen increased oxytocin receptor affinity in the medial preoptic area of the hypothalamus (Caldwell, Walker et al. 1994) and receptor density in the

ventromedial nucleus (Coirini, Schumacher et al. 1991). Additionally, progesterone increased oxytocin receptor density in limbic structures (Patchev, Schlosser et al. 1993), which could subsequently increase the effects of oxytocin in females relative to males. Fluctuations in gonadal hormones may account for the sex differences in motivation to take meth during the PR tests and meth seeking during cue-induced reinstatement.

Oxytocin reduced responding for meth along the PR schedule of reinforcement in females down to a level comparable to males. However, motivation for sucrose was similar in males and females regardless of pretreatment condition. In males, oxytocin did not have an effect on either meth or sucrose taking during the PR. A distinction between drug and natural reward occurred in females, as oxytocin only decreased meth taking, but not sucrose taking, during the PR. Interestingly, females in general demonstrated greater motivated meth taking, which is in line with previous self-administration studies with meth (Roth and Carroll 2004), cocaine (Roberts, Bennett et al. 1989, Carroll, Morgan et al. 2002), and heroin (Cicero, Davis et al. 2002). While we found the ability of oxytocin to reduce motivation to obtain meth was specific to females, a previous study showed that oxytocin (1 mg/kg) decreased responding on a similar PR tasks in male rats (Carson, Cornish et al. 2010). These contrasting findings in males likely arise from several methodological differences between studies. For example, in our study, rats were maintained on a FR schedule of reinforcement and two PR ratio tests were given in a counterbalanced manner with a minimum of two days for intervening stabilization. In contrast, rats in the

earlier study transitioned to and maintained their responding along a PR schedule of reinforcement and oxytocin administration occurred on five consecutive days with ascending doses. As such, these repeated daily doses of oxytocin might have resulted in a sensitized response to oxytocin. Also, rats maintained along a PR schedule received fewer daily meth infusions on average than rats in our study that were maintained along a FR. The increased meth intake for males may have rendered them less susceptible to lower doses of oxytocin (i.e., oxytocin's efficacy may be related to total meth intake).

Oxytocin only reduced cue-induced reinstatement in meth females, indicating some specificity for sex and conditioning of drug-cue associations. For example, in females, oxytocin decreased responding for meth-conditioned cues, but not sucrose. This difference indicates that oxytocin has some degree of specificity to reduce relapse to drug related cues, rather than those associated with natural reward. Alternatively, different response rates between the meth and sucrose studies provide a potential explanation for this difference. Specifically, the sucrose females received on average 561.8 (±25.4) primary and secondary reinforcers (i.e., sucrose pellets and light + tone stimulus complex) during the last 5 days of self-administration as compared to the meth females that received 162.9 (±10.5) reinforcers. Consequently, this difference in response rate and reinforcement history may have rendered sucrose females less susceptible to oxytocin's ability to attenuate cue-induced reward seeking.

In the current study, females responded more to meth and sucrose conditioned cues in comparison to males. Previous studies have shown that

females responded equally to males in response to conditioned cues during reinstatement of cocaine (Feltenstein, Henderson et al. 2011, Zhou, Ghee et al. 2012) or nicotine (Feltenstein, Ghee et al. 2012) seeking. Importantly, these effects were evident when rats were trained and tested along an FR1 schedule of reinforcement. Meth rats in our study were trained and tested on an FR5 schedule of reinforcement, whereby the light + tone stimulus complex occurred after every 5 active lever presses during the reinstatement test. Male meth rats had low levels of reinstatement on this test schedule relative to our experiences typically observed with FR1 cue-induced reinstatement (Reichel and See 2010, Reichel, Chan et al. 2012), suggesting a possible floor effect. In spite of the low reinstatement in males, females demonstrated markedly more robust reinstatement in the identical situation. Surprisingly, females were impervious to the lean reinforcement schedule during cue-induced reinstatement. This enhanced responding to conditioned reinforcers suggests that females may entrain the original associations to a greater degree than males, indicating differences in interpretation of the associative strength of the reward. Associative strength is defined as the degree of strength of learning that occurs between a conditioned stimulus (CS, i.e., light + tone) and the unconditioned stimulus (US, i.e., physiological effects of meth or sucrose). Increased meth-cue and sucrose-cue reinstatement in females may be due to increased salience or value of the US, which imbues the associative strength of the CS. Support for this notion comes from a demonstration that estrogen enhanced conditioned learning in delay and trace conditioning paradigms (Shors, Beylin et al. 2000). Since we

utilized freely cycling females with an intact hormonal system, fluctuations in estrogen and progesterone levels during self-administration may have influenced the original associative processes.

Oxytocin was equally effective at reducing reinforcer-primed reinstatement of meth and sucrose seeking, although females reinstated to a greater extent to a meth prime than males. This sex difference has been seen before and demonstrates that, regardless of session length, females exhibit enhanced meth seeking to a priming injection [current report, (Holtz, Lozama et al. 2012, Reichel, Chan et al. 2012)]. Importantly, meth-primed reinstatement did not depend upon estrous cycle phase for females, nor did cycle interact with the ability of oxytocin to reduce meth seeking. In fact, oxytocin reduced prime-induced meth seeking during all cycle phases for females. Further, oxytocin decreased sucrose seeking during sucrose primed reinstatement tests. Taken together, oxytocin decreased seeking for both meth and sucrose during the reinstatement tests in both sexes. This effect suggests that oxytocin decreased seeking for both drug and natural reward, perhaps through similar mechanisms. Although the underlying mechanisms are beyond the scope of this study, one suggestion relies on the interaction of oxytocin with the dopamine system (Baskerville and Douglas 2010). Oxytocin may decrease the saliency of reward via interactions with dopamine activity in regions known to be involved in drug seeking. For example, oxytocin attenuated meth-induced dopamine efflux in the nucleus accumbens (Qi, Yang et al. 2009), decreased meth-induced Fos expression in the nucleus accumbens core (Carson, Hunt et al. 2010), and

regulated meth-induced changes in extracellular glutamate and γ-aminobutyric acid (GABA) in mouse brain (Qi, Han et al. 2012). Future studies will need to directly elucidate the mechanism by which oxytocin attenuates drug seeking.

In all subjects, oxytocin attenuated yohimbine-induced reinstatement, suggesting a common mechanism for reductions in stress-induced reinstatement. Oxytocin produces anxiolytic effects in humans (Macdonald and Macdonald 2010) and animals (Neumann, Wigger et al. 2000) through inhibition of the hypothalamic-pituitary-adrenal axis, which is a sex independent effect in rats (Neumann, Wigger et al. 2000). The pharmacological stressor, yohimbine, produced similar reinstatement in both sexes of rats with a meth history. This finding was somewhat surprising, given that female cocaine addicts are purportedly more likely to relapse to stressful life events (McKay, Rutherford et al. 1996, Back, Brady et al. 2005). However, results have been somewhat mixed in animal models of relapse with cocaine. While one study showed greater yohimbine-induced reinstatement in females relative to males (Anker and Carroll 2010), our laboratory previously reported that sex differences in reinstatement only emerged when cues were simultaneously presented in combination with yohimbine (Feltenstein, Henderson et al. 2011). In the current study, we also found no sex differences in yohimbine-induced reinstatement of sucrose seeking. Earlier reports showed that yohimbine reinstated sucrose seeking in males (Ghitza, Gray et al. 2006, Richards, Simms et al. 2008), but to our knowledge, this effect has not been previously shown in females.

Although inactive lever presses were uniformly lower than active lever presses, oxytocin reduced inactive lever presses in rats with a history of meth self-administration, but not sucrose. Changes in inactive lever responding could indicate alternative strategies in exploratory drive for reward and/or non-specific motor behavior. These options are unlikely, however, as pilot data from our laboratory has found that oxytocin (1 mg/kg) does not reduce meth-induced or basal motor activity beyond baseline values (data not shown). Furthermore, oxytocin did not decrease responding for sucrose during the PR test, suggesting an effect specific to meth-experienced rats.

In conclusion, we report: 1) oxytocin reduced motivation to self-administer meth in females, but not males; 2) females responded more to conditioned reinforcers during cue-induced reinstatement tests; and 3) sex similarities in the ability of oxytocin to decrease meth and sucrose seeking in response to either a meth prime or a pharmacological stressor. Oxytocin appears to have the greatest influence on meth-experienced females, decreasing motivation and relapse in response to cue, meth prime, and yohimbine-induced reinstatement. In contrast, oxytocin only impacted males in response to meth and yohimbine-primed reinstatement. Taken together, these results indicate that oxytocin or oxytocin receptor agonists may be a promising pharmacotherapeutic treatment strategy for meth addiction in both males and females in response to multiple relapse triggers.

These data indicate that oxytocin differentially affects meth seeking in males and females. Specifically, oxytocin has differential effects during PR and

cue-induced reinstatement. Further studies are needed to more specifically assess the effect of oxytocin on consumption and motivation to seek meth.

Chapter 3: Behavioral Economics of Drug Addiction

Behavioral economics (BE) has been termed “a science of behavior” (Hursh 1984) and a general term used to describe any concept or method of applying economic theory to analyze behavior of individuals (Bickel, Green et al. 1995). This theory came about in the 1950’s-1960’s, although many argue that these concepts existed before then (Hosseini 2003). Over the years behavioral economics has become a broad term used to describe many different methods and theories. For the purposes of this dissertation, one crucial aspect of BE is *consumer demand theory*, which describes how demand for rewards varies by the price a subject is willing to pay to receive the reward (Hursh, Raslear et al. 1988). Evidence suggests that consumption of addictive drugs, like other goods, can be examined in terms of this economic concept. For example, to assess a demand curve, subjects are asked how much drug they would purchase at multiple prices. These data can then be plotted, and typically a negative slope is produced following the *law of demand* (i.e., demand decreases as the prices increases) (Hursh 1980). In humans, drug demand increases with abuse, and

addicts often end up paying not only financial, but also career and relationship costs.

BE paradigms are particularly useful as a measure of drug demand since they allow for parallel quantitative analyses of this addiction-related demand across species (including humans, non-human primates, and rodents)(Fig 3-1).

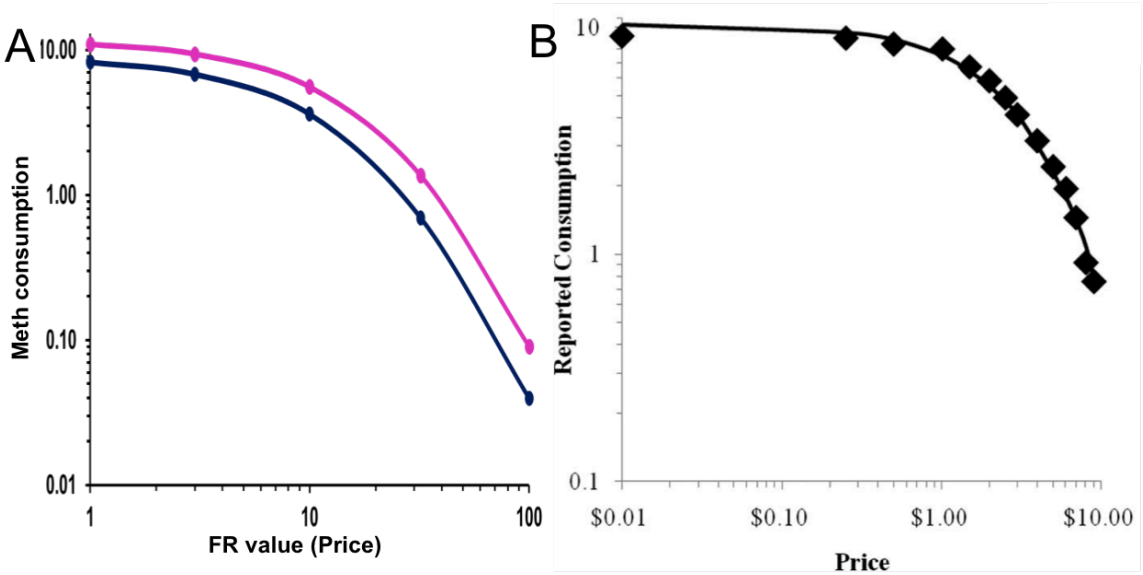

Figure 3-1 Example demand curves for rats and humans.
A) Mean meth demand curve for males (blue) and females (pink) during our BE paradigm for number of meth infusions (consumption) by increasing FR value (cost). B) Mean alcohol demand curve of college students by alcohol consumption with increasing price of drinks. Graph B was adapted from (Murphy, MacKillop et al. 2009).

The BE paradigm measures consumption of drug during both low-effort and high-effort (i.e., low-price and high-price) conditions, providing a quantitative analysis of motivation for meth normalized for differences in baseline intake. These assessments are derived from economic equations that describe drug demand (consumption) as a function of drug price (effort required to obtain drug) (Hursh 1980). A demand curve provides values for the following variables: Q_0, α, *Omax*,

and *Pmax*, (Hursh and Silberberg 2008). Importantly, the same variables can be assessed across species, allowing direct comparison of rewards and manipulations. Q_0, *Omax,* and *Pmax* can be estimated graphically from response curves, while α must be calculated using the exponential demand equation (Equation 1) (Hursh and Silberberg 2008).

$$Log_e(Q) = Log_e(Q_0) + k(e^{-Q_0 \alpha C} - 1)$$

In Equation 1, *Q* is the measure of consumption (i.e., demand), Q_0 is the measure of demand when price or cost (*C*) approaches 0, *k* specifies the range of *Q*, and α is a measure of demand curve elasticity (i.e., how quickly demand falls with increases in price) (Hursh and Silberberg 2008).

As it is known that increased effort will decrease consumption, assessment of Q_0 is a calculated variable which predicts demand for consumption at null effort and is extrapolated from consumption during low effort (i.e., cost) conditions (Hursh and Silberberg 2008). To further expand on the definitions above in terms of drug taking behaviors, in humans Q_0 would be the amount of drug they would choose to take if there were an unlimited amount, or for rodents in a self-administration paradigm, how much drug they would choose to consume in the absence of required effort (e.g., lever presses). In humans, Q_0 has been shown to significantly correlate with several measures of addiction (e.g., motivation to quit smoking, problem drinking) for both alcohol and nicotine (MacKillop and Murphy 2007, Murphy, MacKillop et al. 2009, MacKillop, Miranda et al. 2010, MacKillop, O'Hagen et al. 2010, Murphy, MacKillop et al. 2011)

Additionally, α is a measure of demand curve elasticity, meaning the estimated price an individual is willing to pay for a drug. For example, in humans this can be assessed based on how much money an individual will pay for a drug, or for rodents how much effort (i.e., lever presses) they are willing to put forth to receive the drug. α is calculated based on Equation 1, which normalizes α in respect to Q_0 (Hursh and Winger 1995, Spiga, Martinetti et al. 2005, Hursh and Silberberg 2008, Bentzley, Fender et al. 2013), allowing for direct comparison across reinforcers (Ko, Terner et al. 2002, Winger, Hursh et al. 2002, Winger, Galuska et al. 2006, Wade-Galuska, Winger et al. 2007). This normalized measure of demand is shown to be superior to the measurement of Pmax (the maximum price the individual is willing to pay) in studies of addiction-like behavior. For example, α but not Pmax predicted the number of alcoholic drinks consumed per week, problems that occurred because of alcohol, and the amount college students spent on alcohol (Murphy, MacKillop et al. 2009). Additionally, α but not Pmax predicated subsequent addiction-related behavior, including the number of cigarettes adolescent smokers smoked per day (Murphy, MacKillop et al. 2011). Therefore, this dissertation concentrated on the assessment of Q_0 and α for all BE studies. Figure 3-2 depicts where Q_0 and α are assessed on an example demand curve.

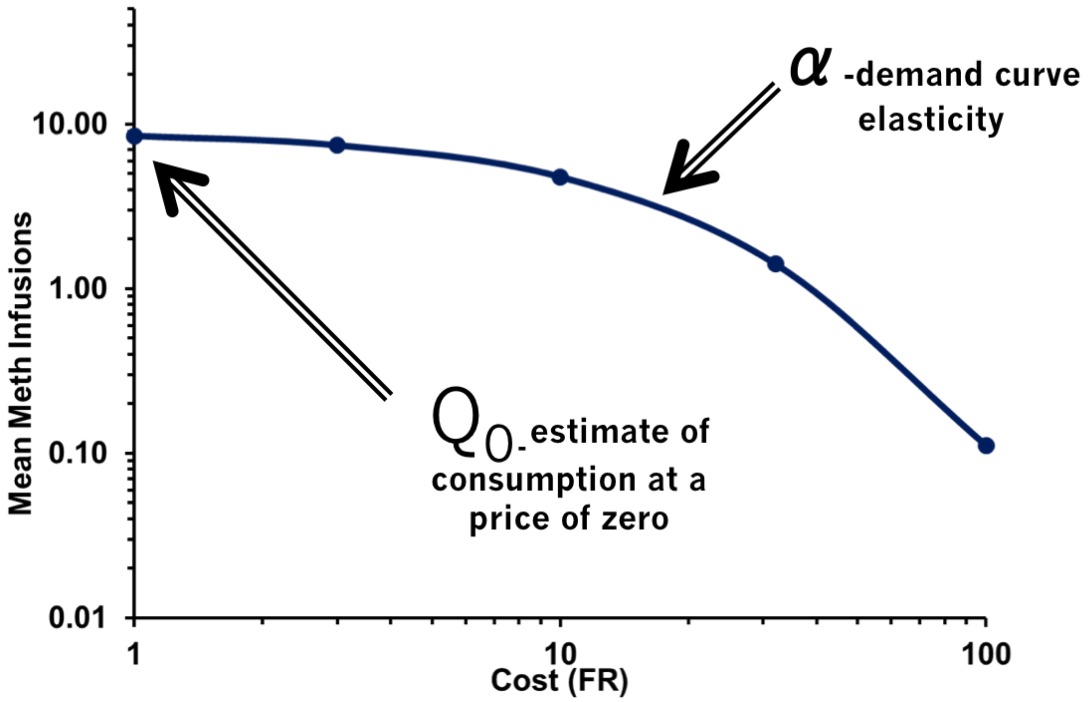

Figure 3-2 Representation of where Q_0 and α are assessed on a demand curve. *α* is a measure of demand curve elasticity and is a calculated variable (normalized to Q_0) that represents the point at which the demand for the drug begins to decrease. Q_0 is an estimate of drug consumption at the price of zero and is calculated by extrapolating consumption during low effort (i.e., FR1) conditions.

Demand curve analysis is a particularly useful method of characterizing drug self-administration, because it can measure intake at low cost (desired intake levels; Q_0) and motivation (α) as separate variables within the same session (Bentzley, Fender et al. 2013). Importantly, these BE measures of motivation and intake show individual differences that predict relapse in both human (Tucker, Vuchinich et al. 2002, Tucker, Vuchinich et al. 2006) and animal studies (Galuska, Banna et al. 2011, Bentzley, Jhou et al. 2014).

Behavioral economics of meth self-administration

Previous models designed to assess demand curves in rodents required animals to stabilize responding at multiple different prices (FR values). This required multiple daily sessions at each price, necessitating weeks of testing to determine a demand curve (Oleson and Roberts 2009, Galuska, Banna et al. 2011). Unfortunately, this conventional method severely limits testing of acutely administered drugs, or brain manipulations (e.g., intracranial microinjections). Fortuitously, this limitation has recently been overcome with the design of the within-session BE procedure (Oleson, Richardson et al. 2011, Oleson and Roberts 2012, Bentzley, Fender et al. 2013). However, this procedure was designed specifically for cocaine self-administration, based on many factors including the half-life of cocaine, and no comparable within-session paradigm had been designed for meth.

In the cocaine within-session paradigm, it becomes increasingly more difficult (i.e., requires more lever presses) throughout the session to obtain the same amount of drug. This design is optimal for cocaine, since the half-life is so short (12-13 min in rats (Booze, Lehner et al. 1997)) rats will increase their effort to maintain their preferred blood level. However, because meth has a significantly longer half-life (~60 min in rats (Milesi-Halle, Hendrickson et al. 2005)), rats learn to take more infusions of meth during low effort conditions at the beginning of the session and not respond under high effort conditions (unpublished observations, data not shown). Thus, we designed a variation of the originally published within-session BE paradigm (Lenoir and Ahmed 2008,

Oleson, Richardson et al. 2011, Oleson and Roberts 2012, Bentzley, Fender et al. 2013) to account for the longer half-life of meth. We validated this novel paradigm by verifying that calculated demand curve variables (α and Q_0) were similar whether they were obtained in a conventional, multi-day BE paradigm or a within-session BE paradigm (data shown in following chapter, Fig 4-1).

In a multi-day BE paradigm, rats self-administer drug for multiple days at each price (e.g., FR 1, 3, 10, 32, 100). When responding is stable at one price for consecutive days, the rat is moved to the next price (in ascending order). The stable values (total active lever presses) at each price are used to calculate a demand curve, thus taking an average of two weeks to determine one demand curve. During our within-session BE paradigm, rats are given 5 min at each price (i.e., FR 100, 32, 10, 3, 1) in descending order, with 20 min time-outs between each price. Therefore all prices are measured in one session, and structured in descending order to assess demand during high effort conditions before increased blood levels of meth confound this measurement. The demand curve is calculated similarly to the multi-day model, except that total lever presses from every 5 min price point are used, allowing a demand curve to be calculated for every session. Importantly, this paradigm allows for repeat testing because responding can be re-stabilized between tests. Figure 3-3 diagrams the differences in the methodology for both the multi-day paradigm (Fig 3-3A) and the within-session paradigm (Fig 3-3B).

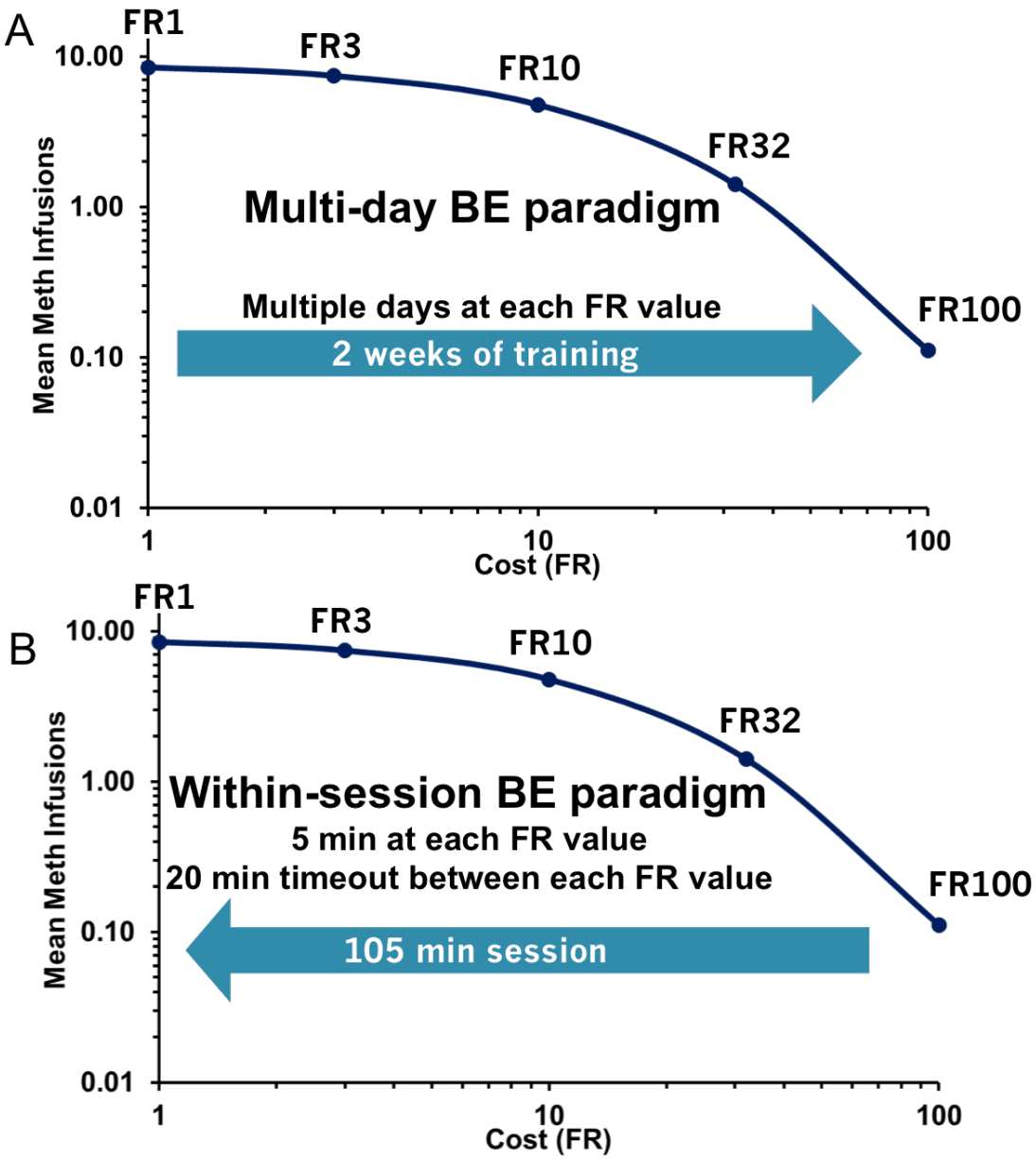

Figure 3-3 Comparison of the multi-day and within-session BE paradigms. A) During the multi-day BE paradigm, rats have multiple self-administration sessions at each price (i.e., FR value) until they reach stable responding. FR values increase in ascending order (FR 1, 3, 10, 32, 100) on subsequent daily sessions, and it takes 2 weeks on average to compute a single demand curve. B) During the within-session BE paradigm, rats have 5 bins (5 min each) at each price (FR 100, 32, 10, 3, 1) in descending order with a 20 min timeout (house light off) between each bin (105 min total). A demand curve is computed for each session.

In summary, behavioral economic analysis of self-administration provides a translational approach for measuring motivation and preferred consumption of a drug within the same session. Additionally, these measures allow for direct comparison between species and/or types of reinforcers and have been shown to correlate with addiction behaviors in humans and animals. Overall, this within-session BE paradigm provided the ideal approach to further examine the possible sex differences in the effects of oxytocin on meth seeking behaviors observed in Chapter 2.

Chapter 4: Economic Demand of Methamphetamine Seeking

Introduction

Evidence suggests that oxytocin, an endogenous peptide well known for its role in social behaviors and childbirth, is a promising addiction pharmacotherapy. We have shown that oxytocin differentially affects meth seeking in males vs. females on a progressive ratio (PR) test. However, in the PR test, meth intake progressively decreased across the course of the session. This change confounds the measurement of drug seeking motivation, as it is affected by tolerance, sensitization, locomotor activation or shifts in the preferred levels of drug consumption, making it unclear as to whether oxytocin is affecting motivation to seek meth in particular. In addition, females have higher levels of baseline meth consumption and because different intake levels could also confound PR measures (e.g., due to locomotor activation), it is difficult to examine sex differences in motivation for drug using this test alone. Therefore, we employed a within-session behavioral economic (BE) paradigm to further investigate sex differences in meth seeking as this paradigm allows independent

assessment of oxytocin effects on motivation (α) and preferred intake levels (Q_0).

The within-session BE paradigm was designed specifically for cocaine self-administration, based on many factors including the short half-life of cocaine (Oleson, Richardson et al. 2011, Oleson and Roberts 2012, Bentzley, Fender et al. 2013). As meth has a much longer half-life compared to cocaine, a variation of the originally published within-session BE paradigm was designed for these studies. The within-session BE paradigm allows measurement of drug demand at high effort (motivation; α), normalized based on intake at low effort (baseline consumption; Q_0) (Bentzley, Fender et al. 2013, Bentzley, Jhou et al. 2014). The paradigm was modeled after BE procedures used to assess motivation for reward in humans (MacKillop and Murphy 2007, Mackillop, Murphy et al. 2009, Murphy, MacKillop et al. 2009, MacKillop, Miranda et al. 2010, MacKillop, O'Hagen et al. 2010). Importantly, the same BE variables (α, Q_0) assessed across humans and animals have been shown to predict later relapse-like behavior (MacKillop and Murphy 2007, Galuska, Banna et al. 2011, Bentzley, Jhou et al. 2014). Therefore, the translational potential of preclinical BE studies is particularly strong.

In Experiment 1, we first validated the within-session BE paradigm designed for meth, and examined if individual variability on meth demand (α) or preferred levels of intake (Q_0), predicts relapse behaviors. Additionally, we assessed the effects of oxytocin on meth demand and on cue-induced reinstatement in order to identify if either motivation (α) or baseline consumption (Q_0) predicts the ability of oxytocin to attenuate cue-induced reinstatement

(relapse behavior). If so, oxytocin could be screened for its efficacy in modulating BE variables in human drug addicts, and thereby inform clinical strategies to reduce the risk of subsequent relapse to drug taking.

In Experiment 2, we examined where oxytocin may act in the brain to decrease meth seeking behaviors. Several studies have shown that oxytocin decreases reward behaviors whether administered systemically (Carson, Cornish et al. 2010, Carson, Hunt et al. 2010) or microinfused directly into the brain (Qi, Yang et al. 2008, Qi, Yang et al. 2009, Baracz, Rourke et al. 2012, Baracz, Everett et al. 2014), indicating that systemic oxytocin is primarily acting in the brain to produces its effects. However, to our knowledge, this has never been directly tested during a drug seeking paradigm. Therefore, we examined whether systemically administered oxytocin effects could be blocked by intracerebroventricular infusion of an oxytocin receptor antagonist (OXA).

We then examined if nucleus accumbens (NAc) is a primary brain region where oxytocin acts to reduce meth seeking in our paradigm, based on numerous studies that show a crucial role of NAc in mediating oxytocin's effects on reward-related behaviors (Liu and Wang 2003, Ross, Freeman et al. 2009, Keebaugh and Young 2011, Burkett and Young 2012, Dolen, Darvishzadeh et al. 2013). Previous studies assessing oxytocin's effects on meth-induced behaviors in the NAc have shown that systemically-administered oxytocin decreased meth-induced Fos activation in NAc core (Carson, Hunt et al. 2010), and oxytocin microinfused into NAc core blocked meth-induced conditioned placed preference (Baracz, Rourke et al. 2012) or meth-primed reinstatement of meth seeking

(Baracz, Everett et al. 2014). Although oxytocin appears to act in the brain to mediate rewarding effects of meth, local effects of oxytocin on meth seeking during BE or cue-induced reinstatement have not been assessed. Thus, we examined if oxytocin in NAc core is both necessary and sufficient to attenuate meth seeking behaviors.

Methods

Subjects

Male and female Sprague Dawley rats (Harlan; initial weight of 200-275 g) were used in all experiments. Rats were individually housed on a reversed 12:12 light-dark cycle in a temperature and humidity controlled vivarium. Water and rat chow (Harlan, Indianapolis, IN, USA) were available *ad libitum* throughout the study. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina, and were in accordance with the eight edition of the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011.

Surgery

Rats were anesthetized with IP injections of ketamine (66 mg/kg; Vedco Inc, St. Joseph, MO, USA), xylazine (1.3 mg/kg; Lloyd Laboratories, Shenandoah, IA,

USA), and Equithesin (0.5 ml/kg; sodium pentobarbital 4 mg/kg, chloral hydrate 17 mg/kg, 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol, 10% ethanol solution). Ketorolac (2.0 mg/kg, IP; Sigma Chemical, St. Louis, MO) was given before surgery as an analgesic. Catheters (constructed with Silastic tubing, Dow Corning Corporation, Midland, MI) were inserted 33 mm into the right jugular vein and secured with silk sutures. During recovery from surgery, catheters were flushed once daily for 5 days with 0.1 ml of Timentin (24 mg/0.1; GlaxoSmithKline, Research Triangle Park, NC) and 10 U/ml of heparinized saline (Elkins-Sinn, Cherry Hill, NJ). During meth self-administration (methamphetamine hydrochloride; Sigma Chemical, St. Louis, MO), catheters were flushed with 0.1 ml of 10 U/ml heparinized saline before and after every self-administration session. Catheter patency was periodically verified with methohexital sodium (10 mg/ml dissolved in 0.9% saline; Sigma Chemical, St. Louis, MO), a short acting barbiturate that produces a rapid loss in muscle tone when administered intravenously.

Methamphetamine self-administration and BE procedures

All self-administration experiments were conducted during the rats' dark cycle in standard Plexiglas self-administration chambers (30 X 20 X 20 cm) that were enclosed in sound attenuating cubicles with a ventilation fan (Med Associates, St. Albans, Vermont) and linked to a computerized data collection program (MED PC, Med Associates). Each chamber was equipped with two retractable levers with a white stimulus light above each lever, house light, and

tone generator. For meth self-administration, infusion tubing enclosed in steel spring leashes (Plastics One Inc., Roanoke, VA) was connected to the infusion harness and a weighted swivel apparatus (Instech, Plymouth Meeting, PA) was suspended above the box to allow for free movement within the chamber.

During self-administration sessions the house light remained on throughout the sessions and a response on the active lever resulted in activation of the pump and delivery of a 2-sec meth infusion (17.5 µg/50 µl bolus for females and 20 µg/50 µl bolus for males) and a 5-sec presentation of a stimulus complex (illumination of the white stimulus light over the active lever and activation of tone generator; 78 dB, 4.5 kHz), followed by a 20-sec time-out. During the time-out period, responses on the active and inactive levers were recorded, but had no scheduled consequences.

Rats acquired meth self-administration in 2-hr sessions along a fixed ratio (FR) 1 schedule. After meeting the acquisition criterion (5 consecutive sessions at >20 infusions/session and <15% variability in the last 2 days), FR values were increased in ascending order (FR 1, 3, 10, 32, 100) in subsequent daily sessions.

After reaching criterion on all FR values (<15% variability in the last 2 days), rats were switched to the within-session BE paradigm. During this session, rats have 5 bins (5 min each) at each price (FR 100, 32, 10, 3, 1) in descending order with a 20 min timeout (house light off) between each bin (105 min total). Cues were presented with infusions in the same manner as during a self-administration session. The rats ran daily on the within-session procedure for a minimum of 5 sessions and until the last 3 sessions produce an α value

(described below) that had <25% variability of the mean of the previous 3 days. All testing occurred in a within subject, counterbalanced manner, with responding re-stabilized (to the same criterion) between each test.

Demand curve analysis

Price is defined here as number of responses needed to obtain 1 meth infusion. Our primary measure of intake when effort required to obtain drug is low was Q_0 (mg/kg/rat), and our primary measure of motivation to self-administer drug when effort required to obtain drug is high was α, the rate at which consumption decreases with increasing effort. Our primary analysis employed values for Q_0 and α derived from the exponential demand equation in parallel with previous literature (Bentzley, Fender et al. 2013). Consumption data for each session and each rat were mathematically fit to the exponential demand equation by determining values for Q_0 and α that minimize the sum of the squares of the logarithmically weighted residuals.

Extinction and Reinstatement

Following testing during the within-session BE paradigm, rats underwent a minimum of seven daily 2 hr extinction sessions to a criterion of <25 active lever presses on 2 consecutive days. During each extinction session, responses were recorded on both levers, but had no programmed consequences. Upon reaching criteria, rats underwent cue- and/or meth-prime induced reinstatement testing. During cue-induced reinstatement, responding on the active lever resulted in

presentation of the light+tone stimulus complex along an FR1 schedule of reinforcement. For drug-primed reinstatement tests, an injection of meth (1 mg/kg dissolved in 0.9% physiological saline, IP) was given immediately prior to testing (Schwendt, Rocha et al. 2009, Reichel, Chan et al. 2012), and responses on either lever were recorded, but did not elicit any programmed consequences. Between all reinstatement tests, rats experienced a minimum of 2 extinction sessions, or until extinction criterion was met.

Estrous cycle monitoring

Female rats were habituated to vaginal cytology procedures during meth self-administration sessions. Vaginal lumen samples were collected by gently flushing 30 µl of ddH_20 with a sterile saline-dipped pipette tip and extracting the sample using a micropipette before rats were placed in the self-administration chambers. Collected samples were smeared on to a glass slide, stained with Quik-Dip Hematology Stain (Mercedes Medical, FL), and examined for classification of cycle phase (estrus, proestrus, and diestrus I/II) based on previously published criteria (Marcondes, Bianchi et al. 2002); Feltenstein et al., 2011) with a light microscope set at 10x magnification.

Fos labeling

Immediately following cue-induced reinstatement or extinction, rats were anesthetized, perfused with saline and 4% paraformaldehyde, and brains extracted, postfixed, cryoprotected, and then sliced at 40 µm. For Fos labeling,

slices were blocked in normal donkey serum (1:500), incubated in rabbit anti-Fos antiserum overnight (1:10,000, Millipore) followed by incubations in a biotinylated donkey-anti-rabbit secondary antibody (1:500) for 2 h at room temperature, and an avidin-biotin complex to amplify signal. Fos immunoreactivity was visualized with 3,3'-Diaminobenzidine (DAB) and 0.6% nickel ammonium sulfate to yield blue-black nuclei in activated cells. After mounting, sections were analyzed to quantify the average number of Fos+ neurons in each hemisphere from the NAc core. Multiple sections (3 slices/animal at 200 μm intervals) were analyzed, and an average for each animal was computed. Areas of interest were identified based on the Paxinos and Watson (2007) brain atlas and Fos+ neurons were quantified using standardized thresholds for size and intensity in Image J (NIH) by an experimenter blind to all conditions.

Stereotactic surgery

Immediately after catheter surgery, subjects were placed in a stereotactic frame (Kopf, Tujunga, CA, USA) and implanted with bilateral stainless steel guide cannulae (22 gauge, 11 mm, Plastics One, Roanoke, VA, USA) into the lateral ventricle at a 10° angle (-.85 posterior, ±2.0 medial–lateral, −3.0 ventral) or the NAc core 6° angle (+1.2 posterior, ±2.4 medial–lateral, −5.2 ventral) based on a rat brain atlas (Paxinos and Watson 1998). Cannulae were secured to the skull with jeweler's screws and dental acrylic. Stylets were placed into the guide cannulae to prevent occlusion.

Drugs

Methamphetamine HCl (Sigma) was dissolved in 0.9% sterile saline. Oxytocin (Cell Sciences, Canton, MA) for systemic administration was dissolved at 1 mg/ml in sterile saline; Cell Sciences, Canton, MA). For intracranial administration, oxytocin was dissolved in aCSF to 1.2 ug/ul for NAc microinfusions. The oxytocin antagonist $d(CH_2)_5$[Tyr$(Me)_2$,Thr4,Orn8,des-Gly-$NH_2$9]-vasotocin (OXA), was a gift from Dr. Maurice Manning, University of Toledo, OH. OXA was dissolved in aCSF to 2 ug/ul for intracerebroventricular (ICV) microinfusions or 1 ug/ul for NAc microinfusions.

ICV microinfusions

To test if systemically administered oxytocin acts within the brain, rats were stabilized on the within-session BE procedure and on subsequent sessions received unilateral microinfusions (28 gauge, Plastics One, Roanoke, VA, USA) via polyethylene tubing connected to gastight 10-µL Hamilton syringes (Hamilton, Reno, NV, USA) set in an infusion pump and delivered 1 µL over a 2 min period; injectors were left in place for an additional 2 min. All rats received 4 microinfusions in a counterbalanced manner of OXA (2 ug/ul) or aCSF into the lateral ventricle immediately followed by a systemic injection of oxytocin (1 mg/kg) or saline 30 min prior to testing. Responding during the BE paradigm was re-stabilized between all tests.

NAc core microinfusions

To acclimate rats to the microinfusion procedure, injection cannulae (28 gauge, Plastics One, Roanoke, VA, USA) extending 2 mm beyond the guide cannulae were bilaterally inserted (no infusions) the day prior to the first microinfusion test. On test days, rats received bilateral microinfusions via polyethylene tubing connected to gastight 10-µL Hamilton syringes (Hamilton, Reno, NV, USA) set in an infusion pump and delivered 0.5 µL/side over a 1 min period; injectors were left in place for an additional minute. To test whether oxytocin in the NAc core decreases meth seeking, rats were pretreated with microinfusions of either oxytocin (0.6 µg/side) or aCSF (in a counter balanced manner) 10 min prior to being tested on the within-session BE paradigm and/or cue-induced reinstatement. To test if oxytocin selectively acts within the NAc core to reduce meth seeking, rats received microinfusions of either OXA (2 µg/µL) or aCSF into the NAc core immediately followed by a systemic injection of oxytocin (1 mg/kg) or saline 30 min prior to testing on the within session BE paradigm. During BE testing, responding was re-stabilized (3 days <25% variability) between all BE tests. Rats tested on cue-induced reinstatement received a maximum of 2 reinstatement tests in a counterbalanced order, and were returned to extinction criterion between tests. All tests occurred in a counterbalanced manner with rats receiving a maximum of 6 microinfusions.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (Version 6.01). Logarithmic transformations produced Gaussian distributions of α and Q_0 (Shapiro-Wilk, $P > 0.05$), and transformed variables were used for all analyses. The effects of treatment and sex were determined using a two-way analysis of variance (ANOVA) with post hoc analyses conducted using Bonferroni's when applicable. Predictions of treatment effects were determined by multiple linear regression and Pearson correlation. Repeated ANOVA with Tukey's multiple comparison correction was used to determine changes in α, Q_0, during meth seeking as a consequence of microinfusion treatment. One-way ANOVA with Tukey's post hoc was used for all data analysis in the Fos experiment.

Results

Experiment 1- Sex differences in the within-session BE paradigm and effects of oxytocin

Multi-day and within-session BE paradigms show similar results

To validate the novel paradigm designed to assess economic demand, male and female rats were first trained on a traditional BE paradigm (multiple days at each FR value). Following training, rats were stabilized on the within-session paradigm to test whether the traditional and novel paradigm produced similar results. In both paradigms, females showed greater motivation to seek meth (lower α) (Fig 4-1 A&C), and higher preferred consumption at null cost (higher Q_0) than males (Fig 4-1 B&D). We further validated our within-session BE paradigm by showing that α calculated during the traditional BE paradigm highly

correlated with the same variables assessed during the within-session BE paradigm in both sexes (Fig 4-1 E&F).

Due to fact that the BE paradigm necessitates many more active lever presses than a typical FR1 self-administration paradigm, inactive lever presses were very infrequent and did not differ between males and females (inactive lever presses-stable baseline BE males vs. females: t_{18}=1.33, p<0.05)(data not shown). Therefore, inactive lever presses were not analyzed for all subsequent studies.

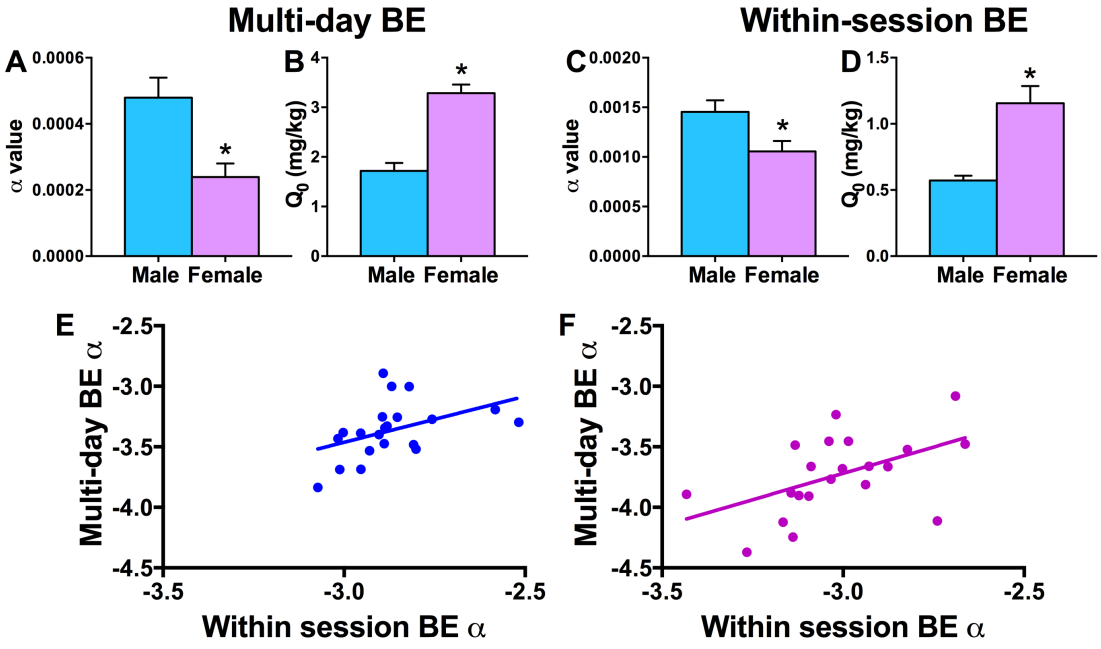

Figure 4-1 Validation of within-session BE paradigm
Demand variables were measured for all rats on the multi-day BE paradigm and then on the within-session BE paradigm. A&B) α and Q_0 values for males (blue bars) and females (pink bars) during the multi-day BE paradigm. C&D) α and Q_0 values during the within-session BE paradigm. In both paradigms, females had lower α values (higher motivation) and higher Q_0 values (higher intake at low FR values or prices) than males (*p<0.05). E&F) Correlations between α values measured during the multi-day and within-session BE paradigms in males (blue) and females (pink). In both sexes, individual α values correlated between the multi-day and within-session BE paradigms (Males r=0.44, p<0.05; Females r=0.51, p<0.05). For all data males: n=22; females n=21.

Demand for meth (α) is related to meth intake (Q_0)

We then assessed whether baseline economic demand for meth (α) was related to meth intake null cost (Q_0) during the within-session BE paradigm. In contrast to cocaine, where these variables were unrelated in males, in both sexes we found a relationship between α and Q_0 (male: r= -0.50, p<0.05 female: r= -0.44, p<0.05)(Fig 4-2A&B) measured during meth self-administration. This relationship also existed between the variables calculated from the multi-day BE paradigm (male: r= -0.51, p<0.05 female: r= -0.45, P<0.05)(data not shown). These results indicate that in both sexes, rats with higher intake at low effort conditions also show higher motivation during high effort conditions.

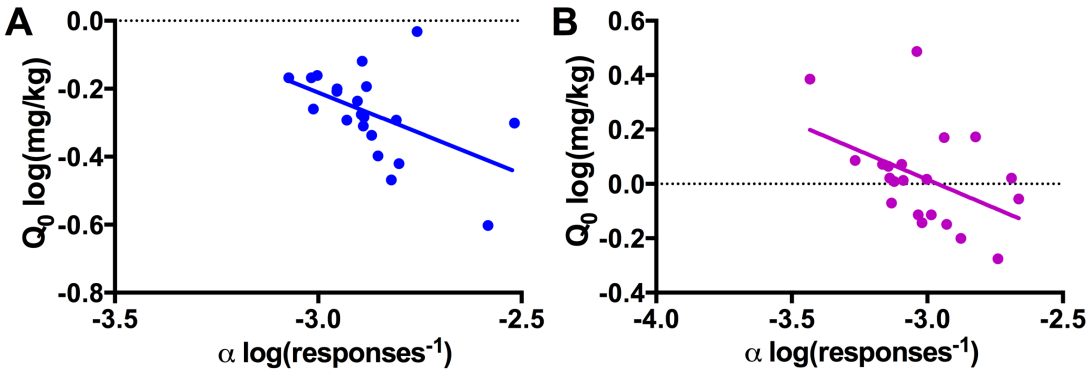

Figure 4-2 Demand variables α and Q_0 are related
Baseline economic values were determined for all rats on the within-session BE paradigm. A) Correlation of α values with Q_0 values in males (n=22 rats; r= -0.50, p<0.05). B) Correlation of α values with Q_0 values in females (n=21 rats; r= -0.44, p<0.05).

Baseline demand (α), but not consumption (Q_0), during BE predicts meth seeking

We next examined whether individual differences in baseline demand (α) or free consumption (Q_0) for meth measured during the within-session paradigm predicted meth seeking during abstinence. Males and females were trained on the within-session BE paradigm and subsequently run on daily extinction sessions followed by cue- and meth prime-induced reinstatement sessions. α predicted meth seeking (number of active lever presses) on the first day of extinction (males: $r=-0.49$, $p<0.05$; females: $r=-0.58$, $p<0.01$) (Fig 4-3 A&B), cue-induced reinstatement (males: $r=-0.43$, $p=0.05$; females: $r=-0.47$, $p<0.05$) (Fig 4-3 C&D), and meth-primed reinstatement (males: $r=-0.32$, $p>0.05$; females: $r=-0.37$, $p>0.05$) (Fig 4-3 E&F). Males and females did not differ in any of these effects. As lower α values indicate greater motivation for meth, these negative correlations show that rats with the higher motivation (measured during BE) exhibited greater meth seeking during abstinence and reinstatement of meth seeking.

In contrast, there was no relationship in either sex between baseline consumption (Q_0) and meth seeking on the first day of extinction (males: $r= 0.07$, $p>0.05$; females: $r= 0.43$, $p>0.05$) (Fig 4-4 A&B), cue-induced reinstatement (males: $r= 0.20$, $p>0.05$; females: $r= 0.19$, $p>0.05$) (Fig 4-4 C&D), or meth-primed reinstatement (males: $r= 0.33$, $p>0.05$; females: $r= 0.23$, $p>0.05$) (Fig 4-4 E&F).

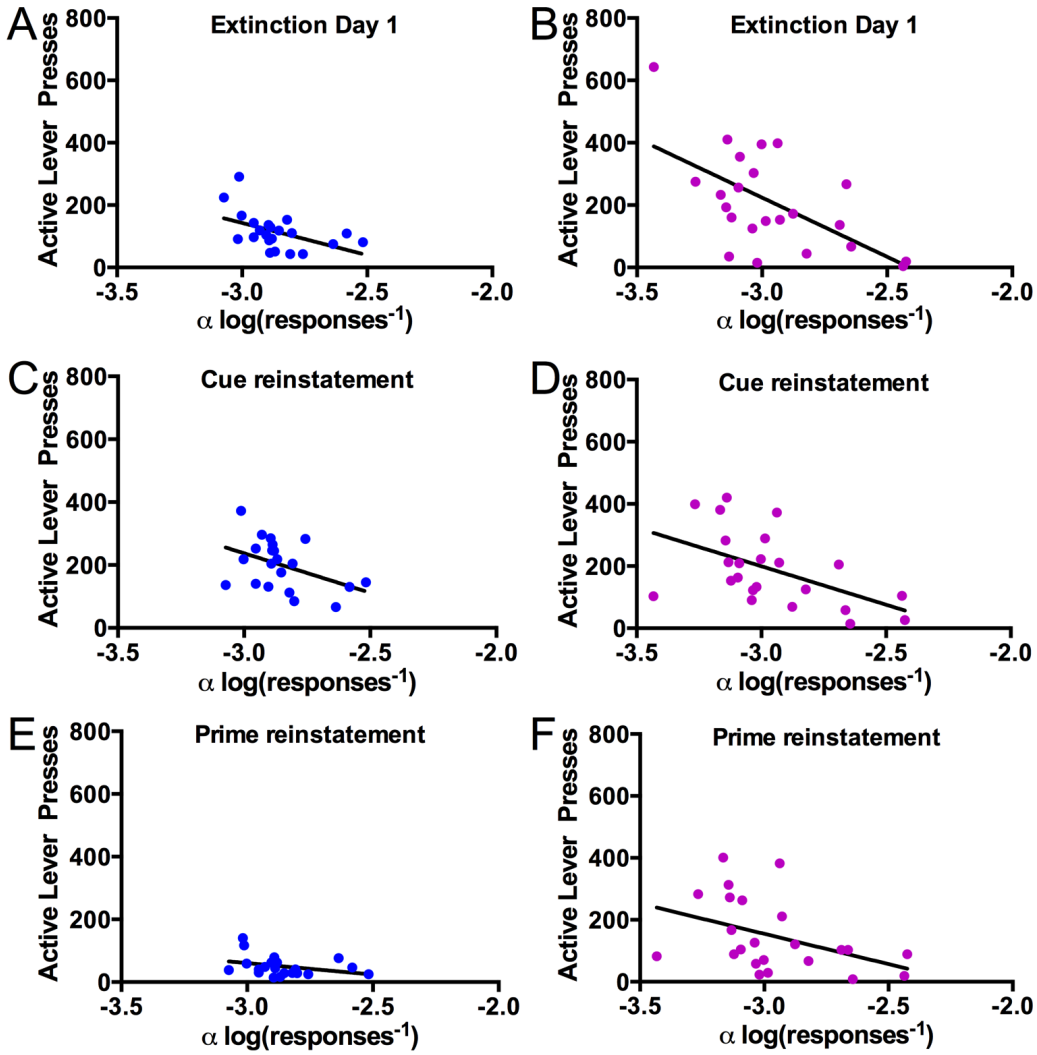

Figure 4-3 Economic demand predicts meth seeking behaviors.
A&B) In both males (blue) and females (pink) α measured during the within-session BE paradigm predicts meth seeking on extinction day one (males: r= -0.49, p<0.05; females: r= -0.51, p<0.05). C&D) Following extinction, α predicted cue-induced reinstatement of meth seeking in both sexes (males: r= -0.56, p<0.05; females: r= -0.44, p<0.05) E&F) α did not predict meth-primed reinstatement in either sex (males: r= -0.35, p>0.05; females: r= -0.32, p>0.05). For all data: males n=22; females n=21.

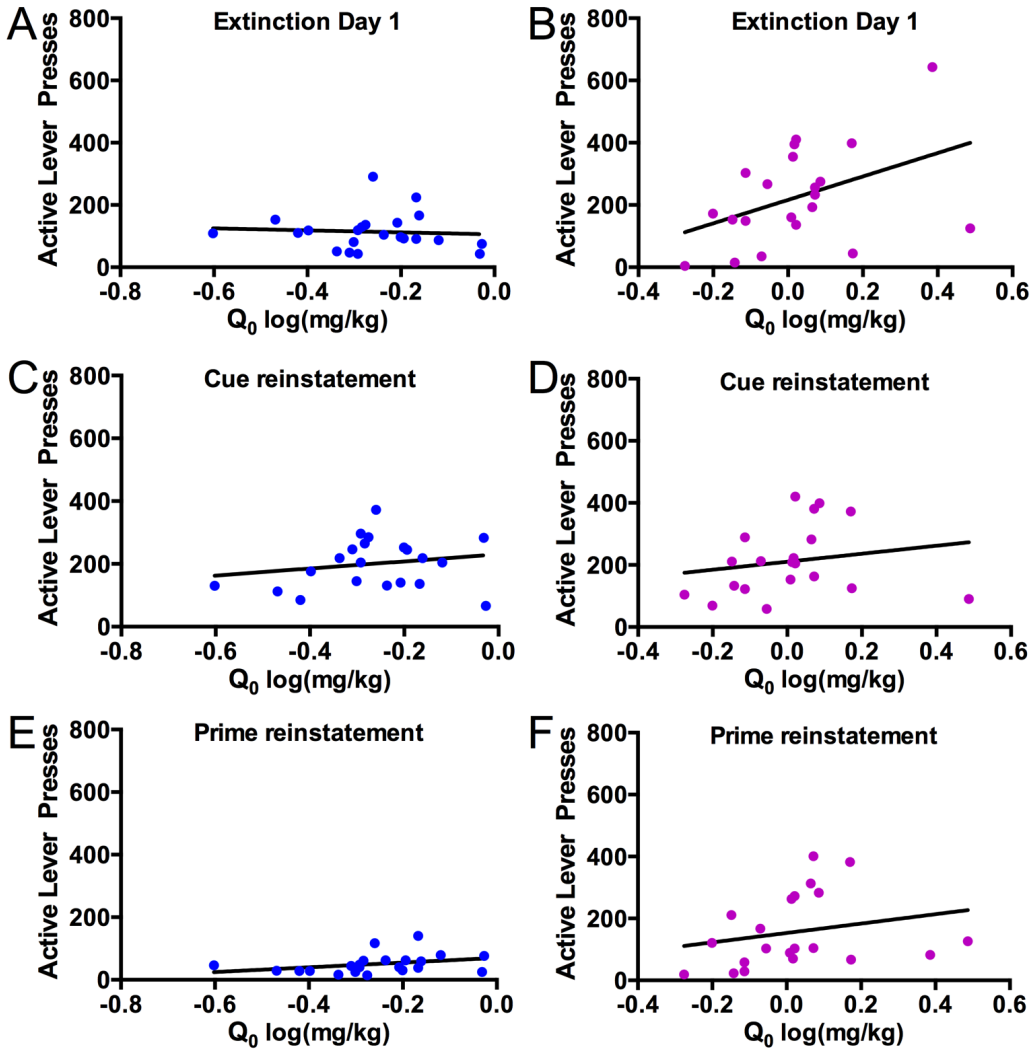

Figure 4-4 Q_0 does not predict meth seeking behaviors.

A&B) In both males (blue) and females (pink) there is no relationship between Q_0 measured during the within-session BE paradigm and meth seeking during extinction day one (males: r= 0.07, p>0.05; females: r= 0.43, p>0.05), C&D) cue-induced reinstatement of meth seeking (males: r= 0.20, p>0.05; females: r= 0.19, p>0.05), (E&F) or meth-primed reinstatement (males: r= 0.33, p>0.05; females: r= 0.23, p>0.05). For all data: males n=22; females n=21.

Oxytocin decreases motivation to seek meth similarly in males and females

We evaluated potential sex differences in the effects of systemic oxytocin on demand for meth and reinstatement of meth seeking using the within-session BE procedure. Rats were stabilized on the within-session paradigm to determine baseline economic demand. On subsequent sessions, rats were pretreated with oxytocin (1 mg/kg i.p.) or saline (counterbalanced) 30 min prior to the BE session. Oxytocin increased α (decreased motivation to seek meth) in both sexes (Two-way ANOVA, treatment main effect $F_{(1,38)} = 52.12$, $p<0.0001$) (Fig 4-5A), but had no effect on Q_0 (preferred intake levels) (Two-way ANOVA, Treatment $F_{(1,38)} = 4.03$, $p=0.052$) (Fig 4-5B). These results demonstrate that oxytocin greatly reduces the motivation to seek meth, but does not affect consumption at low effort. Rats were then extinguished and tested on cue-induced reinstatement. Oxytocin decreased cue-induced reinstatement in both sexes (Two-way ANOVA, treatment main effect, $F_{(1,42)} = 41.05$, $p<0.0001$) (Fig 4-5C). There were no effects of sex in any of the measures, indicating that oxytocin decreases meth seeking similarly in both sexes.

Additionally, there is no effect of estrus cycle phase on BE variables during within-session stabilization (α: $F(2,24)=0.24$, $p>0.05$; Q_0: $F(2,24)=0.02$, $p>0.05$) or cue-induced reinstatement (saline: $F(2,14)=1.93$, $p>0.05$; oxytocin: $F(2,14)=0.68$, $p>0.05$) (data not shown).

To determine if this BE paradigm could predict individual differences in oxytocin's ability to reduce meth seeking, we assessed the relationship between

demand for meth and reinstatement behavior. Individual variability in baseline meth demand (*α)* predicted the efficacy of oxytocin to decrease meth seeking during reinstatement similarly in both males and females (males: r= -0.45, $p<0.05$; female: -0.50, $p<0.05$) (Fig 4-5 D&E). Overall, there were no sex differences in any of the measures, indicating that oxytocin has equal efficacy at reducing meth seeking in both males and females, and is most effective in reducing reinstatement in rats with the highest motivation to seek meth during BE.

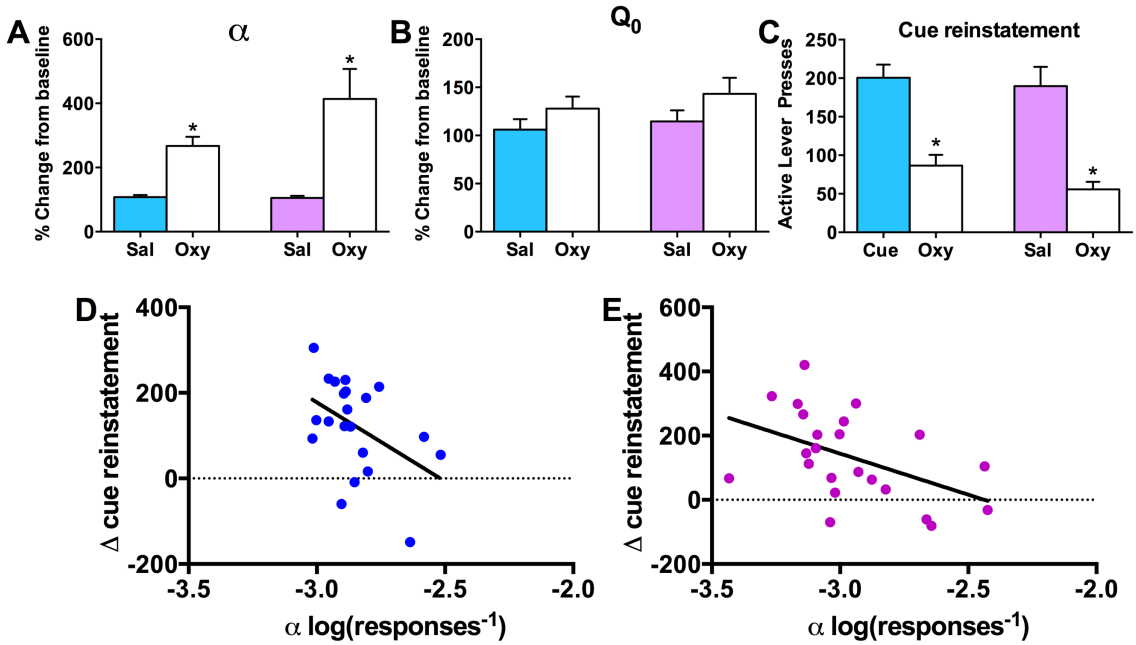

Figure 4-5 Oxytocin decreases meth seeking similarly in both sexes.

Male (blue) and female (pink) rats were tested with oxytocin (oxy, 1 mg/kg i.p.) or saline (sal) during the within-session BE paradigm and cue-induced reinstatement of meth seeking. A) Percent change in α from baseline. In both sexes (male n=22, female n=21), oxytocin robustly increased α (decreased motivation) B) Percent change in Q_0 from baseline. Oxytocin did not affect the percent change of Q_0 in either sex (male n=22, female n=21). C) Number of active lever presses during cue-induced reinstatement of meth seeking. Oxytocin decreased cue-induced reinstatement in both sexes (male n=21, female n=23). Overall, a two-way ANOVA showed no effects of sex in any of these measures, all * indicate a significant treatment effect; *$p<0.05$. D&E) Baseline demand predicted the efficacy of oxytocin to reduce cue-induced reinstatement (change in cue reinstatement from saline test) in both sexes (males n=21: r= -0.45, $p<0.05$; female n=20: -0.50, $p<0.05$).

Expirement 2- Site selectivity of oxytocin in decreasing motivation to seek meth

In the next set of experiments, we examined central mechanisms of oxytocin that produce a decrease meth seeking. Due to the complete lack of sex differences with oxytocin in the previous studies, all subsequent studies were conducted only in male rats.

Oxytocin antagonist ICV blocks the effects of systemic oxytocin

Oxytocin administered systemically has been presumed to attenuate drug seeking via a central mechanism; however, this has not been explicitly tested. To directly test this, rats were first stabilized on the within-session BE paradigm to assess baseline meth demand (α and Q_0). On subsequent sessions, all rats received unilateral microinfusions of OXA (2ug/1ul) or aCSF into a lateral ventricle immediately followed by a systemic injection of oxytocin (1 mg/kg) or saline in a counterbalanced manner 30 min prior to testing.

Overall, systemic oxytocin decreased motivation, and intra-ICV antagonist (OXA) infusion completely blocked this effect (repeated measures ANOVA, $F_{(3,15)}=20.57$, $p<0.0001$)(Fig 4-6A). Specifically, systemic oxytocin significantly increased α (Tukey post hoc: aCSF/sal vs. aCSF/oxy $p<0.0001$). OXA microinfused ICV immediately prior to systemic oxytocin administration blocked the oxytocin-induced inhibition of motivation (increase in α) to seek meth in the within-session BE paradigm (Tukey: aCSF/oxy vs. OXA/oxy $p<0.001$). There was no effect when the OXA was administered ICV the absence of systemic oxytocin (Tukey: aCSF/sal vs. OXA/sal $p>0.05$). There were no significant effects of any of

the treatments on Q_0 ($p>0.05$) (Fig 4-6B). The actions of direct OXA blockade in reversing systemic oxytocin effects indicates that systemic oxytocin is acting in the brain to reduce meth seeking.

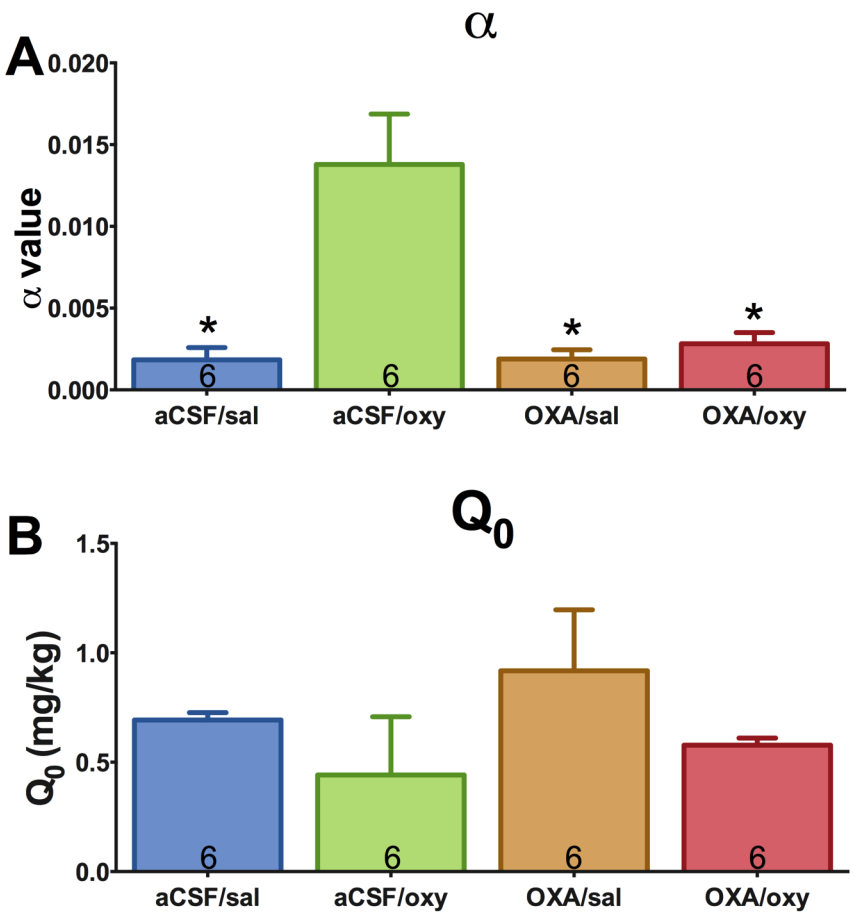

Figure 4-6 Oxytocin antagonist in the brain blocks systemic oxytocin effects.
Rats stabilized on the within-session BE paradigm were tested with either microinfusions of OXA or aCSF (ICV) immediately followed by administration of oxytocin or saline (i.p.) in a counterbalanced manner. A) *α* values assessed during the within-session BE paradigm. Systemic oxytocin (aCSF/oxy, green bar) increased *α* (decreased motivation) compared to controls (aCSF/sal, blue bar). ICV administration of OXA blocked this effect of systemic oxytocin (OXA/oxy, red bar), although did not affect behavior when administered with vehicle (OXA/sal, orange bar). *$p<0.05$ compared with aCSF/oxy B) Q_0 values assessed during the within-session BE paradigm. There were no effects on Q_0 in any of the treatments.

Systemic oxytocin decreases neuronal activation in NAc core

Oxytocin in the NAc modulates reward behaviors (Baskerville and Douglas 2010, Carson, Hunt et al. 2010, Baracz, Rourke et al. 2012, Baracz, Everett et al. 2014). Thus, we determined whether Fos positive neurons in NAc core would reflect the ability of systemically administered oxytocin to attenuate cue-induced reinstatement of meth seeking. Male rats from experiment 1 were pretreated with oxytocin (1 mg/kg i.p.) or saline 30 min prior to a final test of either extinction or cue-induced reinstatement. Compared to extinction, cue-induced reinstatement increased active lever presses (one-way ANOVA, $F_{(2,16)}$=20.82, p,0.0001; Tukey post hoc: EXT vs CUE SAL p<0.0001), and oxytocin attenuated reinstatement behavior (Tukey: CUE SAL vs. CUE OXY, p<0.001)(Fig 4-7A).

Rats were sacrificed immediately after the session to examine neuronal activation in the NAc core via Fos immunohistochemistry. Systemic oxytocin decreased the number of Fos activated cells in NAc core during cue-induced reinstatement [one-way ANOVA, $F_{(2, 16)}$=4.076, p=0.0371] as compared to saline controls (Tukey: CUE SAL vs. CUE OXY p<0.05)(Fig 4-7B). Thus, oxytocin may act to reduce meth seeking via stimulation of previously identified oxytocin receptors in the NAc core.

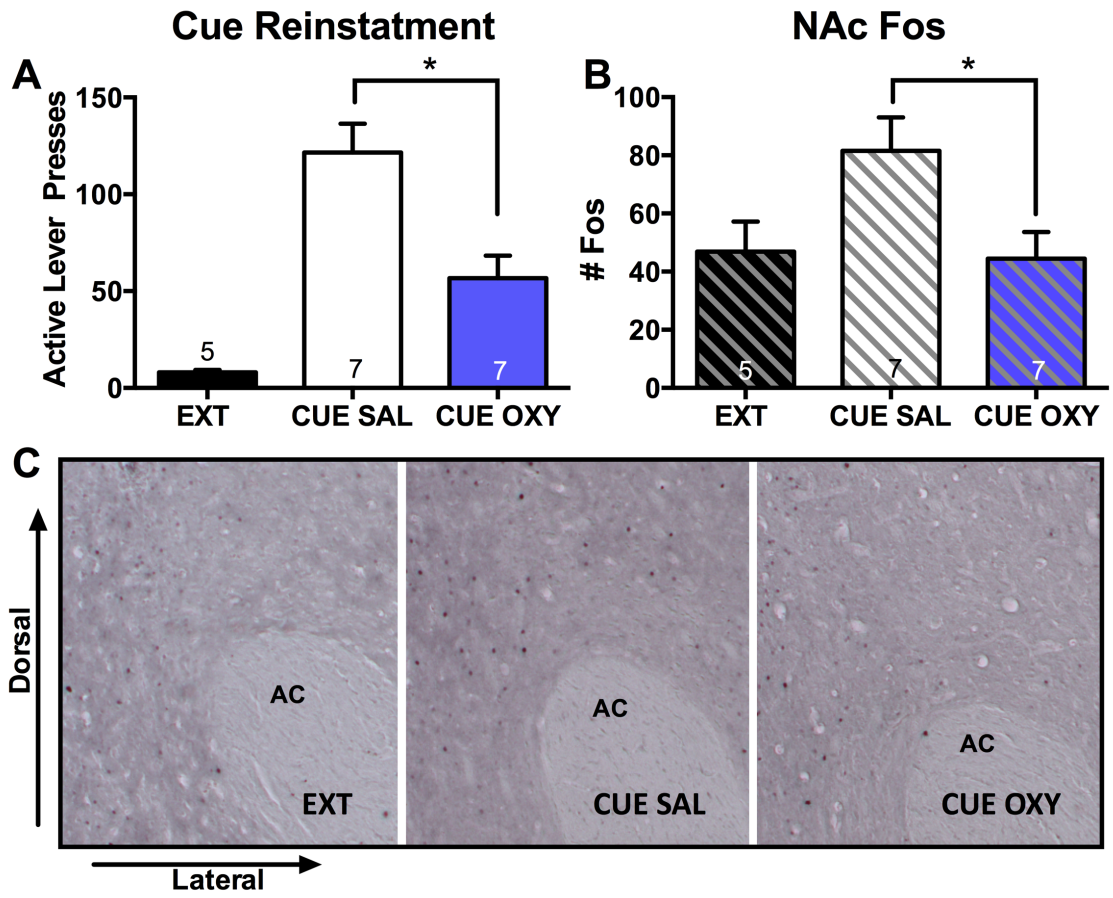

Figure 4-7 Oxytocin decreases reinstatement-induced Fos in NAc core.
A) Number of active lever presses during extinction (EXT, black bar) or cue-induced reinstatement of meth seeking with pretreatment of saline (CUE SAL, white bar) or oxytocin (1mg/kg i.p.; CUE OXY, purple bar). Oxytocin attenuated cue-induced reinstatement of meth seeking (*p<0.05). B) Rats were sacrificed immediately after the session to examine Fos expression in the NAc core of rats tested on either extinction (black hashed bar), cue-induced reinstatement with saline (white hashed bar), or oxytocin (purple hashed bar). There were less Fos positive neurons in the NAc core in rats treated with oxytocin prior to cue-induced reinstatement than saline controls (one way ANOVA *p<0.05). C) Example of Fos staining in each treatment group.

Oxytocin infused into the NAc core decreases meth seeking

To test whether oxytocin directly in NAc core decreases meth seeking, rats received microinfusions of either oxytocin (0.6 ug/ul) or aCSF (in a counter

balanced manner) 10 min prior to being tested on the within-session BE paradigm (Fig 4-8 A&B) and cue-induced reinstatement (Fig 4-8 C). Oxytocin increased *α* compared to aCSF (t_{11}=2.274, p=0.04), but did not alter consumption at low effort Q_0 (t_{11}=0.4679, p=0.07). Oxytocin decreased cue-induced reinstatement of meth seeking (t_{10}=2.940, p=0.015). Overall, intra-NAc core oxytocin decreases meth seeking similar to systemic oxytocin.

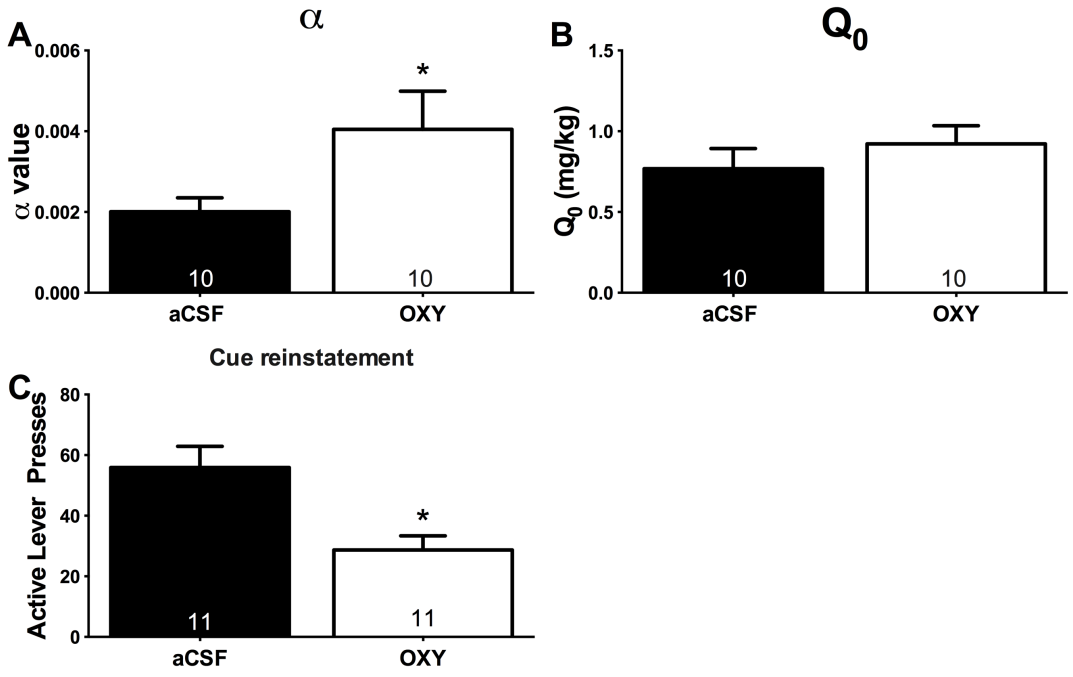

Figure 4-8 Microinfusions of oxytocin into NAc core decreases meth seeking.
A) *α* was increased (decreased motivation) following oxytocin microinfusions as compared to aCSF. B) Oxytocin microinfusions had no effect on Q_0 C) Oxytocin decreased the number of active lever presses during cue-induced reinstatement (*p<0.05).

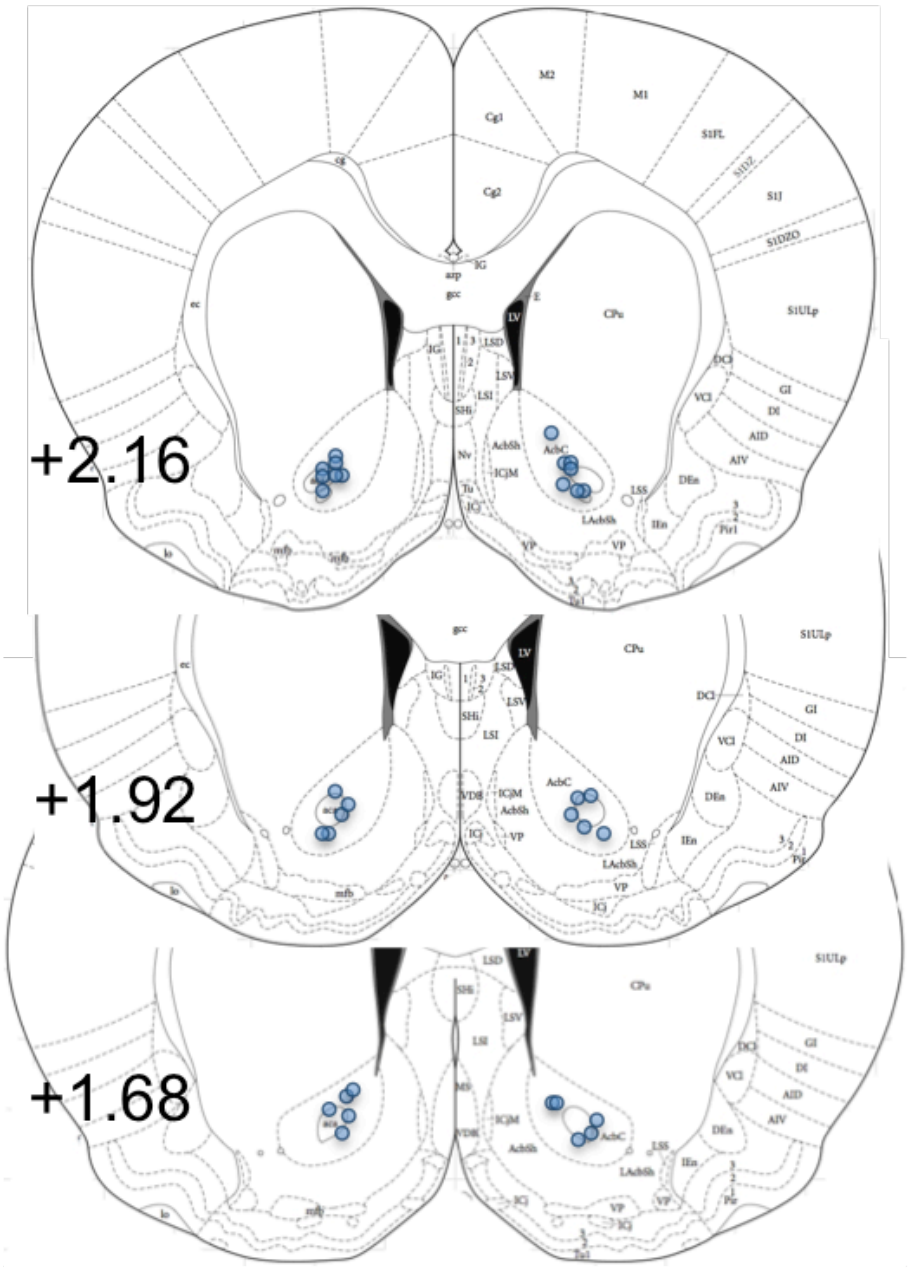

Figure 4-9 NAc core microinjection sites plotted on atlas sections.
NAc core microinjection sites plotted on atlas sections for rats in figures 4-8 and 4-10. Schematic coronal sections were taken from Paxinos and Watson (2007), and numbers refer to location of the coronal slice in mm relative to Bregma.

Systemic oxytocin effects dependent on NAc core

Finally, to further determine whether systemic oxytocin acts within the NAc core to reduce meth seeking, rats were first stabilized on the within-session BE paradigm. On subsequent sessions, all rats received bilateral microinfusions of OXA (2ug/ul) or aCSF into the NAc core immediately followed by a systemic injection of oxytocin (1 mg/kg) or saline 30 min prior to testing. As previously seen, systemic oxytocin significantly increased α (decreased motivation to seek meth) [Repeated measures ANOVA, $F_{(3,21)}$=6.690, p=0.002; Tukey post hoc: aCSF/sal vs. aCSF/oxy p<0.05](Fig 4-10 A). Infusion of OXA into the NAc immediately prior to systemic oxytocin administration blocked the oxytocin-induced inhibition of motivation (α) to seek meth in the within-session BE paradigm (Tukey: aCSF/oxy vs. OXA/oxy p<0.01). OXA had no effects when administered into the NAc in the absence of systemic oxytocin (Tukey: aCSF/sal vs. OXA/sal p>0.05). Assessing Q_0 showed a statistically significant difference between group means [Repeated measures one-way ANOVA $F_{(3,21)} = 5.84$, $p = 0.04$](Fig 4-10 B); however, there were no differences between specific groups (Tukeys, p>0.05) for all comparisons.

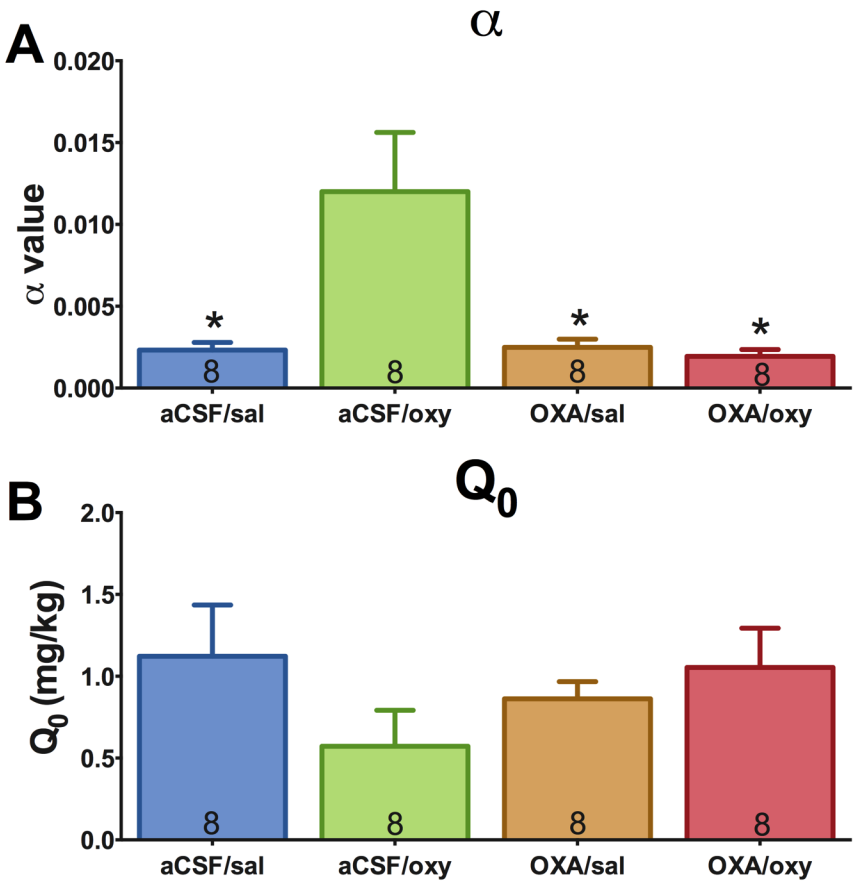

Figure 4-10 Oxytocin antagonist infused into NAc core blocks effect of systemic oxytocin Rats stabilized on the within-session BE paradigm were tested with either microinfusions of OXA or aCSF into the NAc core immediately followed by administration of oxytocin or saline (i.p.) in a counterbalanced manner. A) *α* values assessed during the within-session BE paradigm. Systemic oxytocin (aCSF/oxy, green bar) increased *α* (decreased motivation) compared to controls (aCSF/sal, blue bar). Intra-NAc administration of OXA blocked this effect of systemic oxytocin (OXA/oxy, red bar), although did not affect behavior when administered with vehicle (OXA/sal, orange bar). *p<0.05 compared with aCSF/oxy B) Q_0 values assessed during the within-session BE paradigm. There were no effects on Q_0 between any of the treatments groups.

Discussion

Using a combination of meth economic demand measurement plus direct neural circuitry assessment, we found that oxytocin has excellent potential as a pharmacotherapy for meth addiction via modulation of oxytocin receptor signaling in the NAc core. We first successfully validated a within-session BE paradigm that can predict subsequent relapse-like behaviors in both males and females. Using this model, we determined that systemic oxytocin has similar effects of attenuating meth seeking in both sexes. Importantly, oxytocin has greater effects in rats with the strongest addiction phenotype, regardless of sex, indicating it is an effective pharmacotherapy in both males and females. We then showed that systemic oxytocin acts primarily through a central, rather than peripheral mechanism to decrease meth seeking. Finally, we demonstrated that the NAc core was crucial for oxytocin effects on meth seeking, as it was both necessary and sufficient for the behavioral effects of oxytocin on meth seeking.

Experiment 1

We first validated the within-session BE paradigm for meth by showing similar effects in the traditional multi-day BE paradigm and the within-session paradigm. In both paradigms, we confirmed previous findings that females will take more meth than males when increased effort is needed (i.e., PR schedule) (Roth and Carroll 2004, Cox, Young et al. 2013), and also take more meth on a low-effort FR1 schedule (Reichel, Murray et al. 2009). We also found a

relationship between α and Q_0, indicating that rats with greater meth intake under low-effort conditions also had higher motivation during high-effort conditions. This finding contrasts with a recent report from our laboratory using the cocaine BE paradigm, where no relationship between these variables was observed (Bentzley, Jhou et al. 2014). This could reflect a difference in meth compared to cocaine or methodological differences in the two paradigms. However, it is unlikely to be due to the design of the meth within-session BE paradigm, as this relationship also exists between the variables calculated from the multi-day BE paradigm. This indicates that inherent differences between the two drugs produced the distinction, which could be due to many factors including the unique pharmacokinetic and pharmacodynamic profiles.

Additionally, using the within-session BE paradigm, we replicated findings showing that α (measured during a multi-day BE paradigm) predicts cue-induced reinstatement of meth seeking in males (Galuska, Banna et al. 2011). Similar to results with cocaine (Bentzley, Jhou et al. 2014), α (but not Q_0) predicted meth seeking on extinction day one and during cue-induced reinstatement. However, unlike cocaine, α did not predict drug-primed reinstatement of meth seeking. This difference could be attributed to the different pharmacokinetics of the drugs, or the abnormally low levels of meth-primed reinstatement in the males (as compared to the study in Chapter 2). Future assessment across a range of meth prime doses may be useful for this paradigm.

Previous findings indicated that oxytocin differentially affect meth seeking in males and females. Specifically, systemic oxytocin decreased meth motivation

on a progressive ratio (PR) schedule of reinforcement in females, but not males, but attenuated reinstatement (relapse like behavior) in both sexes (Chapter 2). However, because meth intake during the PR test progressively decreases across the course of the session, the increased blood levels of meth achieved at the beginning of the session confounds the subsequent measurement of drug seeking motivation. Also, because of the longer half-life of meth as compared to other psychostimulants (e.g., cocaine), the PR paradigm can easily be affected by tolerance or other shifts in preferred levels of meth consumption, making it unclear as to whether oxytocin directly affects motivation to seek meth. We solved the problems that confound the PR paradigm by using a normalized measure of demand elasticity to independently measure motivation (α) and preferred intake levels (Q_0) in the within-session BE paradigm (Bentzley, Fender et al. 2013). Interestingly, we saw that in both sexes oxytocin reduced motivation (increased α), but did not affect consumption (Q_0). This indicates that oxytocin attenuates motivation to a similar extent in both males and females, but does not decrease intake under low effort conditions. This result differs from what is seen for cocaine in males, where oxytocin at the same dose decreased both α and Q_0 in a BE paradigm (Bentzley, Jhou et al. 2014), and both PR and FR1 schedules of reinforcement (Zhou, Sun et al. 2014) (note that oxytocin has not been tested on measures of cocaine seeking in females). Interestingly, the fact the oxytocin only decreases motivation but not intake at low effort appears to be a key difference in oxytocin attenuation of cocaine- vs. meth-seeking. Perhaps this difference is due to the increased neurotransmitter release of meth compared to

cocaine (Cruickshank and Dyer 2009). Further studies will be needed to determine the mechanism of oxytocin to help fully understand this difference.

We also found that economic demand for meth predicted the efficacy of oxytocin to reduce subsequent cue-induced reinstatement of meth seeking. Specifically, in both males and females, oxytocin reduced reinstatement most in rats with the strongest addiction phenotype (highest initial motivation to seek meth). Similarly economic demand assessed in alcohol dependent individuals also predicted treatment outcomes (MacKillop and Murphy 2007). As economic demand (α) appears to have predictive validity for addiction-like behavior in rats (Bentzley, Jhou et al. 2014), studies should be directed at assessing pharmacotherapies to see if this predictive relationship occurs in human psychostimulant addicts.

Overall, this model of meth seeking accurately detects sex differences in meth self-administration, and allows for direct comparison of pharmacotheraputic efficacy in both sexes. In addition, this model demonstrates that oxytocin attenuates meth seeking behaviors similarly in both sexes, indicating its potential as an effective pharmacotherapy for meth addiction in both males and females.

Experiment 2

Previous studies have examined the effects of systemic oxytocin on addiction-like behaviors (Carson, Cornish et al. 2010, Carson, Hunt et al. 2010, Cox, Young et al. 2013, Zhou, Sun et al. 2014) with the general assumption that oxytocin is acting through a central (rather than peripheral) mechanism to

produce its behavioral effects. We directly confirmed these assumptions, finding that an ICV infusion of an oxytocin receptor antagonist (OXA) completely blocked the effect of systemic oxytocin on meth seeking. Similarly, an oxytocin antagonist ICV has been shown to reverse the effect of systemic oxytocin on sniffing behavior induced by an acute injection of cocaine (Sarnyai, Babarczy et al. 1991). Additionally, systemically administered oxytocin activates oxytocin neurons in the paraventricular nucleus (PVN) (Carson, Hunt et al. 2010), presumably causing oxytocin release throughout the brain, including regions that modulate addiction behaviors. This evidence suggests that systemic oxytocin is producing its effects via a central mechanism, however it is unclear specifically where systemic oxytocin acts to activate the central oxytocin neurons. Studies show that less than 1% of peripherally administered oxytocin crosses the blood brain barrier, presumably not enough to cause a central effect (Landgraf, Ermisch et al. 1979, Mens, Laczi et al. 1983, Ermisch, Barth et al. 1985). Moreover, the half-life of the oxytocin peptide in the periphery is only 2-10 minutes (Mens, Laczi et al. 1983), necessitating a secondary mechanism that would produce the long-lasting behavioral effects (at least 2½ h) we observed in our self-administration and reinstatement studies. To our knowledge, no clear secondary mechanism has been previously demonstrated to explain how systemic oxytocin acts centrally.

It has been hypothesized that systemic oxytocin may stimulate the release of central oxytocin in the PVN via vagus nerve stimulation (Porges 1998), as the nucleus of the solitary tract (which receives primary visceral afferent inputs) has a direct projection to PVN (Sawchenko and Swanson 1982). Alternatively,

oxytocin could enter the brain via circumventricular organs, as these regions lack the blood brain barrier. Additionally, many of these areas are known to contain oxytocin receptors including area postrema, organum vasculosum of lamina, subfornical organ, and the median eminence (Yoshida, Takayanagi et al. 2009). Once oxytocin neurons in the PVN are activated, local dendritic release of oxytocin can cause a positive-feedback effect allowing for prolonged activation of the oxytocin system (Ludwig, Sabatier et al. 2002, Rossoni, Feng et al. 2008). Further studies are needed to examine the mechanism by which systemically administered oxytocin is acting to decrease meth seeking.

Several studies have examined the effects of oxytocin in NAc on reward and drug related behaviors (Ross, Freeman et al. 2009, Baskerville and Douglas 2010, Carson, Hunt et al. 2010, Baracz, Rourke et al. 2012, Burkett and Young 2012, Baracz, Everett et al. 2014). Therefore, we examined if systemic oxytocin acted through OT receptors in NAc to decrease meth seeking. Oxytocin robustly decreased Fos in NAc core in rats pretreated with oxytocin prior to cue-induced reinstatement. This finding expands on previous work that showed oxytocin caused a similar effect in rats acutely administered meth (Carson, Hunt et al. 2010), by demonstrating this effect in an animal model of relapse.

In previous studies, oxytocin infused into NAc core blocked conditioned place preference for meth (Baracz, Rourke et al. 2012) and decreased meth-primed reinstatement (Baracz, Everett et al. 2014). Here, we furthered our understanding of the role of the NAc in oxytocin effects by demonstrating that intra-accumbens core microinfusions of oxytocin decreased motivation

(increased α) and cue-induced reinstatement for meth seeking. Importantly, intra-NAc core oxytocin attenuated meth seeking behavior in a manner similar to systemic treatment, suggesting a common mechanism.

As a final assessment, we observed that reductions in motivation driven by systemic oxytocin (increased α) depend on the NAc core, as OXA infused into the NAc blocked this effect. Although previous studies have suggested the NAc as a key region for oxytocin driven reduction in drug seeking (Carson, Hunt et al. 2010, Baracz, Rourke et al. 2012, Baracz, Everett et al. 2014), the current study demonstrates that oxytocin in the NAc is both necessary *and* sufficient to do so.

Although systemic oxytocin clearly mediates meth seeking through actions in the NAc core, specific cellular mechanisms are unknown. I will briefly discuss these mechanisms here with a more detailed discussion in the final chapter. Neural tracing studies confirm that oxytocin neurons in the PVN directly project to the NAc (Knobloch, Charlet et al. 2012, Dolen, Darvishzadeh et al. 2013), which when activated by systemic oxytocin may increase oxytocin release in the NAc. However, it is currently unclear whether oxytocin decreases meth seeking via a pre- or postsynaptic mechanism in the NAc core. A recent study showed that social conditioned place preference was dependent on the coordinated release of serotonin via oxytocin interactions with presynaptic receptors on dorsal raphe neurons that project to NAc core (Dolen, Darvishzadeh et al. 2013). Although due to the primary involvement of dopamine (rather than serotonin) release in NAc in mediating psychostimulant self-administration (Roberts, Koob et al. 1980), oxytocin likely drives this effect on meth seeking via an alternative mechanism.

Studies have shown that oxytocin mediates drug and social reward behaviors (at least in part) via interactions with the mesocorticolimbic dopamine system (Qi, Yang et al. 2008, Qi, Yang et al. 2009, Baskerville and Douglas 2010, Yang, Qi et al. 2010, McGregor and Bowen 2012, Baracz and Cornish 2013). For example, systemic oxytocin blocked cocaine-induced dopamine release in the NAc (Kovacs, Sarnyai et al. 1990) and the interaction between dopamine and oxytocin in the NAc are necessary for pair bond formation in prairie voles (Liu and Wang 2003). In addition, a recent study showed that oxytocin and D2 receptors form heteromers in the NAc (Romero-Fernandez, Borroto-Escuela et al. 2013), suggesting oxytocin may increase inhibition via activation of these receptors. In summary, while oxytocin attenuated meth seeking through activity in NAc, future studies will be needed to determine whether the specific mechanism is pre- or postsynaptic and if D2-oxytocin heteromers are involved.

In summary, we used a translational behavioral economic model to demonstrate that demand for meth predicts relapse-like behaviors and the efficacy of oxytocin to reduce these behaviors in both sexes. Moreover, these effects of systemic oxytocin were shown to be mediated through a central rather than a peripheral mechanism, and more specifically dependent on actions in the NAc core. However the specific cellular mechanisms of oxytocin remain to be determined. Together, these results indicate that oxytocin is a promising pharmacotherapy for meth addiction in both males and females, and emphasize the NAc core as the region of interest for further assessment of the mechanism of oxytocin in drug addiction.

CHAPTER 5: Conclusions

Oxytocin as a potential pharmacotherapy for addiction

Currently there are no FDA approved treatments for psychostimulant addiction. Many hurdles or problems from a preclinical standpoint contribute to this fact. Here I will discuss two of the problems, as they relate to the work in this dissertation. First, no established preclinical animal models exist that can accurately predict treatment efficacy of pharmacotherapies for psychostimulant addiction in humans. Second, it is clear that there are sex differences in use patterns and response to treatment in human meth addicts (Dluzen and Liu 2008), indicating that females may respond differently than males to pharmacotherapies. Unfortunately, the vast majority of preclinical studies assessing potential pharmacotherapies for addiction have only used males. The work in this dissertation attempts to address both of these problems by validating a behavioral economic model to assess meth addiction behaviors, and then utilizing the model to examine oxytocin's potential as a pharmacotherapy in both males and females.

. In Chapter 2, we showed that oxytocin decreased responding on a PR schedule of reinforcement in females but not males, but attenuated reinstatement similarly in both sexes. These results indicate that oxytocin may differentially

affect motivation to seek meth during self-administration and abstinence in males and females. However, because intake of meth may have confounded the measure of motivation in the PR paradigm, we further examined this effect of oxytocin during the BE paradigm (Chapter 4) and found that oxytocin decreased motivation to seek meth similarly in both sexes (but did not affect consumption). To understand why the two paradigms produced different results, we have to more carefully consider the differences between the two paradigms. During the PR test, the number of lever presses needed to obtain the next infusion increases throughout the session, whereas during BE the prices (FR value) decrease throughout the session. As a result, rats on a PR schedule receive multiple infusions at low effort conditions before motivation is assessed under high effort conditions. In contrast, during BE, motivation under high effort conditions is measured before rats can attain high blood levels of meth. This difference is especially important when examining effects of a drug with a long-half life such as meth.

Thus when comparing the paradigms, early responding during PR would be most similar to Q_0 and later responding to α. Females showed approximately double the responding during the PR compared to males (increased motivation), and oxytocin only reduced this responding to the level of male controls. This indicates that oxytocin only decreased motivation under high effort conditions, but not at low effort (as females still received an average of 9 meth infusions), consistent with the effects observed in the BE paradigm. In males performing the PR task, we may not have been able to observe an effect of oxytocin due to the

generally low responding on PR, making it difficult to dissociate intake at low and high effort conditions. Alternatively, oxytocin could have different effects in males and females with high systemic meth levels. Specifically, under high blood levels of meth, oxytocin may decrease motivation to a greater extent in females compared to males. This would be consistent with data in Chapter 2 showing oxytocin decreased meth-primed reinstatement to a greater extent in females than males. These results illustrate the importance of separately assessing motivation and consumption.

Importantly, by developing a variation of the within-session paradigm designed for cocaine (Oleson, Richardson et al. 2011, Oleson and Roberts 2012, Bentzley, Fender et al. 2013), we show that the same economic variables can be measured within-session for meth. In addition, the within-session BE model for meth accurately predicted addiction-like behaviors in both males and females. Specifically, α has been shown to predict addiction-like behaviors more accurately than Pmax (Murphy, MacKillop et al. 2009, Murphy, MacKillop et al. 2011), which is similar to 'breakpoint' assessed in the PR schedule of reinforcement. In humans, α but not Pmax predicted addiction behaviors with alcohol and nicotine (Murphy, MacKillop et al. 2009, Murphy, MacKillop et al. 2011). Thus, compared to PR, the BE model more accurately assesses motivation and predicts subsequent drug seeking behaviors. This measure of demand also predicted the efficacy of oxytocin to reduce reinstatement. In human studies economic demand also predicts treatment efficacy. In cigarette smokers, a pharmacotherapy that reduced demand also increased abstinence

(McClure, Vandrey et al. 2013), while in alcohol dependent individuals, demand predicted overall treatment outcomes (MacKillop and Murphy 2007). Since the assessment of economic demand with the BE paradigm allows for direct comparison of rewards and manipulations across animal and human studies of addiction, this paradigm shows promise for use in preclinical studies to screen addiction pharmacotherapies for their efficacy of predicting treatment in human addicts.

This BE paradigm demonstrated that oxytocin has similar efficacy in decreasing motivation to seek meth in males and females. This surprised us, as oxytocin has been shown to have differential effects on males and females for various social and sexual behaviors (Ross, Freeman et al. 2009, Baskerville and Douglas 2010, Keebaugh and Young 2011, Rutherford, Williams et al. 2011, Burkett and Young 2012). However, these differences may be specific to social and sexual behaviors, as males and females have explicitly distinct roles in these behaviors.

In terms of reward related behaviors, a recent study showed that lower doses of oxytocin (e.g., 0.3 mg/kg) decreased sucrose-seeking in females, but not males (Zhou, Ghee et al. 2015), indicating that females may be more sensitive to lower doses of oxytocin. Although our data indicates otherwise for meth, as oxytocin did not decrease meth seeking behavior at that same dose (0.3 mg/kg) in females during meth-primed reinstatement (Fig 2-1). This indicates that oxytocin acts at similar doses in males and females to reduce meth seeking, but additional doses should be tested to rule out dose dependent sex differences.

Nevertheless, this does not preclude the possibility of sex differences in the effects of oxytocin on other drugs or in human addicts.

Overall, the results of this dissertation indicate that oxytocin may be a successful pharmacotherapy for meth addiction in both males and females. Recent clinical studies in alcohol and marijuana dependent individuals also show promise for oxytocin as a treatment for addiction (McRae-Clark, Baker et al. 2013, Pedersen, Smedley et al. 2013); however, specific effects of sex were not examined. Future studies with humans will need to examine oxytocin's potential as a pharmacotherapy for psychostimulant addiction in both sexes.

Theories of oxytocin and addiction

Several theories have been proposed to explain how oxytocin may decrease addiction behaviors. These theories propose that oxytocin modulates neural circuits related to stress, learning and memory, and social behaviors (McGregor and Bowen 2012, Sarnyai and Kovacs 2014). Stress is well characterized as one of the major contributors to relapse behaviors (Sinha 2001, Sinha 2007), and the anxiolytic effects of oxytocin (Neumann and Landgraf 2012, Smith and Wang 2012) may help to decrease relapse. In fact, oxytocin has recently been shown to decrease anxiety triggered by cue-induced reinstatement of cocaine seeking (Morales-Rivera, Hernandez-Burgos et al. 2014). Oxytocin has also been shown to modulate memory consolidation and retrieval (Kovacs and De Wied 1994, Boccia, Kopf et al. 1998, Boccia and Baratti 2000), which could attenuate relapse behaviors by inhibiting memory retrieval or enhancing

consolidation of extinction learning. However, the effect of oxytocin on learning and memory has not been studied in relation to addiction behaviors, thus it is unclear if any of these effects in basic memory performance would affect drug related memories.

Finally, oxytocin facilitates social behavior, and it has been suggested that increased social interaction in human drug addicts may contribute to reductions in addiction behaviors (McGregor and Bowen 2012). A recent study showed that oxytocin mediates social reward through specific mechanisms in the NAc core (Dolen, Darvishzadeh et al. 2013). Similarly, in Chapter 4 we showed that the reduction of meth seeking by systemic oxytocin depends upon the NAc core. Thus, as significant overlap exists for the mechanisms that modulate social/sexual reward and addiction behaviors (Burkett and Young 2012), oxytocin may attenuate of drug seeking through a similar mechanism.

Potential cellular mechanisms of oxytocin in NAc core

Systemic oxytocin activates oxytocin neurons in the PVN (Carson, Hunt et al. 2010) and a subset of those oxytocin neurons project to the NAc core (Knobloch, Charlet et al. 2012, Dolen, Darvishzadeh et al. 2013). Thus, together with our data indicating NAc core dependent effects of systemic oxytocin, it appears that systemic oxytocin activates PVN oxytonergic neurons to increase release of oxytocin into NAc core. However, within the NAc core it is unknown whether oxytocin is acting via a post- or presynaptic mechanism to attenuate meth seeking. Dolen et al. (2013) examined oxytocin receptor localization in NAc

core and found that postsynaptic oxytocin receptors are only found on parvalbumin-positive (Parv+) interneurons and glia cells (GFAP+), and not on MSNs, cholinergic, or nitric oxide interneurons (Dolen, Darvishzadeh et al. 2013).

First, these findings indicate that oxytocin may act on the Parv+ interneurons, and as oxytocin receptors are Gq coupled, this activation would inhibit GABAergic medium spiny neurons (MSNs). Selective inhibition of neurons in the NAc core (which is composed of >90% MSNs) can decrease cocaine seeking (Stefanik, Moussawi et al. 2013). Additionally, Parv+ interneurons in NAc core have been shown to specifically synapse onto MSNs that project to the ventral tegmental area (VTA) (Bennett and Bolam 1994, Meredith 1999). The activation of this specific circuit may contribute to the reduction in cocaine-induced dopamine release observed in the NAc after systemic oxytocin (Kovacs, Sarnyai et al. 1990), and subsequently the reduction in addiction behaviors.

Oxytocin may also act on the astrocytes (GFAP+ glia cells) in the NAc core. These astrocytes contribute extensively to glutamate regulation, which is known to be disrupted after drug self-administration and during reinstatment (Kalivas 2009). Specifically, decreased extrasynaptic glutamatergic tone contributes to increased drug-seeking in a varety of ways. For example, astrocytes normally release extrasynaptic glutamate which activates mGluR2/3 receptors located presynaptically on cortical glutamatergic axons, inhibiting synaptic release of glutamate (Moussawi and Kalivas 2010). However, extrasynaptic glutamate is disrupted in addiction, causing over-activation of the glutamatergic inputs to NAc MSNs, which increases drug seeking behavior

(Stefanik, Moussawi et al. 2013). Thus, oxytocin may act by activating astrocytes to increase extracellular glutamate release and restore glutamatergic tone. A recent study showed a similar mechanism, whereby specific activation of astrocytes (via a Gq coupled DREADDs) in the NAc core decreased cue-induced reinstatement of cocaine seeking, and this was shown to act via a mGlur2/3 dependent mechanism (Scofield, Boger et al. 2015). Thus, as oxytocin receptors are also Gq coupled and on astrocytes in NAc core, it is tempting to speculate that oxytocin may reduce relapse via this gilal mechanism.

Additionally, oxytocin acts on presynaptic receptors located on NAc core afferents. Since inhibition of the NAc core decreases drug seeking (Stefanik, Moussawi et al. 2013), it is plausible that oxytocin preferentially facilitates presynaptic GABAergic afferents, thereby inhibiting MSNs. Dolen et al (2013) examined oxytocin receptor localization on presynaptic terminals of regions that project to the NAc core. These regions included PVN, anterior olfactory nucleus, paraventricular thalamus, basolateral amygdala, cortex of the amygdala, ventral hippocampal CA1 region, dorsal raphe nucleus, caudal VTA, central amygdala, and ventral subiculum (Dolen, Darvishzadeh et al. 2013). However, most of these regions are known to send predominately glutamatergic projections to NAc. However, some oxytocin receptor expressing inputs are predominately GABAergic, including central amygdala and caudal VTA. VTA projections to NAc are primarily dopaminergic, but a GABAergic projection to NAc core also exists (Van Bockstaele and Pickel 1995, van Zessen, Phillips et al. 2012). As the GABAergic neurons in VTA lie primarily in the caudal portion (Nair-Roberts,

Chatelain-Badie et al. 2008), it's possible that oxytocin receptors in NAc could primarily be found on presynaptic GABAergic projections from VTA. Thus, oxytocin could attenuate drug seeking behaviors by increasing GABA release from either the central amygdala or VTA to inhibit MSNs.

Finally, a recent study showed the existence of a dopamine D2-oxytocin heteromer in NAc (Romero-Fernandez, Borroto-Escuela et al. 2013). These receptors were shown to have facilitatory allosteric interactions, meaning that oxytocin increased D2 binding and Gi/o coupling. This suggests that oxytocin could potentially inhibit glutamate release in NAc core via activation of presynaptic D2 receptors. However, the distribution and specific cellular localization of these heteromers has not been determined, making it difficult to draw conclusions about their contributions to oxytocin effects in meth seeking. However, interactions between oxytocin and dopamine are known to be required for many reward related behaviors (Liu and Wang 2003, Baskerville, Allard et al. 2009, Baskerville and Douglas 2010, Burkett and Young 2012, Baracz and Cornish 2013), indicating that this mechanism could be plausible.

Although a clear synaptic mechanism remains to be determined, it is evident that oxytocin acts within the NAc to reduce meth seeking. Oxytocin may attenuate drug seeking through direct interactions with the dopamine, glutamate or GABAergic neurotransmitter systems in the NAc core. Future studies should focus on examining whether oxytocin is attenuating meth seeking via a pre- or postsynaptic mechanism in the NAc core and if these effects extend to other addictive drugs.

In summary, drug addiction is associated with long-lasting neurobiological changes that increase relapse vulnerability. Thus, we utilized a behavioral economic model to specifically examine the relationship between motivation to seek a meth and relapse vulnerability. Additionally, we used this model to assess the efficacy of oxytocin as a pharmacotherapy for addiction with the hope of increasing the translational potential of this data set. Specifically, oxytocin appears to be a promising pharmacotherapy for meth addiction because it decreases motivation to seek meth and attenuates relapse-like behavior in rats with the strongest addiction phenotype. Although few sex differences were observed in the effects of oxytocin, this dissertation emphasizes the importance of focusing on sex and individual differences for the development of efficacious pharmacotherapies for addiction in all populations of humans. Cumulative results from this dissertation indicate that oxytocin would be a promising treatment for meth addiction in humans, and highlight the NAc core as the region of interest for further assessment of the mechanism of oxytocin in drug addiction.

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