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Effects of methamphetamine on sexual behavior

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EFFECTS OF METHAMPHETAMINE ON SEXUAL BEHAVIOR

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ABSTRACT

Methamphetamine (Meth) is a highly addictive psychostimulant associated with enhanced sexual desire, arousal, and sexual pleasure. Moreover, Meth abuse is frequently linked with the practice of sexual risk behavior and increased prevalence of Human Immunodeficiency Virus (HIV). Currently, the neurobiological basis for this drug-sex nexus is unknown. Moreover, there is a lack of studies investigating the effects of Meth on sexual behavior and more importantly, compulsive sex-seeking behavior, under controlled experimental settings in animal models. First, using immuhistochemistry for mating- and Meth-induced neural activation it was demonstrated that Meth administration in male rats activates neurons in brain regions of the limbic system that are involved in the regulation of sexual behavior. Specifically, Meth and mating co-activated neurons in the nucleus accumbens (NAc) core and shell, basolateral amygdala (BLA), anterior cingulate (ACA) and orbitofrontal (OFC) cortices. Second, the effects of acute or chronic administration of Meth on different aspects of sexual behavior were tested including motivation and performance, compulsive behavior, and reward. Results showed that high doses of Meth inhibited sexual motivation and performance. Next, to investigate Meth effects on compulsive sexual behavior a paradigm was established in which visceral illness induced by lithium chloride (LiCl) was paired with sexual reward. A low Meth dose (1mg/kg; s.c.) that does not impair sexual function had long-term effects on compulsive sexual behavior. Specifically, two weeks following the last Meth administration, Meth-pretreated males displayed sex-seeking behavior despite having learned the adverse consequences of mating. This effect was dependent on Meth

administration being concurrent with sexual experience. Finally, using a conditioned place preference (CPP) paradigm, it was shown that concurrent Meth and sex experience was required for enhanced CPP for mating with Meth and for Meth alone. In contrast, reward for mating alone was decreased. Together, these findings illustrate that Meth can activate the same neurons as sexual behavior and in turn may alter this natural reward behavior. Moreover, these data indicate that the association between drug use and mating may be required for expression of compulsive sex behavior reported by Meth users and is correlated with increased reward seeking for concurrent Meth exposure and mating.

Keywords: nucleus accumbens, basolateral amygdala, prefrontal cortex, orbitofrontal cortex, substance abuse, psychostimulant, methamphetamine, compulsive behavior, reproductive behavior, sexual risk behavior, impulse control.

CO-AUTHORSHIP

Chapter 1 entitled “Mixing pleasures: Review of the effects of drugs on sexual behavior in humans and animal models” was written by Karla S. Frohmader and Kyle K. Pitchers with inputs by Dr. Lique Coolen, data included was completed by Karla S. Frohmader, Kyle K. Pitchers, and Margaret E. Balfour. Chapter 2 entitled “Methamphetamine acts on subpopulations of neurons regulating sexual behavior in male rats” was written by Karla S. Frohmader with inputs by Dr. Lique Coolen, experimental procedures and data analysis were performed was by Karla S. Frohmader and Joost Wiskerke. Drs. Roy A. Wise, Michael N. Lehman, and Lique Coolen provided intellectual input. Chapter 3 entitled “Effects of Methamphetamine on sexual performance and compulsive sex behavior in male rats” was written by Karla S. Frohmader with inputs by Dr. Lique Coolen, experimental procedures and data analysis were performed was by Karla S. Frohmader and Katherine L. Bateman. Drs. Michael N. Lehman and Lique Coolen provided intellectual input. Chapter 4 entitled “Concurrent exposure to methamphetamine and sexual behavior enhances subsequent drug reward and causes maladaptive sexual behavior in male rats” was written by Karla S. Frohmader with inputs by Dr. Lique Coolen, experimental procedures and data analysis were performed was by Karla S. Frohmader. Drs. Michael N. Lehman, Steven R. Laviolette, and Lique Coolen provided intellectual input.

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LIST OF ABBREVIATIONS

ABC, avidin-biotin–horseradish peroxidase complex

ACA, anterior cingulate area

Amph, D-amphetamine

BLA, basolateral amygdala

BNSTpl, posterolateral bed nucleus of the stria terminalis

BNSTpm, posteromedial bed nucleus of the stria terminalis

BT, biotinylated tyramide

CeA, central amygdala

CPA, conditioned place aversion

CPP, conditioned place preference

CPu, caudate putamen

DA, dopamine

E, ejaculation

EL, ejaculation latency

EOP, endogenous opioids

ERK, extracellular-regulated protein kinase

GABA, gamma-aminobutyric acid

HIV, Human Immunodeficiency Virus

IF, infralimbic area

IL, intromission latency

IM, intromission

LiCl, lithium chloride

LAC, locomotor activity chamber

M, mount

MDMA, 4-methylenedioxy-methamphetamine

MEApd, posterodorsal medial amygdala

Meth, methamphetamine

ML, mount latency

MOR, mu-opioid receptor

mPFC, medial prefrontal cortex

MPN, medial preoptic nucleus

NAc, nucleus Accumbens

PB, phosphate buffer

PBS, phosphate buffered saline

PEI, post ejaculatory interval

pERK, phosphorylated MAP Kinase

PKMzeta, Protein Kinase M zeta

PL, prelimbic area

STD, sexually transmitted disease

THC, Δ^9-tetrahydrocannabinol

VTA, ventral tegmental area

ZIP, zeta inhibitory peptide

CHAPTER 1: INTRODUCTION

Mixing pleasures: Review of the effects of drugs on sexual behavior in humans and animal models[1]

[1] Sections 1.1-1.6 were published in Hormones and Behavior (2010) 58,149-162

1.1 INTRODUCTION

It is well recognized that drugs of abuse act on the circuits in the brain that regulate motivation and reward associated with natural behaviors, including sexual behavior, eating, drinking, aggression, and maternal behavior (Anselme, 2009; Carlezon and Thomas, 2009; Hyman et al., 2006; Kalivas et al., 2009). Drugs act on the receptors of the endogenous neurotransmitter systems that regulate these natural rewarding behaviors, such as dopamine, opioid, endocannabinoid, serotonin, and noradrenaline systems (Hull et al., 2002; Pfaus, 2009). The current review will summarize literature describing the effects of drugs of abuse on male and female sexual behavior in humans and animal models. Next, potential sites of where drugs may act to influence sexual behavior are reviewed, with emphasis on action in the mesolimbic system. Finally, we will review recent studies on how sexual behavior and sexual experience may affect responses to drugs of abuse.

1.2 Human studies

It is well recognized that psychoactive drugs (both prescribed and otherwise) impact sexual function, performance, libido, and arousal (Pfaus, 2009). Interest in the effects of these drugs on sexual behavior has primarily originated from a prevalence of sexual health related diseases within the addict population. Chronic use of drugs in general leads to “risky” sexual behavior in substance abusers causing high rates of sexually transmitted diseases, including HIV, although different drugs are associated with variable degrees of sexual risk behavior (Crowe and George, 1989b; Fisher et al., 2011; Peugh and Belenko,

2001; Raj et al., 2007a; Rawson et al., 2002; Sanchez et al., 2002). This review will focus on the more commonly used substances of abuse: psychostimulants, opiates, alcohol, and marijuana (Δ^9-tetrahydrocannabinol; THC). Before discussing several studies below, it should be noted that human studies are complicated by several factors. All data are based on self-reports from chronic drug users, often while in rehabilitation programs, using questionnaires; hence they lack a reliable instrument for measurements of relationships between drug use and sexual function. Most users abuse multiple drugs (polydrug use) that can independently cause different changes in sexual function. Subjects have variable drug histories, such as variations in numbers of exposures to a drug or rate of administration (daily, weekly, etc). Moreover, studies on sexual behavior in addicted individuals do not consider sexual function before drug use, which can easily be addressed in animal models, but is currently not the case. Finally, gender is not always considered as a variable, and most studies include only male subjects while few studies have examined effects of drugs on sexual function in men and women separately. Nonetheless, there is a consensus that there is a relationship between drugs and sexual function and main findings are summarized below.

1.2.1 Psychostimulants

Methamphetamine (Meth) increases dopamine neurotransmission by blocking dopamine transporters and increasing vesicular dopamine release. Meth has been declared one of the most abused illicit drugs in the world (Ellkashef et al., 2008; NIDA, 2006) and has a profound effect on sexual risk behaviors. Meth users (men and women) report heightened sexual desire and arousal, and enhanced sexual pleasure (Green and Halkitis, 2006;

Schilder et al., 2005; Semple et al., 2002). However, Meth is also associated with decreased sexual function as chronic Meth abuse results in a condition termed "crystal dick" or an inability to reach full erection and delayed ejaculation and orgasm (Bell and Trethrowan, 1961; Choe and Wang, 2002; Frosch et al., 1996; Peugh and Belenko, 2001). Concurrent use of Meth and Viagra, to further enhance sexual performance, has increased in popularity (Semple et al., 2009). Meth abuse is commonly associated with loss of sexual inhibition and subsequent risk-taking behavior (Green and Halkitis, 2006; Halkitis et al., 2001; Mckirnan et al., 2001; Rawson et al., 2002) including numerous sexual partners and lack of protection (Frosch et al., 1996; Somlai et al., 2003; Springer et al., 2007). The effects of Meth on sexual risk-taking behaviors are greater compared to other drugs of abuse (Peugh and Belenko, 2001; Rawson et al., 2002).

Cocaine is a strong psychostimulant that increases dopamine by blocking dopamine transporters and has been commonly associated with increased sexual arousal and risk-taking behavior in humans (Rawson et al., 2002). Cocaine has been reported to prolong sexual arousal or to cause spontaneous erections and orgasms (Buffum, 1982). In contrast, other research has shown that chronic cocaine use diminishes sexual desire and the ability for both males and females to be able to reach orgasm (Reviewed by Peugh and Belenko, 2001).

Methylphenidate is a weaker psychostimulant commonly prescribed for attention-deficit disorder, which like cocaine, increases dopamine by blocking dopamine transporters (Volkow et al., 1995, 1999). Volkow et al. (2007) conducted a study to examine the

effects of methylphenidate administered to cocaine abusers and cocaine-naïve subjects on sexual desire. Both cocaine-experienced and -naïve participants reported enhanced sexual desire when administered methylphenidate compared to saline, although the environment in which the drug was given was devoid of sexual stimuli. Methylphenidate only affected sexual desire when administered intravenously and not orally, the latter of which results in smaller dopamine increases.

MDMA (4-methylenedioxy-methamphetamine) or "ecstasy" first became popular among college students in the 1980s. In a recent study, 10% of college students at a large US university reported MDMA use, with only alcohol and marijuana used more commonly (Boyd et al., 2003). MDMA users report enhanced pleasure in touching and physical closeness rather than sexual experience (Beck and Rosenbaum, 1994). MDMA impairs sexual performance, including erectile dysfunction and delayed orgasm (Buffum and Moser, 1986; Zemishlany et al., 2001) and inhibited sex drive (Parrott et al., 2001; Passie et al., 2005; Topp et al., 1999).

1.2.2 Opiates

Opiates such as morphine and heroin act on receptors in the brain that normally bind endogenous opioid peptides. Similar to effects of psychostimulants, heroin users report high risk sexual behaviors in both men and women. Subjects frequently report having multiple sexual partners, inconsistent or no condom use, and prevalence of STD/HIV diagnosis (Raj et al., 2007b; Sánchez et al., 2002). Although small doses of heroin may enhance sexual desire and performance (Miller and Gold, 1988), chronic opiate use,

including methadone and buprenorphine, synthetic and semisynthetic opiates prescribed for opiate addiction treatment, is characterized by decreases in sexual desire, response, and orgasms for both men and women and cause erectile and ejaculatory dysfunction (Buffum, 1982; Miller and Gold, 1988; Peugh and Belenko, 2001).

1.2.3 Alcohol

Alcohol acts on gamma-aminobutyric acid (GABA) receptors resulting in the inhibition of neural excitability. Alcohol is an important drug to discuss as it is often available in many social scenarios throughout multiple cultures and is commonly associated with disinhibited social behaviors. Human studies report confounding effects of alcohol on the human libido. While some research shows that alcohol enhances sexual behavior and desire, other studies report alcohol-induced impairments of sexual function. Potential factors contributing to the controversial findings are the circumstances under which the drinking takes place, studies in laboratory settings versus self-report studies from users, as well as the doses of alcohol consumption (Reviewed by Crowe and George, 1989a; Peugh and Belenko, 2001). Laboratory studies have shown that in men, low blood alcohol levels do not affect or slightly enhance sexual arousal and responsiveness (measured by peak penile circumference), but higher blood alcohol levels result in decreases in erectile responsiveness, reduction in arousal, and impaired ability to ejaculate (Crowe and George, 1989a; Peugh and Belenko, 2001). However, other laboratory studies did not show major effects of either low or high blood alcohol levels on measures of arousal (George et al., 2006). Women show reduced vaginal flow responses even with moderate alcohol consumption. In apparent contrast, women self-

report increased sexual arousal and pleasure with greater alcohol consumption and are more likely to engage in sexual activities with someone either than their partner while under the influence (Peugh and Belenko, 2001). Chronic alcohol consumption has detrimental effects on sexual and reproductive function, erectile and ejaculatory dysfunctions in men and diminished sexual arousal, interest, and orgasm in women (Crowe and George, 1989a).

1.2.4 THC/marijuana

THC binds to cannabinoid receptors in the brain and is the active ingredient in marijuana which is the most commonly used illicit drug (NIDA, 2005). Limited to self-report studies, THC has been associated with increased sexual arousal and function. Specifically 70% of users reported marijuana as an aphrodisiac, 81% reported enhanced sexual pleasure and satisfaction (Halikas et al., 1982). However, other studies have shown that chronic marijuana smoking may lower testosterone levels and other reproductive hormones leading to erectile dysfunction in men (Grinspoon and Bakalar, 1997).

Thus, in summary, based on studies in chronic users, psychostimulants and opiates have well documented effects on sexual risk behaviors, while generally decreasing sexual performance in men and women. Chronic use of THC and alcohol is associated with decreased sexual performance, but has also been reported to increase sexual arousal. Currently, the neurobiological basis of this drug-sex nexus is not fully understood and there is a need for controlled human studies using validated methods to measure indicators of sexual function as well as non-human animal studies. Therefore,

the next portion of this review will discuss studies on effects of systemic administration of drugs of abuse on sexual behavior in animals. Since rodents have been the model of choice in the majority of studies, the review is largely restricted to data in rodents.

1.3 Animal studies: Sexual performance and motivation

Studies in rodents have utilized various behavioral paradigms to allow measures of appetitive, precopulatory or proceptive (sexual motivation), and copulatory behaviors (sexual performance) in males and females. In male rats, approach to or active seeking of receptive females are indicators of sexual motivation. Several paradigms are used to investigate this measure including recording of approach behaviors and amount of time spent investigating females that are presented behind a barrier. A paradigm used to determine alterations in sexual motivation is the bilevel chamber, consisting of two vertically interconnected levels that allows for measurement of anticipatory searching activity before the entrance of the female partner (Mendelson and Pfaus, 1989; Pfaus et al., 1990b). Furthermore, latencies to first mount and intromission are also considered indicators of sexual motivation (Agmo, 1997; Pfaus et al., 1990b). Male sexual performance is indicated by percentages of animals that display mounts, intromissions, or ejaculations; as well as frequencies of these behaviors (Agmo, 1997). In female rodents, copulatory behavior consist of lordosis behavior; the reflex dorsiflexion of the spine in response to stimulation of the flanks (Pfaus et al., 2007). Appetitive, precopulatory behaviors consist of solicitations, and hops and darts (Pfaus et al., 2007). An elegant paradigm to investigate female appetitive behavior and thus female sexual motivation, is the paced mating paradigm, in which female rats have the ability to actively seek or avoid

the male (Erskine, 1989). The next section will review animal studies testing effects of acute or chronic systemic administration of drugs of abuse on male and female sexual behavior.

1.3.1 Psychostimulants

Amphetamine

The use of *D-amphetamine* (Amph) as a recreational drug is not common practice. Therefore, the effects of Amph on human sexual behavior have not been well documented. However, a vast majority of research implements Amph administration in the rodent animal model to study the effects of psychostimulants on sexual behavior. Previous research includes studies on the effects of both acute and repeated Amph administration on sexual motivation and performance in both male and female rats.

Males: Studies on effects of acute administration of Amph have yielded contradictory results and have either shown facilitation of sexual behavior (Bignami, 1966; Butcher et al., 1969) or no effect (Agmo and Fernandez, 1989; Agmo and Villalpando, 1995). In particular, acute administration of Amph (i.p. 0.5 and 1 mg/kg) or amfonelic acid (0.25 and 0.5 mg/kg; i.p.), which like Amph increases dopaminergic neurotransmission, resulted in significant reductions in latencies to mount and intromit in sexually naïve male rats (Agmo and Picker, 1990). However, amfonelic acid administration resulted in significantly increased frequency of sniffing and rearing behavior while behaviors associated with sexual behavior (female pursuit and mounting attempts) were not altered (Agmo and Picker, 1990). Acute administration of Amph in a test for sexual motivation

also failed to have effects (Agmo, 2003). Therefore, Agmo and coworkers have suggested that increased dopaminergic neurotransmission caused by psychostimulants may indirectly stimulate sexual behavior in male rats by augmenting behavioral arousal.

In contrast, other studies have shown effects of repeated Amph preexposure on sexual motivation and performance in male rodents. Fiorino and Phillips (1999a,c) demonstrated facilitatory effects of repeated Amph (10 injections of 1.5 mg/kg Amph every 2 days) on sexual motivation and performance. Sexual behavior was tested in a drug-free state after a 21-day withdrawal period during 10 mating tests, and males were sexually naïve at the start of testing. Higher percentages of Amph-pretreated sexually naïve males displayed mounts and intromission and with shorter latencies to mount and intromission compared to controls. No effects on ejaculatory behavior or other parameters of sexual performance were observed. The Amph-induced effects on sexual behavior were independent of drug-associated contextual cues and occurred regardless of whether mating was tested in the same or different environment of drug administration. Furthermore, anticipatory searching activity before the entrance of the female was assessed using a bilevel apparatus (Fiorino and Phillips, 1999c). Using this measure as an indicator of sexual motivation (Mendelson and Pfaus, 1989), it was shown that drug pretreatment also enhanced sexual motivation as the Amph group made significantly more level changes in anticipation of the receptive female compared to controls on the last mating test. These results suggested that Amph pretreatment enhanced the attribution of the incentive salience of the rewarding stimulus (the female).

Nocjar and Panskepp (2002) also reported effects of chronic Amph pretreatment on sexual motivation, but reported a complex interaction between drug history and drug-paired environment. It was reported that rats preexposed to Amph injections and tested after 2 weeks of drug abstinence showed enhanced or decreased sexual pursuit dependent on the drug history. Animals treated on alternate days (5.0 mg/kg; i.p.; twice daily during 5 alternate days) displayed enhanced sexual pursuit, but only if drug was administered in an environment other than the home cage. In contrast, daily injections resulted in decreased sexual pursuit regardless of the drug-history environment. In these studies, male rats were sexually naïve; hence, effects were independent of consummation of reward or learning of the incentive value of the female stimulus. Together, these studies demonstrate that Amph-sensitizing treatments can result in cross-sensitization of sexual behavior and cause increased sexual motivation and performance. However, the time of drug abstinence appears to be of influence as male rats that were tested 12 hours after withdrawal from an escalating dose schedule of Amph displayed decreased anticipatory activity (measured in the bilevel apparatus) and longer post ejaculatory intervals, reflecting a decrease in sexual motivation and initiation, without affecting other measures of sexual performance (Barr et al., 1999). Thus, drug history and drug history environment, as well as time of drug abstinence, are factors that influence effects of Amph on male rat sexual behavior.

Females: In female rats, acute or chronic administration of Amph seems to have opposite effects on female copulatory behavior and sexual motivation. Acute Amph decreased lordosis behavior and proceptive behaviors in hormone-primed female rats (Guarraci and

Clark, 2003; Pednekar and Mascarenhas, 1993). Moreover, acute Amph affects sexual motivation: Guarraci and Clark (2003) conducted a study to test the effects acute doses of Amph on pacing mating behavior in ovariectomized and hormone-primed sexually experienced female rats. It was shown that an acute dose of Amph (0.5, 1, or 2 mg/kg; i.p.) increased the likelihood that a female would withdraw from a male after mounting and intromitting compared to saline-injected females and decreased the percentage of time females spent with the male. These results suggest decreased sexual motivation induced by acute Amph.

In general, chronic administration of Amph facilitates sexual function in female rats. Guarraci and Clark (2003) tested the effects of repeated intermittent Amph exposure on paced mating behavior. Amph-pretreatment resulted in decreased latencies for females to return to the male after sexual stimulation when tested 1 week following the last drug injection. Afonso et al. (2009) showed that Amph-pretreated females tested following a 21-day drug abstinence period, displayed increased solicitations and proceptive behaviors (hops and darts, as well as pacing) without altering lordosis behavior. Effects of repeated Amph were independent from the drug history environment. These data together with those discussed above for males, suggest that Amph sensitization results in cross-sensitization of sexual behavior, with selective activation of appetitive sexual behavior as sexual performance was not enhanced.

Methamphetamine

Although there is abundant research looking at various aspects of Meth abuse on human sexuality, there are only few studies investigating the effects of Meth on sexual behavior under controlled settings. In male Japanese quails (Bolin and Akins, 2009), chronic administration of Meth (1 or 3 mg/kg daily for 10 consecutive days) followed by a 10-day withdrawal period reduced sexual motivation but did not affect sexual performance. In contrast, Meth administration in females rats subchronically pretreated with Meth (total of 3 daily injections of 5 mg/kg) increased proceptive and receptive behaviors (Holder et al., 2010). It should be noted that studies using chronic Meth to examine male rodent sexual behavior are lacking. Therefore, studies in my thesis will investigate the effects of acute and repeated Meth on sexual motivation and performance, social interactions, as well as changes in sexual and/or drug reward.

Cocaine

Data in animal studies have shown that acute and chronic cocaine induces penile erections and facilitates male sexual behavior. Acute cocaine in sexually naive male rats stimulates spontaneous penile erections when males are examined in all-male groups (7.5 mg/kg) (Andersen et al., 2000). In addition, cocaine stimulates spontaneous penile erections and ejaculations when administered following 96 hours of paradoxical sleep deprivation (7 mg/kg) (Andersen et al., 2003). Acute cocaine also decreased intromission frequency and increased mount frequency before ejaculation in sexually experienced males (7.5-15 mg/kg) (Ferrari and Giuliani, 1997). Since increased mounting can be indicative of increased appetitive behavior or alternatively of impaired copulation, these

data suggest that acute cocaine may have complex actions and not simply act as a sexual stimulant. However, chronic treatment (15 mg/kg; 7 daily injections) caused a slight decrease in mount and intromission frequency as well as ejaculation frequency (Ferrari and Giuliani, 1997), suggesting that chronic cocaine treatment facilitated sexual behavior. This effect was dose-dependent as acute or chronic treatment with a higher dose of cocaine (30 mg/kg) severely disrupted sexual behavior (Ferrari and Giuliani, 1997). In support, chronic cocaine treatment in male Japanese quails (6 daily injections 10 mg/kg) displayed enhanced sexual motivation and performance when tested 10 days following last cocaine exposure (Levens and Akins, 2004).

MDMA

Studies using rodents as an animal model mimic findings of MDMA effects on human sexual behavior. Specifically, acute low doses of MDMA (3 mg/kg) administered before mating resulted in impaired sexual performance, reflected by decreased percentages of males that mated as well as increased intromission and ejaculation latencies (Cagiano et al., 2008). Similar to acute administration of MDMA, male rats treated with a neurotoxic multiple-dose regimen of MDMA (40 mg/kg) administered every 12 hours for 4 consecutive days displayed impaired sexual performance 10 days following the first MDMA exposure. Specifically, MDMA pretreatment reduced the percentage of males that mated as well as increased ejaculation and post ejaculatory interval latencies. However, these effects were transient as the inhibitory effects of MDMA on sexual behavior did not persist 2 weeks after the last drug injection (Dornan et al., 1991).

Finally, a recent study from our laboratory (Straiko et al., 2007) investigated the effects of repeated MDMA administration on sexual reward using a conditioned place preference (CPP) paradigm for mating. Male rats were treated with a neurotoxic multiple-dose regimen of MDMA (10 mg/kg) administered every 2 hours for a total of four injections. Twelve days following the last drug injections males were subjected to a CPP test for sex. Although MDMA pretreatment did not alter sexual performance, it significantly impaired the acquisition of mating-induced CPP suggesting an altered sexual reward or associative memory for sexual reward following MDMA treatment.

1.3.2 Opiates

Morphine

Although most the available research on the effects opiates on human sexual behavior is focused on heroin abuse, experimental research has exclusively focused on the effects of morphine on mating behavior. Acute and chronic administration of morphine severely inhibited sexual behavior in male rats and mating remained suppressed for at least 7 days after last morphine injection (Cui et al., 2004). Moreover, acute morphine administration (5-10 mg/kg) reduced spontaneous genital reflexes induced by cocaine in paradoxical sleep-deprived male rats (Andersen et al., 2004). Finally, chronic treatment in male rats resulted in a reduction of successful impregnation of females, suggesting a potential effect of chronic morphine treatment on male fertility (Kalivas, 2009). Mitchell and Stewart (1990) tested the effects of conditioned stimuli previously associated with systemic injections of morphine on sexual behavior in male rats. Males were subjected to a total of four conditioning trials, during which males received 10 mg/kg morphine and

placed in the mating area for 1 hour (paired group) or in the home cage (unpaired group). Exposure to mating environment that males had learned to associate with morphine resulted in facilitation of appetitive measures of sexual behavior, including increased anogenital exploration, pursuit activity, and shorter mount latencies. These data suggests that stimuli associated with opiate reward result in facilitation of sexual arousal or motivation. Furthermore, Nocjar and Panskepp (2002) reported that chronic morphine (10 mg/kg; 10 daily injections) enhanced preference for a receptive female placed in a stimulus box (only allowed interaction without copulation) when preference was tested following a 2-week, but not 3-day drug abstinence period. Hence, together, these studies show that acute or chronic morphine treatments impairs sexual performance and fertility; yet following chronic morphine exposure and a withdrawal period sexual motivation or arousal is enhanced. In contrast, high-dose methadone maintenance regimen was shown to decrease sexual motivation in male rats, consistent with reports of sexual dysfunction in methadone-maintained individuals (Leri et al., 2007) suggesting that opiates may exert differential effects on male sexual behavior.

1.3.3 Alcohol

Recent animal studies investigating the effects of alcohol on sexual behavior are limited. However, previous studies in rodents parallel human findings as acute alcohol administration impairs sexual performance but increases sexual motivation in male rats (Ferraro and Kiefer, 2004; Pfaus and Pinel, 1989). In contrast to the effects of acute alcohol, rats appear to develop tolerance to the effects of repeated alcohol administration on sexual performance. Males receiving repeated injections of alcohol (1 g/kg)

administered before mating eventually show unaffected sexual performance (Pinel et al., 1992) and chronic intake of low doses of alcohol only slightly affects sexual motivation (Cagiano et al., 1998).

1.3.4 THC/marijuana

Acute and repeated THC administration affects neuroendocrine responses and sexual performance in rats. Acute administration of THC (5 mg/kg) has been shown to suppress increases in plasma luteinizing hormone and prolactin levels in male rats exposed sexually receptive females (Murphy et al., 1994). Acute or subchronic (7-14 days of daily injections) administration of THC or cannabinoid receptor agonist HU 210 (25-100 μg/kg) resulted in a dose-dependent impairment of male rat sexual motivation and performance (Hyman et al., 2006; Murphy et al., 1994). Acute HU 210 also inhibits female rat sexual behavior (Hyman et al., 2006). In summary, acute and chronic alcohol and THC impair sexual motivation and performance, although chronic alcohol exposure in low doses only slightly affects sexual behavior as animals appear to develop a tolerance to the effects of alcohol.

1.4 Animal studies: Future directions and sexual risk taking

The studies listed above clearly indicate that drugs of abuse affect expression of male and female sexual behavior, but the interactions are complex and can not be summarized easily. Moreover, there are procedural differences between studies that may be crucial for the outcome of the experiments. First, studies that tested a variety of doses of a particular

drug have demonstrated different effects of the various doses. Unfortunately, most studies listed above only included one particular dose, partially explaining some of the controversial results. Second, studies that tested effects of acute versus chronic exposure to drugs of abuse often yielded opposite effects. In the studies that chronically exposed animals to drugs, mostly animals were tested in drug-free conditions. Very few studies tested the effects of a drug of abuse in animals that are chronically exposed to the drug, which would make translation to the human studies more obvious. Moreover, it is well established that repeated exposure to drugs causes plasticity in the brain pathways that mediate natural reward, including sexual behavior. Hence, this is an important factor to consider. Finally, sexual history or experience may also play a factor in these studies; drugs may affect behavior differently in animals that are sexually naïve versus experienced. Hopefully, future studies will be designed to incorporate these factors and provide a more translational picture that can be used to better understand the drug-sex nexus identified in the human studies.

The animal studies reviewed above have focused almost exclusively on the effects of drugs of abuse on sexual motivation and performance. Although such effects are indeed reported in humans, the human studies have indicated that one of the largest and most problematic effects of drugs on sex behavior is the increase in risk-taking behaviors related to sexual activity. Yet, very few of the animal studies have attempted to investigate this phenomenon, in part probably due to a lack of an established paradigm to study sexual risk behaviors in rodents. Pfaus and Pinel (1989) examined whether alcohol administration facilitates sexual behavior by releasing it from inhibitory control. First,

male rats were trained to inhibit copulatory behavior when presented with a non-receptive female, while maintaining baseline copulatory rates with receptive females. It was then tested if alcohol had the ability to override the learned inhibition of sexual behavior induced with sexually non-receptive females. Indeed, a low, but not high dose of alcohol resulted in significantly increased percentages of males that displayed mounts and ejaculations with non-receptive females, despite the uncooperative behavior of the females. Hence, the use of this conditioning paradigm allowed for testing the facilitation by disinhibition of sexual behavior by a drug of abuse. One of the main goals of my thesis is to examine compulsive sex-seeking behavior induced by Meth use.

1.5 Brain sites of drug action

The endogenous neurotransmitter systems that are affected by drugs of abuse include dopamine, opioid, noradrenergic, serotonin, and endocannabinoid systems and are critically involved in regulation of sexual behavior as extensively reviewed (Hull et al., 2002, 2006; Pfaus, 2009). Hence, drugs can act in numerous brain areas to influence various neurotransmitter systems to affect different components of sexual behavior. Drugs may act in the medial preoptic area, the paraventricular nucleus and ventromedial nucleus of the hypothalamus to affect sexual performance and penile erection in males or lordosis in females (Hull et al., 2002; 2006; Pfaff et al., 2006; Pfaus, 2009). Drugs may also act at the level of the lumbosacral spinal cord to influence erectile and ejaculatory function (Allard et al., 2005; Coolen, 2005; Coolen et al., 2004a; Coolen and Hull, 2004; Giuliano and Rampin, 2004; Hull et al., 2006; Truitt and Coolen, 2002). However, the

current review will focus primarily on the mesolimbic system as the main areas where drugs of abuse can affect sexual motivation and risk-taking behaviors.

1.5.1 Mesolimbic system

The mesolimbic dopamine (DA) circuit has been implicated in the regulation of motivational/appetitive, rewarding, and reinforcing aspects of natural rewarding behaviors including mating (Balfour et al., 2004; Kohlert and Meisel, 1999; Pfaus et al., 1990a), aggression (Pucilowski and Kostowski, 1983), feeding (Avena et al., 2008, 2009; Hernandez and Hoebel, 1988; Martel and Fantino, 1996; Noel and Wise, 1995), drinking (Yoshida et al., 1992), and social bonding (Young et al., 2001; Young and Wang, 2004). The mesolimbic system is comprised of dopaminergic projection neurons in the ventral tegmental area (VTA) that innervate the nucleus accumbens (NAc), amygdala, and medial prefrontal cortex (mPFC) (Kelley, 2004a). In male rats, the mesolimbic system is activated via release of endogenous opioids (EOP) in the ventral tegmental area (VTA) where they inhibit GABAergic interneurons, consequently causing the disinhibition of dopaminergic neurons and subsequent dopamine efflux in the nucleus accumbens (NAc) (Balfour et al., 2004). The NAc encompasses the ventral portion of the striatum and is primarily composed of GABAergic medium spiny neurons (90-95%) (Meredith, 1999) which project to the ventral pallidum and VTA; the other cells being GABAergic and cholinergic interneurons. The NAc is the primary target of mesolimbic dopamine release and is believed to integrate glutamate, GABA, opioid, and other neurotransmitters (including serotonin and substance P), for which the medium spiny neurons have been shown to express receptors (Berridge, 2007; Choe et al., 2006; Kwok et al., 1997;

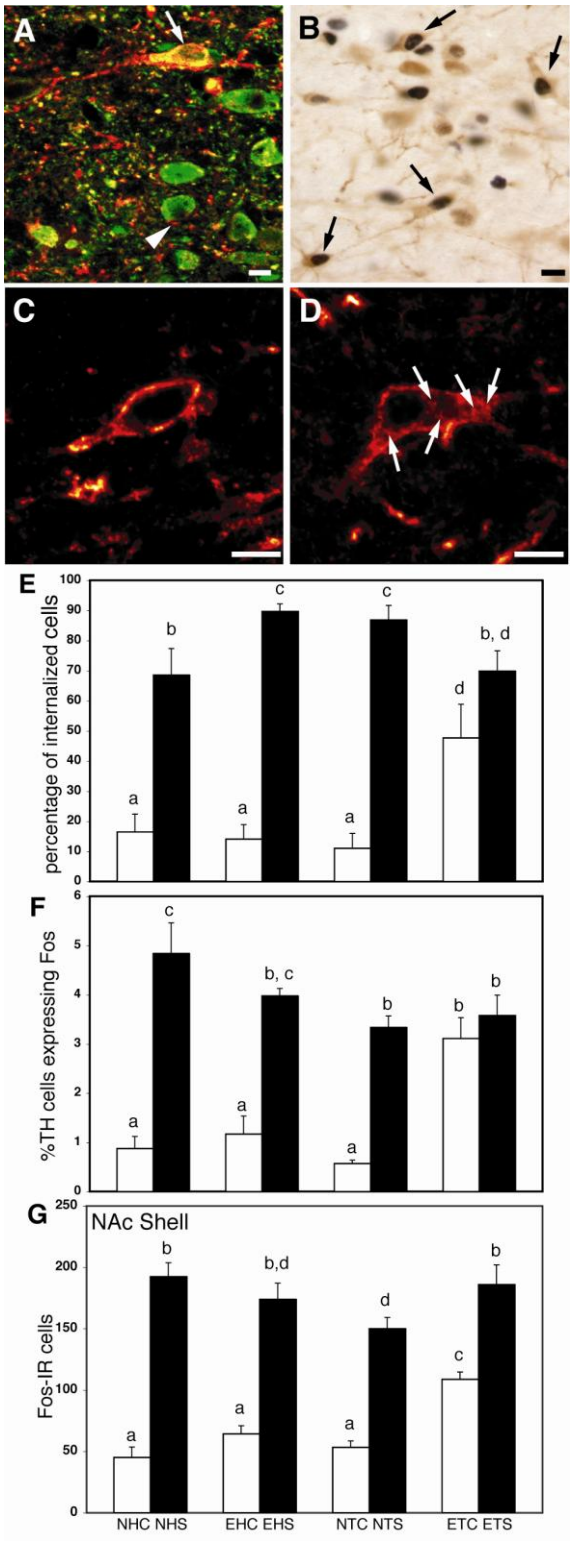
Mansour et al., 1995; Martin et al., 1993). Specifically, dopamine signaling is hypothesized to modulate synaptic plasticity in the NAc by affecting the response to glutamate released primarily from the mPFC (Kelley, 2004b; Mangiavacchi and Wolf, 2004). This integration of both excitatory (glutamate) and inhibitory (DA) signals is believed to play a role in attaching valence to stimuli, and regulating mood and goal-mediated behavior (Kelley, 2004b; Meredith, 1999).

Ventral tegmental area

The VTA has been implicated in sexual motivation or the anticipation of sexual reward. Bilateral intra-VTA morphine injections facilitate sexual motivation in castrated male rats (Mitchell and Stewart, 1990). Naloxone, an opioid receptor antagonist, infused into the VTA inhibits dopamine release into the NAc and blocks anticipatory sexual behavior (van Furth and van Ree, 1996) and the formation of a conditioned place preference for sexual reward (Agmo and Berenfeld, 1990), whereas the infusion of morphine facilitates behavior (Mitchell and Stewart, 1990). Hence, opiates act in the VTA to facilitate sexual motivation. The VTA is a mesencephalic brain region primarily composed of GABAergic interneurons and DA projection neurons, located adjacent to a functionally separate population of DA neurons in the substantia nigra. VTA dopaminergic neurons are under tonic inhibition by local GABAergic interneurons. Stimulation of the $G_{i/o}$-coupled mu-opioid receptor (MOR) results in the inhibition of these GABAergic neurons, which in turn leads to disinhibition of dopaminergic projection neurons and the release of DA into NAc (Ikemoto et al., 1997; Johnson and North, 1992; Klitenick et al., 1992; Matthews and German, 1984). MORs are expressed postsynaptically on both GABAergic cell

bodies (Balfour et al., 2004) (Figure 1.1) and presynaptically in enkephalin-containing axon terminals in close proximity to GABA neurons (Garzon and Pickel, 2001). MORs are activated by endogenous ligands in male rats during sexual behavior or during anticipation of sexual behavior following exposure to conditioned contextual cues that predict mating (Balfour et al., 2004) (Figure 1.1). MOR activation in these studies was determined by visualization of receptor endocytosis, a powerful marker for endogenous ligand-induced activation of G-protein coupled receptors, including MOR (Coolen et al., 2004b; Sinchak and Micevych, 2003). Similar to, and presumably as a consequence of the activation of MOR, VTA DA neurons are activated (using c-Fos expression as marker of neural activation) during sexual behavior, or following exposure to predictive cues in male rats (Balfour et al., 2004) (Figure 1.1). In male birds, VTA DA neurons are involved in production of song specifically for courtship (Goodson et al., 2009; Heimovics and Riters, 2008; Huang and Hessler, 2008). The VTA has also been shown to play role in female sexual behavior as progesterone and its metabolite 3α,5α–THP act in this brain area via D1 receptors to activate lordosis (Frye, 2007) and VTA neurons are activated during female sexual behavior (Coria-Avila and Pfaus, 2007). Moreover, estradiol affects VTA DA neurons as well as basal and stimulated release of DA in the striatum (Reviewed by Becker, 2009).

Figure 1.1 VTA mu-opioid receptor (MOR)-containing neurons and dopamine neurons are activated by mating behavior as well as exposure to predictive cues that were learned to be associated with sexual behavior in male rats. Confocal images (A) illustrating MOR (red) located on GABA-IR (green) cell bodies (arrow) and MOR-IR fibers in close apposition to GABA-IR cells (triangle) in the VTA. Photomicrograph (B) illustrating mating-induced Fos-IR (black) and tyrosine hydroxylase-IR (brown) in the VTA. Double-labeled cells are indicated by arrows. Confocal images (C and D) illustrating MOR-IR in the VTA of a non-mated control animal (C) or following sexual behavior (D). Arrows indicate MOR-IR endosome like particles. Scale bars indicate 10 μm. E-G illustrate quantitative data showing activation of VTA MOR, VTA dopamine, and NAc shell neurons in eight experimental groups. Animals were either exposed to the home environment, lacking predictive cues (naïve home cage (NH) and experienced home cage (EH)), exposed to the environmental cues predictive of sexual behavior (experienced test cage (ET)), or environmental cues that were unpaired and not predictive of sexual behavior (NT). Activation was examined following sexual behavior (S) or in unmated controls (C). Mating (NHS, EHS, NTS, ETS) as well as exposure to contextual cues that are associated with sexual behavior (ETC) induce internalization of VTA MOR neurons (E), induce Fos in VTA TH-immunoreactive cells (F), and Fos in NAc shell neurons (G). Data are presented as mean ± SEM. Solid bars represent groups that mated on the test day, and open bars represent control groups that did not mate on the test day. The statistical relationship between the groups is indicated by lowercase letters; groups that share a common letter do not differ significantly. Modified from Balfour et al. (2004).


A
B
C
D
E
percentage of internalized cells
F
%TH cells expressing Fos
G
NAc Shell
Fos-IR cells
NHC NHS
EHC EHS
NTC NTS
ETC ETS

Few studies have investigated the effect of VTA lesions on female sexual behavior, whereas there is no known literature concerning the effects of VTA lesions on male rodent sexual behavior. Non-specific lesions of the VTA decrease sexual behavior in female rats (Herndon, 1976). In contrast, 6-OHDA lesions show no effect on female rat sexual behavior (Hansen et al., 1991; Nance, 1983). More recently, it has been shown that 6-OHDA lesions in the VTA increase the lordosis quotient and total lordosis duration in female rats and hamsters respectively (Frye et al., 2009). Concomitant effects on motor behavior with 6-OHDA lesions in the VTA and its projection sites have opposite effects in rats and hamsters, despite similar lesion effects on female sexual behavior (Frye et al., 2009).

Nucleus accumbens

Microdialysis studies in male rats have shown that extracellular levels of DA in the NAc are escalated and maintained at an elevated level upon introduction of a receptive female, throughout mating, and return to baseline shortly after termination of copulation (Damsma et al., 1992; Fiorino and Phillips, 1999b; Pfaus et al., 1990a, 1995; Wenkstern et al., 1993). By contrast, DA and its metabolites do not increase significantly in male rats exposed to non-receptive females (Wenkstern et al., 1993). Interestingly, sexually experienced animals that failed to mate also showed no DA release into the accumbens (Pleim et al., 1990; Wang et al., 1995) suggesting a prerequisite for DA in engaging copulatory acts. The female-induced elevation in extracellular dopamine levels are low or absent as a male continues to copulate to satiety with the same female, but upon introduction of a novel female, the DA efflux is renewed, as is copulation (Phillips et al.,

2008). Sexual behavior by female hamsters and rats shows similar elevations in extracellular DA levels during mating (Meisel et al., 1993; Pfaus et al., 1995). Notably there is an effect of sexual history on mating-induced elevation of NAc DA levels. Sexually experienced female hamsters displayed an exaggerated elevation of DA levels, which persisted throughout mating compared to sexually naïve females and females with only three prior mating sessions (Kohlert and Meisel, 1999; Meisel et al., 1993). The escalated extracellular DA in sexually experienced female hamsters is in line with a similar response following chronic administration of drugs of abuse (Robinson and Berridge, 2003). Moreover, NAc DA levels in female rats only increase when the females receive copulatory stimulation at a preferred rate (Becker et al., 2001; Jenkins and Becker, 2001; Mermelstein and Becker, 1995). These accumbens dialysis studies support the notion that extracellular DA levels may reflect the motivational state of the female.

Limited studies have investigated the effect of NAc lesions on sexual behavior. Lesions of the NAc in female rats increase the rejection of male rats and increase the length of post-ejaculatory interval without affecting copulatory behavior of the male sexual partners (Rivas and Mir, 1990, 1991). In male rats, excitotoxic lesions of the NAc suggest that this brain area plays a role in mediating sexual arousal. Specifically, NAc lesioned male rats failed to intromit or rarely copulated to ejaculation, as well as displayed impaired noncontact erections when exposed to an inaccessible receptive female. However, when simultaneously exposed to inaccessible receptive and non-receptive females, these males spent more time in the proximity of receptive females compared to non-receptive females (Kippin et al., 2004). 6-OHDA lesions in the NAc

cause an increase in lordosis in both female hamsters and rats (Frye et al., 2009). The effects of NAc lesions on human sexual behavior are relatively unknown. One human male case reported a loss of interest in pleasure and sex (Goldenberg et al., 2002). There have been a number of studies locally infusing DA pharmacological agents into the NAc to observe effects on sexual behavior. The DA agonist apomorphine was found to induce a small decrease in intromission latency (Hull et al., 1990). To a greater extent Amph significantly decreased mount and intromission latencies (Everitt, 1990). In contrast with systemic administration, intra-accumbens DA antagonists (haloperidol and raclopride) had no effect on behavior latencies (Everitt, 1990). Moses et al. (1995) bilaterally infused a number of DA agonists and antagonists into the accumbens with little to no effect of copulatory behavior; findings that are supported by additional dopamine antagonist studies (Ahlenius and Larsson, 1990; Everitt, 1990; Pfaus and Phillips, 1991). It has become increasingly clear that DA in the NAc is not critical for the consummatory aspect of male copulatory behavior (Reviewed by Paredes and Agmo, 2004). However, a number of studies have looked at the anticipatory phase of male sexual activity using bilevel chambers (Mendelson and Gorzalka, 1987). Using this paradigm, DA (D1 and D2) antagonists have been found to significantly decrease the number of level changing (Pfaus and Phillips, 1991) indicating a facilitatory role for DA in the anticipation for sexual behavior. In support, Fos expression is induced in the NAc of sexually experienced males by exposure to conditioned contextual cues that the males learned to associate with sexual behavior (Balfour et al., 2004). Hence, NAc neurons are activated in anticipation of sexual behavior (Figure 1.1).

The role of DA for female sexual behavior is not completely clear with DA agonists and antagonists administered systemically having both inhibitory and stimulatory influence on sexual receptivity (Reviewed by Melis and Argiolas, 1995). However, DA receptor antagonists block conditioned preference for an environment associated with mating, albeit only if mating actually occurred in that environment (Meisel et al., 1996) and not when conditioned place preference was induced by mating that occurred in a separate chamber (Paredes and Martinez, 2001). In summary, there is an abundance of evidence using pharmacological agents suggesting DA in the NAc plays a limited role in copulation, yet may be critical for sexual motivation.

Medial prefrontal cortex

The prefrontal cortex is divided into three regions: dorsolateral, medial and orbitofrontal (Spinella, 2007). The medial prefrontal cortex (mPFC) can be further broken down into anterior cingulate, prelimbic, infralimbic, and precentral regions (Heidbreder and Groenewegen, 2003). Collectively, these regions and associated subcortical structures mediate motivational aspects of behavior and may play a role in sexual arousal (Sewards and Sewards, 2003). People with lesions to the anterior cingulate region of mPFC show a reduction in initiation and motivated behaviors, including sex (Devinsky et al., 1995; Mega and Cohenour, 1997; Nemeth et al., 1988). Moreover, the mPFC has been found to play a critical role in reward and punishment (Tzschentke, 2000). Human neuroimaging studies show that secondary reinforcers activate the mPFC (Gehring and Willoughby, 2002; Knutson et al., 2000). Using anterograde tracer biotinylated dextran amine injected into mPFC, many brain areas have been identified to be innervated by the mPFC (i.e.,

nucleus accumbens) (Balfour et al., 2006; Gorelova and Yang, 1997). A significant finding from Balfour et al. (2006) was that the majority of the sex-activated Fos-positive cells in the VTA receive putative contacts from the mPFC. Anterior cingulate activity increases in estrous (but not anestrous) female sheep when exposed to males (Ohkura et al., 1997).

As discussed above, in the presence of a female before initiation of contact, male rats experience a DA efflux in the NAc. There is similar increase in extracellular DA release in the mPFC in the presence of a potential mate (Fiorino and Phillips, 1999b). Further supporting its role in the initiation of behavior, the mPFC is involved in the anticipation and execution of a T maze task in sexually motivated males (Hernandez-Gonzalez et al., 2007). In male rats, mPFC lesions have been found to increase mount and intromission latencies 1 week after operation, an effect which dissipated in time (Agmo et al., 1995). Similar impairments to sexual behavior were partially reversed with the central infusion of dopaminergic stimulants (Agmo and Villalpando, 1995). Recent studies conducted by (Davis et al., 2010) reported that mPFC lesions had no effect on sexual behavior, yet show that mPFC lesions block the animal's ability to inhibit sexual behavior in a copulation-contingent aversion paradigm. This effect seems to be independent of a learning impairment because lesion animals are capable of forming a conditioned place preference for sex and a conditioned place aversion for LiCl. These studies support a role for the mPFC in the initiation, persistence and compulsive seeking of sexual reward.

1.5.2 Neural activation by sex and drugs in the mesolimbic system

As discussed above, there is a large body of evidence that drugs of abuse and sexual behavior converge on the mesolimbic DA system (Berhow et al., 1996; Pierce and Kalivas, 1997). Further evidence that drugs and sex are both acting in this system was provided by an examination of neural activation induced by sex behavior or drugs of abuse, using neuronal activity markers such as Fos and phosphorylation of MAP Kinase (pERK) (Balfour et al., 2004; Robertson et al., 1991; Valjent et al., 2000, 2004, 2005). However, it is unclear if sex and drugs are activating the same neurons in the mesolimbic system. Therefore, this question will be addressed in Chapter 2 of my thesis.

1.5.3 Drug-induced neuroplasticity in the mesolimbic system

The mesolimbic DA circuit exists to mediate natural behaviors, yet it appears that repeated drug administration exerts its harmful effects by accroaching the in-place reward system (Hyman et al., 2006). A number of alterations at the levels of the gene, synapse, morphology, and behavior have been identified following chronic drug exposure (Hyman et al., 2006; Kalivas et al., 2009). These changes are hypothesized to underlie the development and maintenance of drug addiction and increase the susceptibility to drug relapse. The effect of repeated drug administration has been shown in many accounts to have an effect on subsequent drug exposure (Grimm et al., 2001). The behavioral effects of repeated drug administration include a sensitized locomotor response to psychostimulants and opiates (Kalivas and Stewart, 1991; Segal and Mandell, 1974), an enhanced drug-related reward value (Lett, 1989; Shippenberg and Heidbreder, 1995; Shippenberg et al., 1996), and increased operant responses for cues associated with prior

drug intake (Reti et al., 2008). Moreover, there are several examples of cross sensitization between families of drugs both with respect to a sensitized locomotor response (Bonate et al., 1997; Cunningham and Kelley, 1992; Leri et al., 2003; Pierce and Kalivas, 1997) and enhanced drug reward (Lett, 1989). In addition, repeated drug administration results in long-lasting changes in dendritic spine density and morphology in the nucleus accumbens (Brown and Kolb, 2001; Li et al., 2003; Robinson et al., 2002; Robinson and Kolb, 2004), prefrontal cortex (Li et al., 2003; Robinson et al., 2002; Robinson and Kolb, 1997, 1999, 2004), and ventral tegmental area (Sarti et al., 2007), and induces alterations in gene expression (Bowers et al., 2004; Lu et al., 2005; McClung and Nestler, 2003, 2008). Finally, repeated drug administration alters synaptic strength at excitatory and inhibitory synapses on midbrain dopamine neurons (Kauer, 2003; Liu et al., 2005; Nugent et al., 2007, 2008; Saal et al., 2003) and neurons in the NAc (Thomas and Malenka, 2003; Thomas et al., 2000; Tzschentke, 2001).

Until recently, it was generally hypothesized that non-compulsive natural behaviors did not induce neuroplasticity (Chen et al., 2008; Kalivas et al., 2009), suggesting that the changes being observed throughout the mesolimbic system were specific for compulsive behavior (i.e., addiction), thus not related to reinforcement in general. Yet, it is becoming increasingly clear that neuroplasticity occurs in the mesolimbic system in response to natural behaviors and not necessarily linked to compulsive seeking of reinforcers.

1.5.4 Effects of experience on the locomotor-inducing effects of amphetamine

Recent studies from our laboratory (Pitchers et al., 2010) have demonstrated that sexual experience in male rats induces a robust sensitized locomotor response to a challenge dose of Amph (0.5 mg/kg; s.c.). This effect was observed as early as 1 day and up to 28 days after final mating session, hence cross-sensitization was long-lasting and did not require a long withdrawal period (Figure 1.2A-C). Additional experiments were conducted to determine the role of the environment in which sexual experience was obtained (same or different than drug administration) and mating paradigm (consecutive or intermittent) was demonstrated that behavioral sensitization induced by sexual experience is independent of context and mating paradigm. Moreover, ejaculation was shown to be critical for behavioral sensitization. Thus, sexual experience caused long-lasting alterations to the locomotor responses induced by Amph, supporting the hypothesis that sexual experience caused neural adaptations in the mesolimbic system.

A behavioral sensitization study has also been conducted in female hamsters (Bradley and Meisel, 2001). Behavioral sensitization was tested 1 week after gaining sexual experience. Females were placed in the behavioral apparatus for 10 minutes to record baseline activity. After 10 minutes, the female hamsters were taken out, injected with a low-dose Amph challenge (1.0 mg/kg) and then placed back in the apparatus. Both sexually experienced and naive hamsters receiving Amph displayed a significant increase in number of crossovers in comparison to the initial 10 minutes before receiving drug dose. However, sexually experienced animals showed increased locomotor response at a

significantly shorter time interval after injection (20 minutes) compared to naïve (30 minutes).

1.5.5 Effects of sexual experience on drug reward

To date, there has been little work conducted testing the effect of sexual experience on drug reward. A study recently conducted in our laboratory composed drug dose-reward curves in sexually experienced and naive animals, utilizing the CPP paradigm (Pitchers et al., 2010). Indeed, sexually experience affected drug-induced CPP, such that sexually experienced males formed a significant CPP for lower doses (0.5 and 1.0 mg/kg) of Amph, that did not induce CPP in sexually naïve animals (Figure 1.2D). These results indicated an enhanced or sensitized amphetamine reward value. Interestingly, this enhancement of drug reward appeared dependent on a period of abstinence from sexual behavior, as no difference existed between sexually experience and naive animals when tested for Amph CPP 1 day after the last mating session, and effects of sexual experience were only evident in animals tested 10 days following last mating experience. Together, these studies support the notion that repeated sexual behavior (sexual experience) in males and females causes alterations in the mesolimbic system that in turn alter the responses to Amph, and increase Amph-reward. The latter has only been shown in males and appears dependent not only on sexual experience but also on subsequent removal of sexual reward.

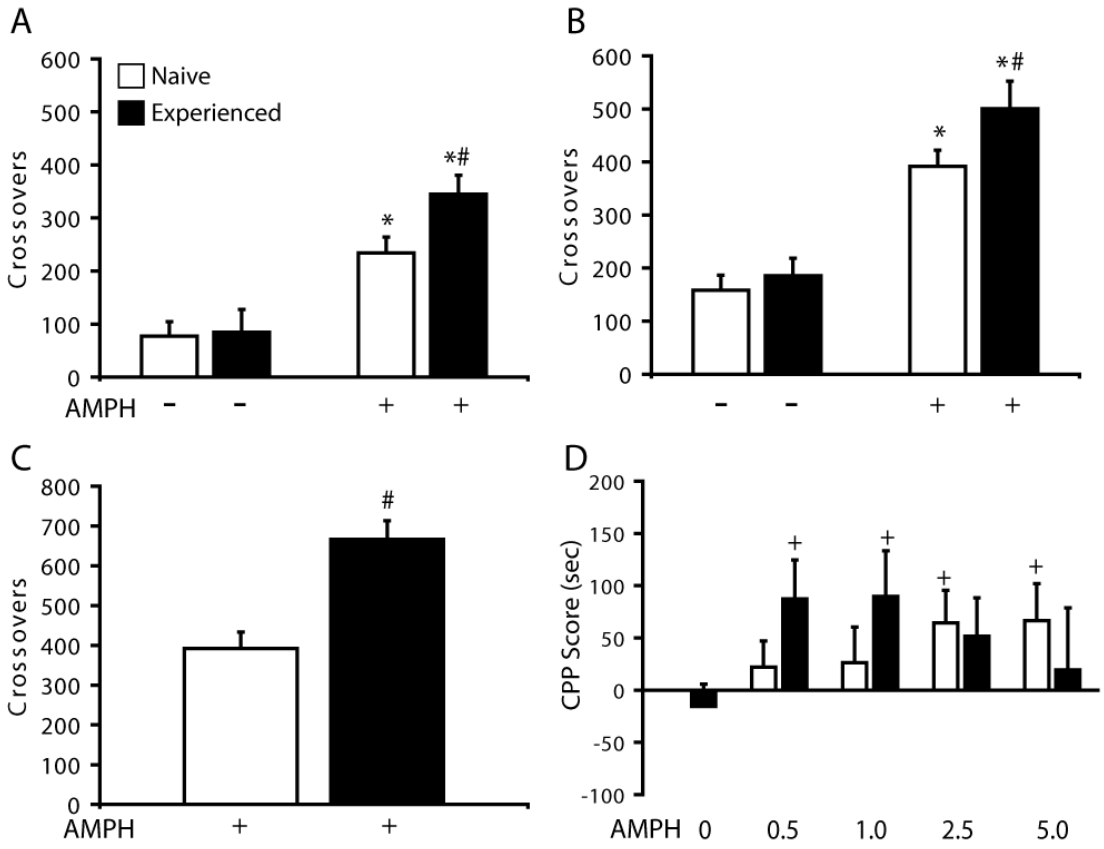


Figure 1.2 Sexually experienced male rats show an immediate and long-lasting sensitized locomotor response to amphetamine in addition to an enhanced amphetamine conditioned reward following a period of sex abstinence. Locomotor response of sexually experienced and naïve animals to saline or amphetamine administered 1 day (A), 1 week (B) or 1 month (C) following the last mating session. Mean ± SEM of total number of crossovers over 31-60 minutes after injection. Amphetamine-induced conditioned place preference of sexually experienced and naïve male rats (D). Mean ± SEM of CPP score, defined as the time spent in the AMPH-paired chamber in the posttest minus the pretest (seconds). * indicate significant differences between AMPH and saline. # indicate significant differences between experience and naïve groups. + indicate significant differences from males receiving saline. Modified from Pitchers et al. (2010).

1.6 SUMMARY

In summary, we discussed the effects of commonly abused drugs including psychostimulants, opiates, marijuana/THC, and alcohol on male and female sexual behavior in humans and animal studies. In general, drug use affects sexual motivation, arousal, and performance, and is commonly associated with increased sexual risk behaviors. We identified a disconnect between human and animal studies, as the latter generally do not investigate effects of drugs on sexual risk behaviors, many do not examine the effects of chronic drug exposure on sexual behavior, and fail to incorporate sexual experience as a factor that influences drug responsiveness. Currently, there are no standardized paradigms to investigate risk taking related to sexual behavior in animal models. Moreover, few studies have investigated the effects of drugs of abuse on sexual behavior in animals that are chronically exposed to the drug, which would allow better translation to human studies. Finally, sexual history is another factor that is not currently taken into account in drug addiction research. Here, we discuss recent studies that in addition to drugs affecting sexual behavior, sexual experience in turn can affect drug responsiveness. Therefore, future studies should attempt to further examine sexual-risk behaviors in animal models of drug abuse, the effects of chronic drug administration on sexual behavior, and the effects of sexual experience on drug responsiveness.

1.7 THESIS RATIONALE AND OBJECTIVES

1.7.1 Rationale

As mentioned above, Meth is a highly addictive psychostimulant and is commonly associated with the practice of sexual risk behavior and increased prevalence of Human Immunodeficiency Virus. Specifically, Meth users report heightened sexual desire, arousal, and sexual pleasure and report having unprotected sex more often than other drug users (Rawson et al., 2002; Schilder et al., 2005; Semple et al., 2002; Somlai et al., 2003; Springer et al., 2007). Currently, the neurobiological basis for this drug-sex association is unknown. Unfortunately, human reports demonstrating the relationship between Meth use and sexual behavior are based on self-report and lack a reliable instrument to measure the effects of Meth on sexual function. Furthermore, studies using animal models to investigate the effects of acute or chronic administration of Meth on sexual behavior are limited. More importantly, there are no studies investigating the effects of Meth on compulsive sexual behavior. In line with this, an investigation into the cellular basis of Meth-induced alterations as well as changes in sex behavior itself using an animal model and under controlled experimental settings is required for understanding the complex connection between Meth use and sexual behavior.

The animal model utilized in this thesis is the male rat. Male rat sexual behavior is highly rewarding and reinforcing (Pfaus et al., 2001). Male rats display conditioned place preference (CPP) for copulation (Agmo and Berenfeld, 1990; Martinez and Paredes, 2001; Tenk, 2009) and perform tasks to gain access to a sexually receptive female

(Everitt et al., 1987; Everitt and Stacey, 1987). Drugs of abuse are also rewarding and reinforcing in this animal model. Male rats also form CPP for and self-administer substances of abuse (Feltenstein and See, 2008; Pierce and Kumaresan, 2006; Wise, 1996). Moreover, it is well known that both naturally rewarding behaviors such as sexual behavior (Balfour et al., 2004; Fiorino et al., 1997) as well substances of abuse (Chang et al., 1997; Di Chiara and Imperato, 1988; Ranaldi et al., 1999) are mediated by the same neural circuits, the limbic system. The limbic system is an interconnected network of the brain areas including the ventral tegmental area, nucleus accumbens, prefrontal cortex, and amygdala, and is responsible for mediating motivation and reward in general (Kalivas and Volkow, 2005; Kelley, 2004a).

1.7.2 Hypothesis

Meth acts on the same neurons that mediate sexual behavior in limbic brain structures and results in altered sexual behavior including, decreased performance, increased motivation and reward, and maladaptive sex-seeking behavior.

1.7.3 Objectives

1. Investigate concurrent neural activation of the limbic circuitry by sexual behavior and Meth administration using immunohistochemical visualization of the immediate early genes Fos and phosphorylated Map Kinase (pERK), respectively.

2. Test the effects of acute Meth on sexual motivation, performance, investigative behavior, and compulsive sexual behavior.
3. Examine the effects of repeated Meth on sexual motivation and performance, compulsive sexual behavior, and sex and/or Meth reward.
4. Investigate the neural substrates involved in compulsive sex-seeking behavior.

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CHAPTER 2:

Methamphetamine acts on subpopulations of neurons regulating sexual behavior in male rats[1]

[1] Research article published in Neuroscience (2010) 166,771-784

2.1 INTRODUCTION

Motivation and reward are regulated by the mesolimbic system, an interconnected network of the brain areas comprised by the ventral tegmental area (VTA) nucleus accumbens (NAc), basolateral amygdala, and medial prefrontal cortex (mPFC) (Kalivas and Volkow, 2005; Kelley, 2004). There is ample evidence that the mesolimbic system is activated in response to both substances of abuse (Chang et al., 1997; Di Chiara and Imperato, 1988; Ranaldi et al., 1999) and to naturally rewarding behaviors such as sexual behavior (Balfour et al., 2004; Fiorino et al., 1997). Male sexual behavior, and in particular ejaculation, is highly rewarding and reinforcing in animals models (Pfaus et al., 2001). Male rodents develop a conditioned place preference (CPP) to copulation (Agmo and Berenfeld, 1990; Martinez and Paredes, 2001; Tenk, 2009), and will perform operant responses to gain access to a sexually receptive female (Everitt et al., 1987; Everitt and Stacey, 1987). Drugs of abuse are also rewarding and reinforcing, and animals will learn to self-administer substances of abuse, including opiates, nicotine, alcohol, and psychostimulants (Feltenstein and See, 2008; Pierce and Kumaresan, 2006; Wise, 1996). Although it is known that both drugs of abuse and sexual behavior activate mesolimbic brain areas, it is currently unclear whether drugs of abuse influence the same neurons that mediate sexual behavior.

Electrophysiological studies have demonstrated that food and cocaine both activate neurons in the NAc. However, the two reinforcers do not activate the same cells within the NAc (Carelli et al., 2000; Carelli and Wondolowski, 2003). Moreover, food and sucrose self-administration do not cause long term alterations of electrophysiological

properties as are induced by cocaine (Chen et al., 2008). In contrast, a collection of evidence suggests that male sexual behavior and drugs of abuse might indeed act on the same mesolimbic neurons. Psychostimulants and opioids alter the expression of sexual behavior in male rats (Fiorino and Phillips, 1999a,b; Mitchell and Stewart, 1990). Recent data from our lab showed that sexual experience alters the responsiveness to psychostimulants as evidenced by a sensitized locomotor responses and sensitized reward perception to D-amphetamine in sexually experienced animals (Pitchers et al., 2010). A similar response has previously been observed with repeated exposure to amphetamine or other drugs of abuse (Lett, 1989; Shippenberg and Heidbreder, 1995; Shippenberg et al., 1996; Vanderschuren and Kalivas, 2000). Together, these findings suggest that sexual behavior and responses to drugs of abuse are mediated by the same neurons in the mesolimbic system. Hence, the first objective of the present study is to investigate neural activation of the mesolimbic system by sexual behavior and drug administration in the same animal. In particular, we tested the hypothesis that the psychostimulant, methamphetamine (Meth), acts directly on neurons that normally mediate sexual behavior.

Meth is one of the most abused illicit drugs in the world (Ellkashef et al., 2008; NIDA, 2006) and it has been frequently linked to altered sexual behavior. Interestingly, Meth users report heightened sexual desire and arousal, as well as enhanced sexual pleasure (Schilder et al., 2005; Semple et al., 2002). Moreover, Meth abuse is commonly associated with sexually compulsive behavior (Rawson et al., 2002). Users often report having numerous sexual partners and are less likely to use protection than other drug

abusers (Somlai et al., 2003; Springer et al., 2007). Unfortunately, studies indicating Meth use as a predictor of sexual risk behavior are limited as they rely on unconfirmed self-reports (Elifson et al., 2006). Therefore, an investigation into the cellular basis of Meth-induced changes in sexual behavior in an animal model is required for understanding this complex drug-sex nexus.

In view of the above outlined evidence suggesting that drugs of abuse, and particularly Meth, may act upon neurons normally involved in mediating sexual behavior, the objective of the present study was to investigate neural activation by sexual behavior and administration of the psychostimulant Meth. This study implemented a neuroanatomical technique, utilizing immunohistochemical visualization of the immediate early genes Fos and phosphorylated MAP Kinase (pERK) to detect concurrent neural activation by sexual behavior and Meth respectively. Fos is only expressed within the nucleus of cells, with a maximal expression level 30-90 minutes after activation of the neuron. There is ample evidence that sexual activity induces Fos expression in the brain (Pfaus and Heeb, 1997; Veening and Coolen, 1998), including the mesocorticolimbic system (Balfour et al., 2004; Robertson et al., 1991). There is also evidence that drugs of abuse induce pERK expression within the mesocorticolimbic system (Valjent et al., 2000, 2004, 2005). In contrast to the expression of Fos, phosphorylation of ERK is a highly dynamic process and only occurs 5-20 minutes after neuronal activation. The distinct temporal profiles of Fos and pERK makes them an ideal set of markers for subsequent neuronal activation by two different stimuli.

2.2 MATERIALS AND METHODS

2.2.1 Subjects

Adult male Sprague-Dawley rats (210-225 g) obtained from Charles River Laboratories (Montreal, QC, Canada) were housed two per cage in standard Plexiglas cages (home cages). The animal room was maintained at a 12/12 h reversed light cycle (lights off at 10.00 h). Food and water were available *ad libitum.* All testing was performed during the first half of the dark phase under dim red illumination. Stimulus females used for sexual behavior were bilaterally ovariectomized under deep anaesthesia (87 mg/kg ketamine and 13 mg/kg xylazine) and received a subcutaneous implant containing 5% estradiol benzoate (EB) and 95% cholesterol. Sexual receptivity was induced by subcutaneous (s.c.) administration of 500 μg progesterone in 0.1 ml sesame oil 4 h prior to testing. All procedures were approved by the Animal Care Committee at the University of Western Ontario and conform to the guidelines outlined by the Canadian Council on Animal Care.

2.2.2 Experimental designs

2.2.2A Experiments 1 and 2

Male rats (n=37) were allowed to mate with a receptive female to one ejaculation (E) or for 30 min, which ever came first in clean test cages (60 x 45 x 50 cm) during five twice-weekly pretest mating sessions, to gain sexual experience. During the latter two sessions, all standard parameters for sexual performance were recorded, including: mount latency (ML; time from introduction of the female until the first mount), intromission latency (IL; time from introduction of the female until the first mount with vaginal penetration),

ejaculation latency (EL; time from the first intromission to ejaculation), post-ejaculatory interval (PEI; time from ejaculation to first subsequent intromission), number of mounts (M), and number of intromissions (IM) (Agmo, 1997). All males received 1 ml/kg daily injection of 0.9% NaCl (saline; s.c.) 3 consecutive days prior to the test day, for habituation to handling and injections. One day before the test day, all males were single housed. In experienced males, Fos can be induced by conditioned contextual cues associated with prior sexual experience (Balfour et al, 2004). Therefore, all mating and control manipulations during the final tests were conducted in the home cage (devoid of predictive conditioned cues) to prevent conditioned-cue induced activation in the unmated control males. Males were distributed into eight experimental groups that did not differ in any measure of sexual performance during the last two mating sessions (data not shown). During the final test, males were either allowed to mate in their home cage until they displayed an ejaculation (sex) or did not receive female partner (no sex). All mated males were perfused 60 minutes following the onset of mating to allow for analysis of mating-induced Fos expression. Males received an injection of 4 mg/kg Meth or 1 ml/kg saline (s.c.) (n=4 each), either 10 (Experiment 1) or 15 (Experiment 2) min prior to perfusion, for analysis of drug-induced phosphorylation of MAP Kinase. Dosage and time before perfusion were based on previous reports (Chen and Chen, 2004; Choe et al., 2002; Choe and Wang, 2002; Ishikawa et al., 2006; Mizoguchi et al., 2004).Control groups included males that did not mate, but received Meth 10 (n=7) or 15 (n=5) min prior to sacrifice, or saline injections 10 (n=5) or 15 (n=4) min prior to sacrifice. Following sacrifice, brains were processed for immunohistochemistry.

2.2.2B Experiment 3

Since a high dose of Meth was used in experiment 1 and 2, an additional neuroanatomical experiment was performed to investigate if sexual behavior and a lower dose of Meth induce dose dependent patterns of overlapping neural activation. This study was carried out in an identical manner as experiments 1 and 2. However, on the final test, mated and unmated groups (n=6 each) received 1 mg/kg Meth (s.c.) 15 min prior to sacrifice.

2.2.2C Experiment 4

To test if neural activation caused by sex and Meth is specific for Meth, this experiment investigated whether similar patterns of overlapping neural activation could be seen with the psychostimulant D-amphetamine (Amph). This experiment was carried out in an identical manner as experiments 1 and 2. However, on the final test, males were administered either Amph (5 mg/kg) or saline (1 mg/kg) (s.c.) 15 min prior to sacrifice (n=5 each). Control unmated males received saline or Amph 15 minutes prior to sacrifice. Overview of the experimental groups utilized in experiments 1-4 is provided in Table 2.1.

2.2.3 Tissue preparation

Animals were anesthetized with pentobarbital (270 mg/kg; i.p., Bimeda-MTC, Animal Health Inc., Cambridge, ON, Canada) and perfused transcardially with 5 ml of saline followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed for 1 h at room temperature in the same fixative, then immersed in 20% sucrose and 0.01% Sodium Azide in 0.1 M PB and stored at 4°C. Coronal sections (35 μm) were cut on a freezing microtome (H400R, Micron, Germany),

Sexual Behavior	Treatment	Dose	Time point	Group size
Experiment 1				
No Sex	Saline	1 ml/kg	10	n=5
Sex	Saline	1 ml/kg	10	n=4
No Sex	Meth	4 mg/kg	10	n=7
Sex	Meth	4 mg/kg	10	n=4
Experiment 2				
No Sex	Saline	1 ml/kg	15	n=4
Sex	Saline	1 ml/kg	15	n=4
No Sex	Meth	4 mg/kg	15	n=5
Sex	Meth	4 mg/kg	15	n=4
Experiment 3				
No Sex	Meth	1 mg/kg	15	n=6
Sex	Meth	1 mg/kg	15	n=6
Experiment 4				
No Sex	Saline	1 ml/kg	15	n=5
Sex	Saline	1 ml/kg	15	n=5
No Sex	Amph	5 mg/kg	15	n=5
Sex	Amph	5 mg/kg	15	n=5

Table 2.1 Overview of experimental groups included in experiments 1-4. During final tests, experimental groups were based on mating behavior (No Sex or Sex), the dose and treatment administered (1 ml/kg saline, 1 or 4 mg/kg Meth, or 5 mg/kg Amph), and the time point of the treatment injection prior to perfusion (10 or 15 min). In addition, group sizes are included for each experiment.

collected in four parallel series in cryoprotectant solution (30% sucrose and 30% ethylene glycol in 0.1 M PB) and stored at 20°C until further processing.

2.2.3A Immunohistochemistry

All incubations were performed at room temperature with gentle agitation. Free floating sections were washed extensively with 0.1 M Phosphate-buffered saline (PBS) between incubations. Sections were incubated in 1% H_2O_2 for 10 min, then blocked in incubation solution (PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100) for 1 h.

pERK/Fos: Tissue was incubated overnight with a rabbit polyclonal antibody against p42 and p44 MAP Kinases ERK1 and ERK2 (pERK; 1:400 experiment 1 lot 19; 1:4.000 experiment 2 and 3 lot 21; Cell Signaling Technology, Danvers, MA, USA Cat # 9101), followed by a 1 h incubations with biotinylated donkey anti-rabbit IgG (1:500; Jackson Immunoresearch Laboratories, West Grove, PA, USA) and avidin-horseradish peroxidase complex (ABC Elite; 1:1000; Vector Laboratories, Burlingame, CA, USA). Then, the tissue was incubated for 10 min with biotinylated tyramide (BT; 1:250 in PBS + 0.003% H_2O_2; Tyramid Signal Amplification Kit, NEN Life Sciences, Boston, MA, USA) and for 30 min with Alexa 488 conjugated strepavidin (1:100; Jackson Immunoresearch Laboratories, West Grove, PA, USA). Next, tissue was incubated overnight with a rabbit polyclonal antibody against c-Fos (1:500; SC-52; Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by a 30 min incubation with goat anti-rabbit Alexa 555 (1:200; Jackson Immunoresearch Laboratories, West Grove, PA, USA). Following staining, the sections were washed thoroughly in 0.1 M PB, mounted onto glass slides with 0.3%

gelatin in ddH_20 and coverslipped with an aqueous mounting medium (Gelvatol) containing anti-fading agent 1,4-diazabicyclo(2,2)octane (DABCO; 50 mg/ml, Sigma-Aldrich, St. Louis, MO, USA). Immunohistochemical controls included omission of either or both primary antibodies, resulting in absence of labeling in the appropriate wavelength.

2.2.4 Data analysis

2.2.4B Sexual behavior

For all four experiments, standard parameters for sexual performance were recorded as described above and analyzed using analysis of variance (ANOVA). Data analysis of sexual behavior during the final test day revealed no significant differences between groups in any of the parameters of sexual performance.

2.2.4B pERK/Fos cell counts

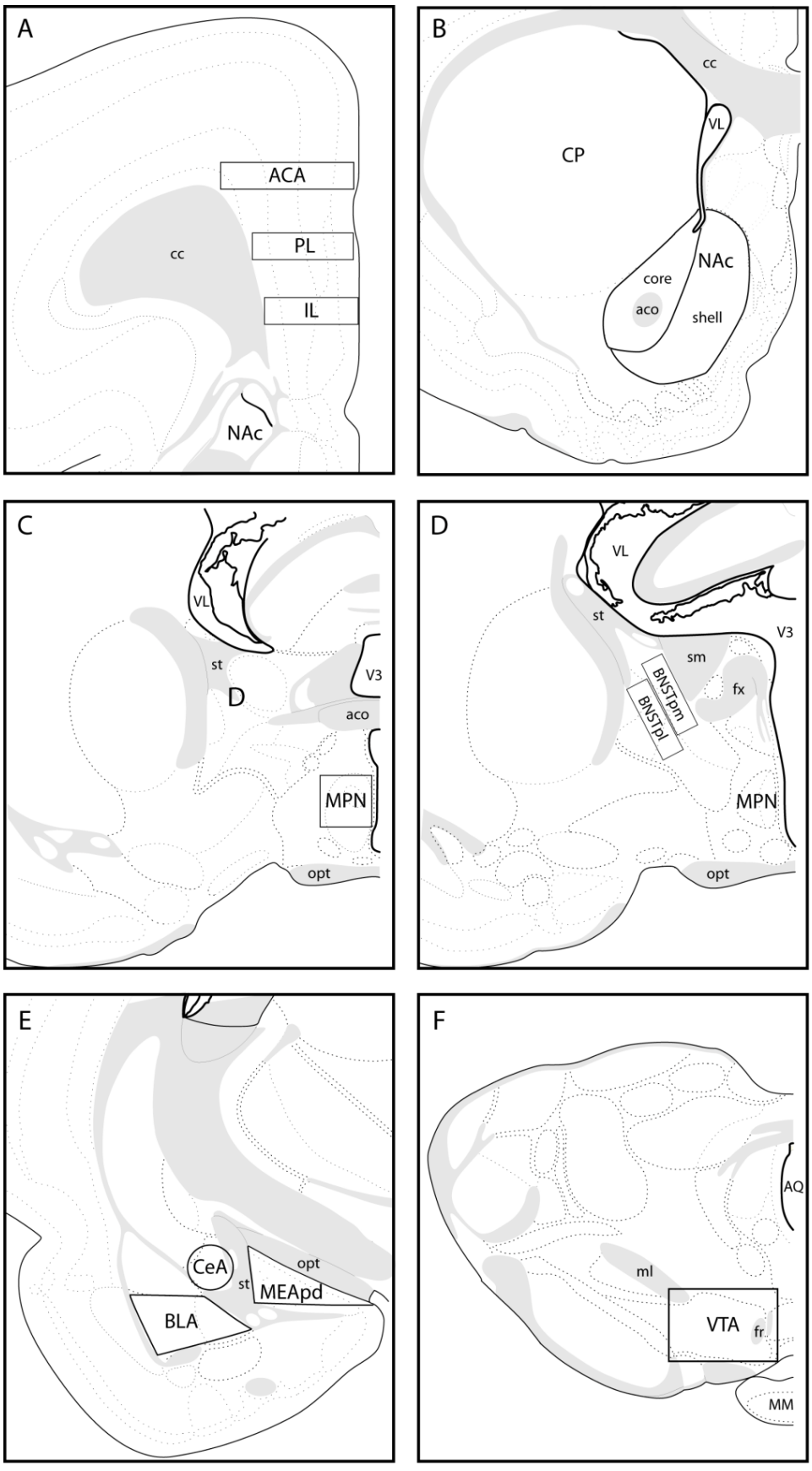
Single and dual labeled cells for Fos and pERK were counted in the caudal levels of NAc core and shell subregions, basolateral amygdala (BLA), posterodorsal medial amygdala (MEApd), central amygdala (CeA), medial preoptic nucleus (MPN), posteromedial and posterolateral bed nucleus of the stria terminalis (BNSTpm and BNSTpl), and the anterior cingulate area (ACA), prelimbic (PL), and infralimbic (IL) subregions of the mPFC. Images were captured using a cooled CCD camera (Microfire, Optronics, Goleta, CA, USA) attached to a Leica microscope (DM500B, Leica Microsystems, Wetzlar, Germany) and Neurolucida software (MicroBrightfield Bioscience, Williston, VT, USA) with fixed camera settings for all subjects (using 10X objectives). Using Neurolucida

software, areas of analysis were defined based on landmarks (Swanson, 1998) unique for each brain region (see Figure 2.1). Standard areas of analysis were used in all areas except NAc core and shell. In the latter areas, pERK and Fos expression was not homogeneous and appeared in patch-like patterns. Therefore, the entire core and shell were outlined based on landmarks (lateral ventricle, anterior commisure, and islands of Calleja). The areas of analysis did not differ between experimental groups, and were 1.3 mm^2 in the NAc core and shell. Standard areas of analysis for the remaining areas were: 1.6 mm^2 in the BLA, 2.5 and 2.25 mm^2 in the MEApd and CeA respectively, 1.0 mm^2 in the MPN, 1.25 mm^2 in the BNST and mPFC subregions, and 3.15 mm^2 in the VTA. Two sections were counted bilaterally for each brain region per animal, and number of single and dual labeled cells for pERK and Fos as well as the percentages of pERK cells that expressed Fos marker were calculated. For experiments 1, 2, and 4, group averages were compared using two way ANOVA (factors: *mating and drug*) and Fisher's LSD for *post hoc* comparisons at a significance level of 0.05. For Experiment 3, group averages were compared using unpaired t-tests at a significance level of 0.05.

2.2.5 Images

Digital images for Figure 2.3 were captured using CCD camera (DFC 340FX, Leica, Wetzlar, Germany) attached to a Leica microscope (DM500B) and were imported into Adobe Photoshop 9.0 software (Adobe Systems, San Jose, CA, USA). Images were not altered in any way except for adjustment of brightness.

Figure 2.1 Schematic drawings and images illustrating brain areas of analysis. Areas of analysis indicated were based on landmarks unique for each brain region, did not differ between experimental groups, and were 1.25 mm^2 in mPFC subregions (A), 1.3 mm^2 in the NAc core and shell (B), 1.0 mm^2 in the MPN (C), 1.25 mm^2 in the BNST subregions (D), 1.6, 2.25, and 2.5 mm^2 in the BLA, CeA, and MEApd respectively (E), and 3.15 mm^2 in the VTA (F). Abbreviations: aco, anterior commisure; AQ, cerebral aqueduct; cc, corpus callosum; CP, caudate putamen; fr, fasciculus retroflexus; fx, fornix; ml, medial lemniscus; MM, medial mammillary nucleus; opt, optic tract; V3, third ventricle; sm, stria medullaris; st, stria terminalis; VL, lateral ventricle. Brain drawings were modified from Swanson (1998).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2.3 RESULTS

2.3.1 Neural activation of the limbic system by sexual behavior and Meth administration

2.3.1A Experiment 1

Analysis of single and dual labeled cells for mating-induced Fos and Meth-induced pERK in males that received Meth 10 minutes prior to sacrifice revealed mating-induced Fos in the MPN, BNSTpm, NAc core and shell, BLA, VTA, and all subregions of mPFC, consistent with prior studies demonstrating mating-induced Fos expression in these areas (Baum and Everitt, 1992; Hull et al., 1999; Pfaus and Heeb, 1997; Veening and Coolen, 1998). Meth administration 10 minutes prior to sacrifice induced pERK in NAc core and shell, BLA, MeApd, CeA, BNSTpl, and regions of mPFC, consistent with activation patterns induced by other psychostimulants (Valjent et al., 2000, 2004, 2005).

Moreover, three patterns of co-expression of neural activation by sexual behavior and Meth were observed: First, brain areas were identified where sex and drugs activated non-overlapping neural populations (Table 2.2). Specifically, in the CeA, MEApd, BNSTpl, and mPFC, significant increases in both drug-induced pERK ($F(1,16)=7.39$-48.8; $p=0.015$-<0.001) and sex-induced Fos ($F(1,16)=16.53$-158.83; $p<0.001$) were observed. However, in these regions there were no significant increases in dual labeled neurons in mated Meth-treated males. The only exception was the MEApd, where an effect of mating on numbers of dual labeled cells were found ($F(1,16)=9.991$; $p=0.006$). However, there was no overall effect of drug treatment and dual labeling in Meth treated groups was not significantly higher than in saline treated groups, thus was not caused by

the drug (Table 2.2). Second, brain areas were identified where neural activation was only induced by mating (Table 2.3). Specifically, the MPN, BNSTpm, and VTA were activated only by mating, and contained significant increases in mating-induced Fos ($F(1,16)=14.99\text{-}248.99$; $p\leq0.001$), but no Meth-induced pERK.

Finally, brain areas were found where sex and drugs activated overlapping populations of neurons (Figure 2.2 and 2.3). In the NAc core and shell, BLA, and ACA, there were overall effects of mating ($F(1,16)=7.87\text{-}48.43$; $p=0.013\text{-}<0.001$) and drug treatment ($F(1,16)=6.39\text{-}52.68$; $p=0.022\text{-}<0.001$), as well as an interaction between these two factors ($F(1,16)=5.082\text{-}47.27$; $p=0.04\text{-}<0.001$; no significant interaction in ACA) on numbers of cells expressing both mating-induced Fos and Meth-induced pERK. Post hoc analysis revealed that numbers of dual labeled neurons were significantly higher in mated Meth-injected males compared to unmated Meth-treated ($p=0.027\text{-}<0.001$), or mated saline-treated ($p=0.001\text{-}<0.001$) males (Figure 2.2 and 2.3). When data were expressed as the percentages of drug-activated neurons, $39.2 \pm 5.3\%$ in the NAc core, $39.2 \pm 5.8\%$ in the NAc shell, $40.9 \pm 6.3\%$ in the BLA, and $50.0 \pm 5.3\%$ of ACA neurons were activated by both mating and Meth.

An unexpected observation was that sexual behavior affected Meth-induced pERK. Although Meth significantly induced pERK levels in both mated and unmated Meth-injected groups, in the NAc, BLA, and ACA, pERK labeling was significantly lower in mated Meth-injected males when compared to unmated Meth-injected males (Figure 2.2B, E, H, and K; $p=0.017\text{-}<0.001$).

Brain areas	Group	Fos	pERK	Dual	% Dual/pERK
Amygdala					
CeA	Sal10	8.1 ± 2.0	33.7 ± 6.1	3.3 ± 0.8	11.1 ± 3.1
	Sex+Sal10	22.9 ± 2.7*	31.0 ± 5.7	13.9 ± 2.1*	43.2 ± 4.3
	Meth10	10.3 ± 2.2	95.3 ± 8.3**	5.1 ± 1.1	5.5 ± 1.6
	Sex+Meth10	21.6 ± 5.9*	77.6 ± 6.6**	17.3 ± 4.6*	20.7 ± 4.6
MEApd	Sal10	72.1 ± 13.5	40.2 ± 7.2	5.1 ± 1.9	11.4 ± 2.7
	Sex+Sal10	295.3 ± 48.1*	47.3 ± 6.7	13.8 ± 2.5*	29.4 ± 1.1
	Meth10	99.1 ± 6.4	84.5 ± 11.1**	9.1 ± 1.4	12.2 ± 2.2
	Sex+Meth10	403.5 ± 3.5*,**	56.5 ± 7.1	11.4 ± 0.5	21.3 ± 3.9
BNST					
BNSTpl	Sal10	19.9 ± 3.3	13.9 ± 5.9	2.5 ± 1.0	15.2 ± 5.4
	Sex+Sal10	116.8 ± 14.9*	6.8 ± 1.1	3.3 ± 0.8	49.3 ± 9.6
	Meth10	23.1 ± 1.7	35.4 ± 4.1**	2.9 ± 0.6	8.6 ± 1.2
	Sex+Meth10	113.3 ± 16.5*	20.6 ± 4.7*	4.9 ± 1.1	24.5 ± 10.5
mPFC					
PL	Sal10	69.5 ± 13.4	31.5 ± 2.2	5.9 ± 2.1	17.9 ± 6.1
	Sex+Sal10	168.6 ± 32.4*	28.3 ± 1.6	12.0 ± 1.4*	41.7 ± 4.9
	Meth10	70.9 ± 11.9	49.7 ± 2.4**	8.1 ± 1.6	16.4 ± 3.3
	Sex+Meth10	148.1 ± 9.6*	32.3 ± 3.5*	13.0 ± 1.4	41.4 ± 5.9
IL	Sal10	59.8 ± 7.9	31.9 ± 2.0	5.2 ± 1.6	15.8 ± 4.4
	Sex+Sal10	129.8 ± 20.5*	23.5 ± 2.9	9.3 ± 2.3	39.4 ± 3.6
	Meth10	62.9 ± 9.0	47.6 ± 2.7**	6.1 ± 1.5	12.8 ± 3.3
	Sex+Meth10	108.5 ± 12.1*	30.1 ± 2.6*	8.3 ± 1.6	27.8 ± 3.7

Table 2.2 Overview of mating-induced Fos and Meth-induced pERK expression in brain areas where sex and drugs activate non-overlapping neural populations. Mean numbers ± SEM of single and dual labeled neurons for pERK and Fos, as well as percentage of Meth activated neurons co-activated by sexual behavior are listed for each brain area for all four experimental groups. * indicate significant differences from unmated males of the same saline- or Meth-injected group ($p<0.05$); ** indicate significant differences from saline-injected groups of the same sex or no sex treatment ($p<0.05$).

Brain areas	Group	Fos	pERK	Dual	% Dual/pERK
MPN					
	Sal10	14.1 ± 3.2	7.4 ± 4.2	4.8 ± 3.6	52.7 ± 10.8
	Sex+Sal10	214.3 ± 36.8*	8.4 ± 3.2	7.3 ± 3.1	83.1 ± 10.2
	Meth10	10.3 ± 4.6	11.8 ± 1.5	2.7 ± 0.9	20.0 ± 5.7
	Sex+Meth10	211.8 ± 10.0*	10.4 ± 3.3	8.7 ± 3.0	73.7 ± 12.5
BNST					
BNSTpm	Sal10	13.3 ± 1.3	13.3 ± 1.3	0.9 ± 0.3	7.3 ± 2.9
	Sex+Sal10	216.8 ± 8.4*	4.6 ± 1.6*	2.4 ± 1.0	36.5 ± 15.0
	Meth10	15.2 ± 1.1	13.3 ± 2.1	1.8 ± 0.5	13.1 ± 4.0
	Sex+Meth10	171.1 ± 27.3*	9.1 ± 2.9	4.9 ± 1.1*	49.0 ± 1.3
VTA					
	Sal10	35.6 ± 4.6	37.2 ± 11.2	14.9 ± 3.6	39.7 ± 7.7
	Sex+Sal10	114.3 ± 39.9*	46.3 ± 9.6	23.6 ± 7.0	24.2 ± 4.9
	Meth10	42.6 ± 4.7	50.2 ± 5.5	14.9 ± 3.5	34.1 ± 4.9
	Sex+Meth10	101.0 ± 16.7*	42.0 ± 8.5	19.5 ± 2.5	22.8 ± 4.2

Table 2.3 Overview of mating-induced Fos and Meth-induced pERK expression in brain areas where neural activation was induced only by mating. Mean numbers ± SEM of single and dual labeled neurons for pERK and Fos, as well as percentage of Meth activated neurons co-activated by sexual behavior are listed for each brain area or all four experimental groups. * indicate significant differences from unmated males of the same saline- or Meth-injected group ($p<0.05$).

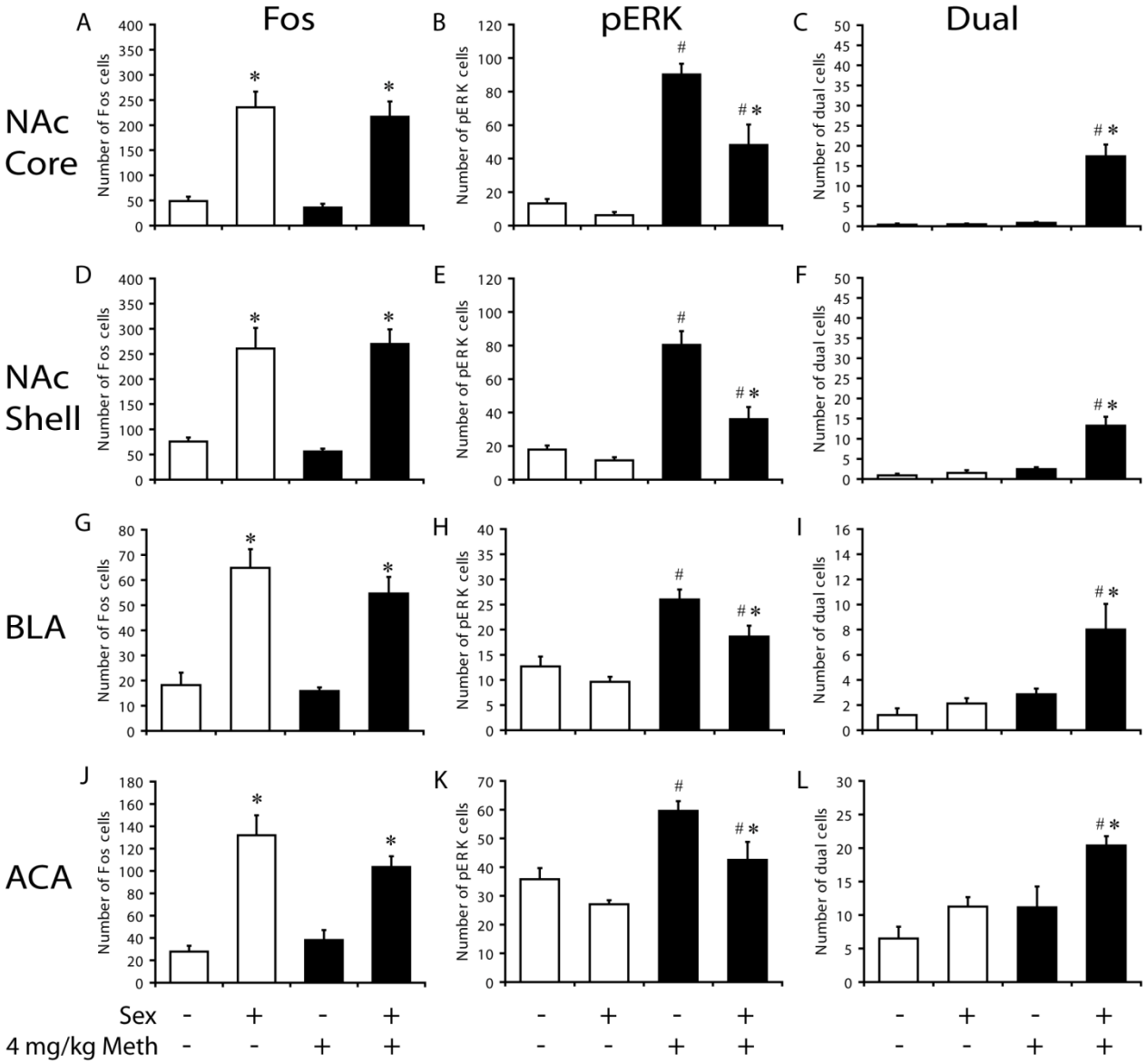

Figure 2.2 Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 10 min following administration of 4 mg/kg Meth. Mean numbers ± SEM of Fos (A, D, G, and J), pERK (B, E, H, and K), and dual (C, F, I, and L) labeled cells in the NAc core (A-C) and shell (D-F), the BLA (G-I), and ACA (J-L). * indicate significant differences from unmated males of the same saline- or Meth-injected groups ($p<0.05$); # indicate significant differences from saline-injected groups of the same sex or no sex treatment ($p<0.05$).

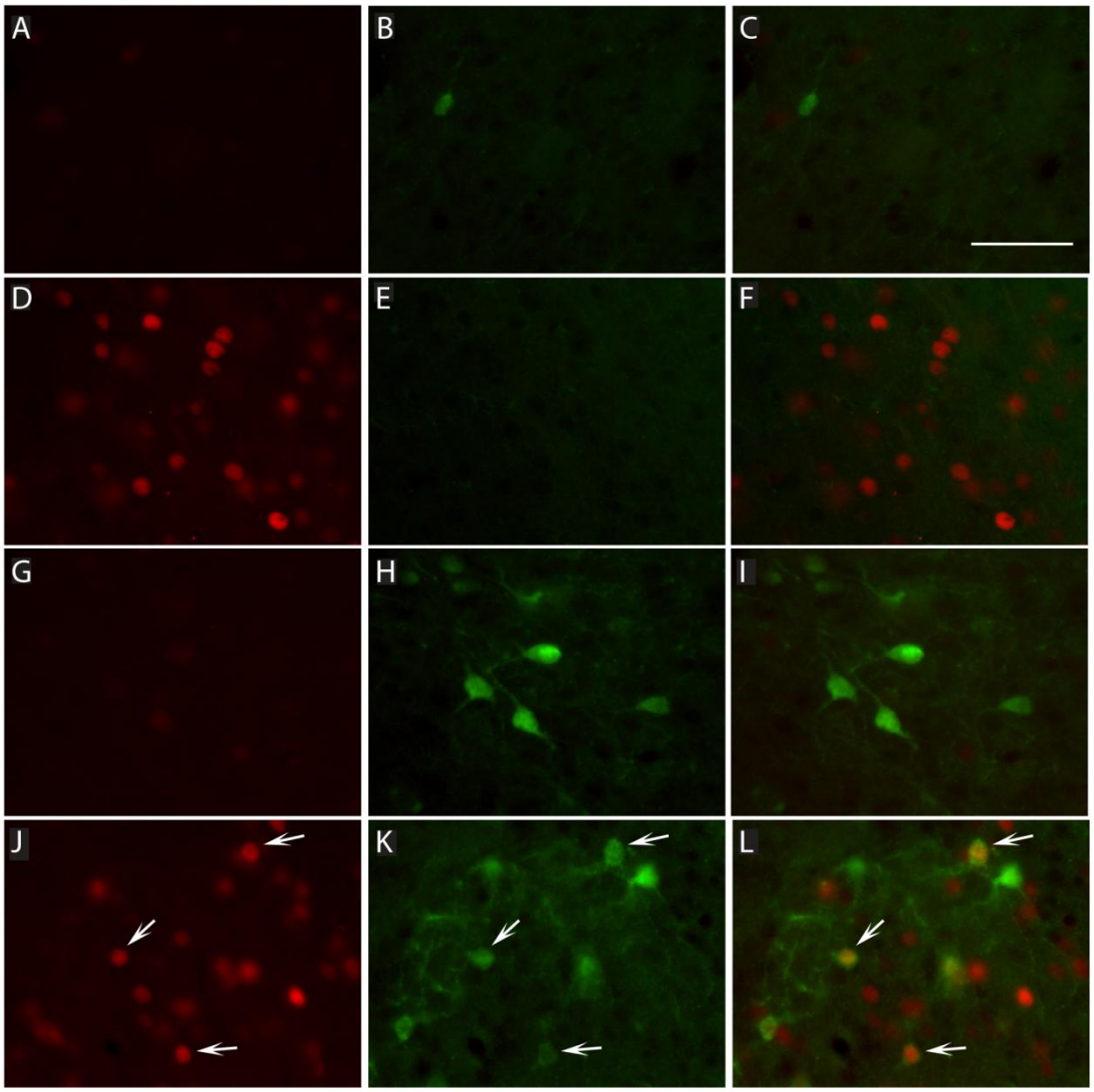

Figure 2.3 Representative images of NAc sections immunostained for Fos (red; A, D, G, and J) and pERK (green; B, E, H, and K) of animals of each experimental group: No Sex+Sal (A-C), Sex+Sal (D-F), No Sex+Meth (G-I), and Sex+Meth (J-L). Right panels are merged images illustrating co-localization of Fos and pERK. Arrows indicate dual labeled cells. Scale bar indicates 50 μm.

This finding may further support the hypothesis that sex and drugs act on the same neurons, but it may also be indicative of mating-induced alterations in drug uptake or metabolism that in turn cause altered neural responses to Meth. To investigate if sexual behavior causes a different temporal pattern of drug-induced activation, sections of the NAc, BLA, and ACA were stained for males sacrificed at a later time point (15 min) following drug administration (Experiment 2).

2.3.1B Experiment 2

Analysis of single and dual labeled cells confirmed the findings described above that sexual behavior and subsequent exposure to Meth 15 minutes prior to sacrifice resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell, BLA, and ACA. In addition, significant co-expression of mating-induced Fos and Meth-induced pERK were again found in these areas (Figure 2.4; mating effect: $F(1,12)=15.93$-76.62; $p=0.002$-<0.001; drug effect: $F(1,12)=14.11$-54.41; $p=0.003$-<0.001). Number of dual labeled neurons in mated Meth-injected males was significantly higher compared to unmated Meth-treated ($p<0.001$) or mated saline-treated ($p<0.001$) males. When data was expressed as the percentages of drug-activated neurons, $47.2 \pm 5.4\%$ (NAc core), $42.7 \pm 7.6\%$ (NAc shell), $36.7 \pm 3.7\%$ (BLA), and $59.5 \pm 5.1\%$ (ACA) of neurons activated by mating were also activated by Meth. Moreover, drug-induced pERK did not differ between mated and unmated animals (Figure 2.4B, E, H, and K), in all areas except for the ACA ($p<0.001$). These data indicate that sexual behavior indeed causes an alteration of the temporal pattern of pERK induction by Meth.

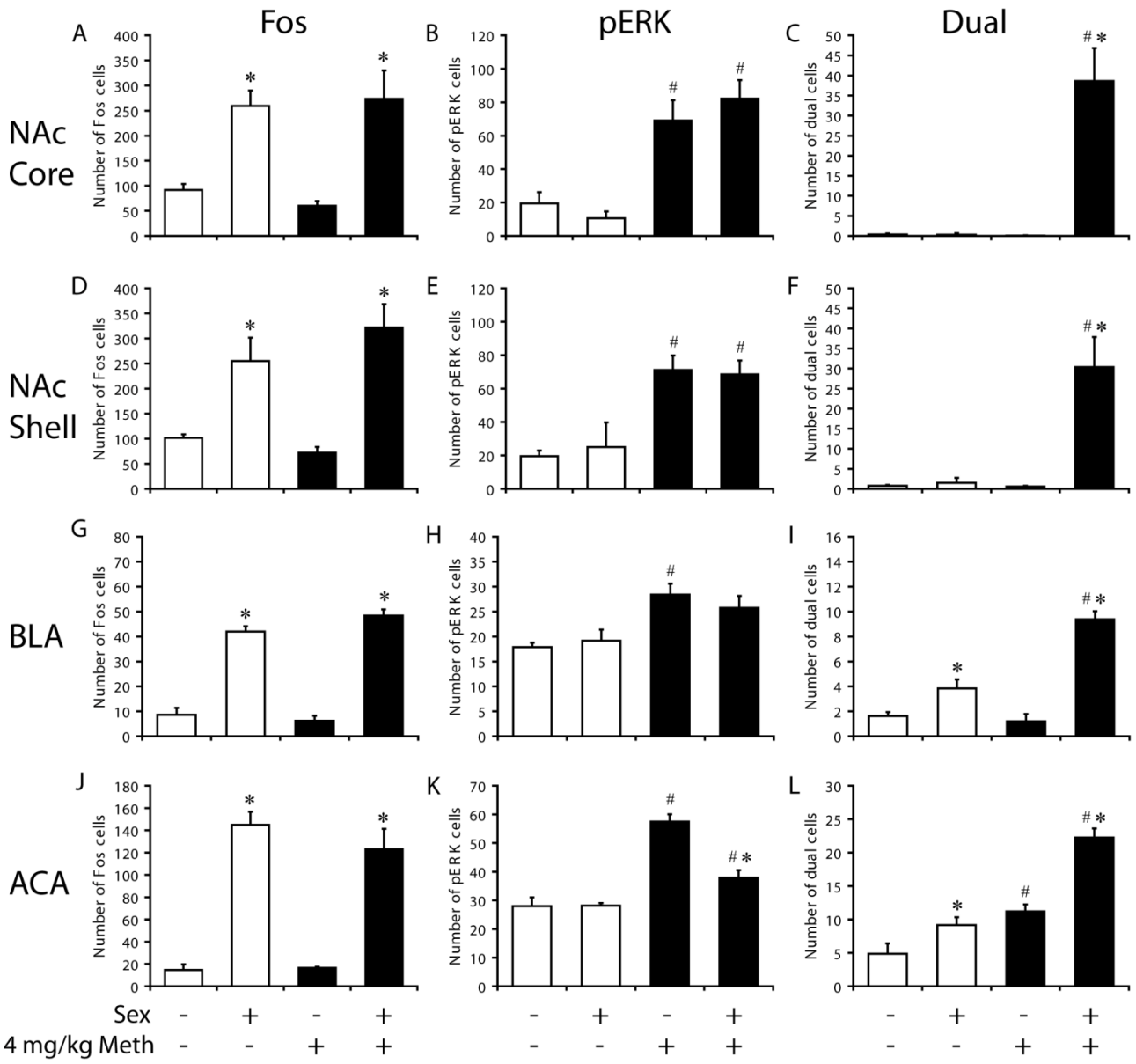

Figure 2.4 Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 4 mg/kg Meth. Mean numbers ± SEM of Fos (A, D, G, and J), pERK (B, E, H, and K), and dual (C, F, I, and L) labeled cells in the NAc core (A-C) and shell (D-F), the BLA (G-I), and ACA (J-L). * indicate significant differences from unmated males of the same saline- or Meth-injected groups ($p<0.05$); # indicate significant differences from saline-injected groups of the same sex or no sex treatment ($p<0.05$).

2.3.2 Neural Activation following Sexual Behavior and 1 mg/kg Meth

2.3.2A Experiment 3

Thus far results revealed that sexual behavior and 4 mg/kg Meth activated overlapping populations of neurons in the NAc core and shell, BLA, and ACA. To investigate the influence of drug-dosage on this overlap in activation, patterns of neural activation were also studied using a lower dose of Meth. The NAc core and shell, BLA, and ACA were analyzed for activation induced by sex and Meth. Indeed, sexual behavior and subsequent exposure to Meth resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell subregions, the BLA, as well as neurons in the ACA region of the mPFC (Figure 2.5). Interestingly, the lower dose of Meth resulted in similar numbers of pERK labeled neurons as induced by 4 mg/kg Meth in the four brain regions analyzed. More importantly, the NAc core and shell, BLA, and ACA displayed significant increases in the number of dual labeled cells (Figure 2.5C, F, I, and L) compared to unmated Meth-injected males ($p=0.003-<0.001$). When data was expressed as the percentages of drug-activated neurons, $21.1 \pm 0.9\%$ and $20.4 \pm 1.8\%$ in the NAc core and shell respectively, $41.9 \pm 3.9\%$ in the BLA, and $49.8 \pm 0.8\%$ of ACA neurons were activated by sex and Meth.

2.3.3 Neural Activation following sexual behavior and administration of D-Amphetamine

2.3.3A Experiment 4

To test whether the above results were specific for Meth, an additional experiment was conducted to study mating- and Amph-induced neural activation. Analysis of single and

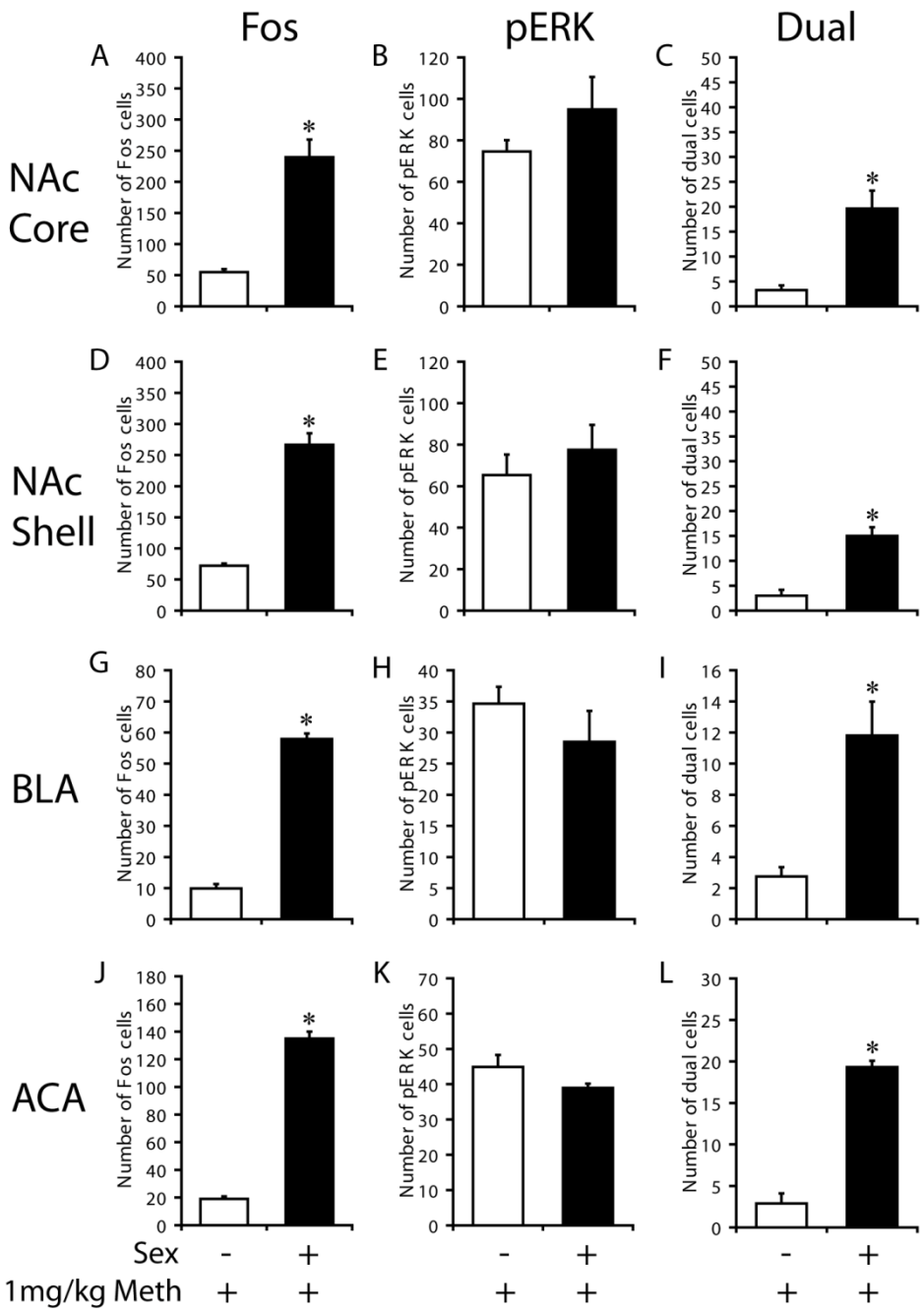

Figure 2.5 Sex-induced Fos and Meth-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 1 mg/kg Meth. Mean numbers ± SEM of Fos (A, D, G, and J), pERK (B, E, H, and K), and dual (C, F, I, and L) labeled cells in the NAc core (A-C) and shell (D-F), the BLA (G-I), and ACA (J-L). * indicate significant differences from unmated males of the same Meth injected groups ($p<0.05$).

dual labeled cells for pERK and Fos showed that sexual behavior and subsequent exposure to Amph resulted in significant increases of Fos and pERK immunolabeling in the NAc core and shell and BLA (Figure 2.6; mating effect: $F(1,15)=7.38\text{-}69.71$; $p=0.016\text{-}<0.001$; drug effect: $F(1,15)=4.70\text{-}46.01$; $p=0.047\text{-}<0.001$). Moreover, the numbers of dual labeled neurons were significantly higher in mated Amph-treated compared to unmated Amph-treated ($p=0.009\text{-}<0.001$), or mated saline-treated ($p=0.015\text{-}<0.001$) males (Figure 2.6C, F, and I). When data was expressed as the percentages of drug-activated neurons, 25.7 ± 2.8% and 18.0 ± 3.2% in the NAc core and shell respectively, and 31.4 ± 2.0% of BLA neurons were activated by both mating and Amph. The ACA region of the mPFC displayed significant levels of mating-induced Fos (Figure 2.6J; $F(1,15)=168.51$; $p<0.001$). However, unlike Meth, Amph did not result in significant increases in drug-induced pERK levels in the ACA (Figure 2.6K) or numbers of dual labeled neurons in the ACA (Figure 2.6L) when compared to both mated and unmated saline-injected males.

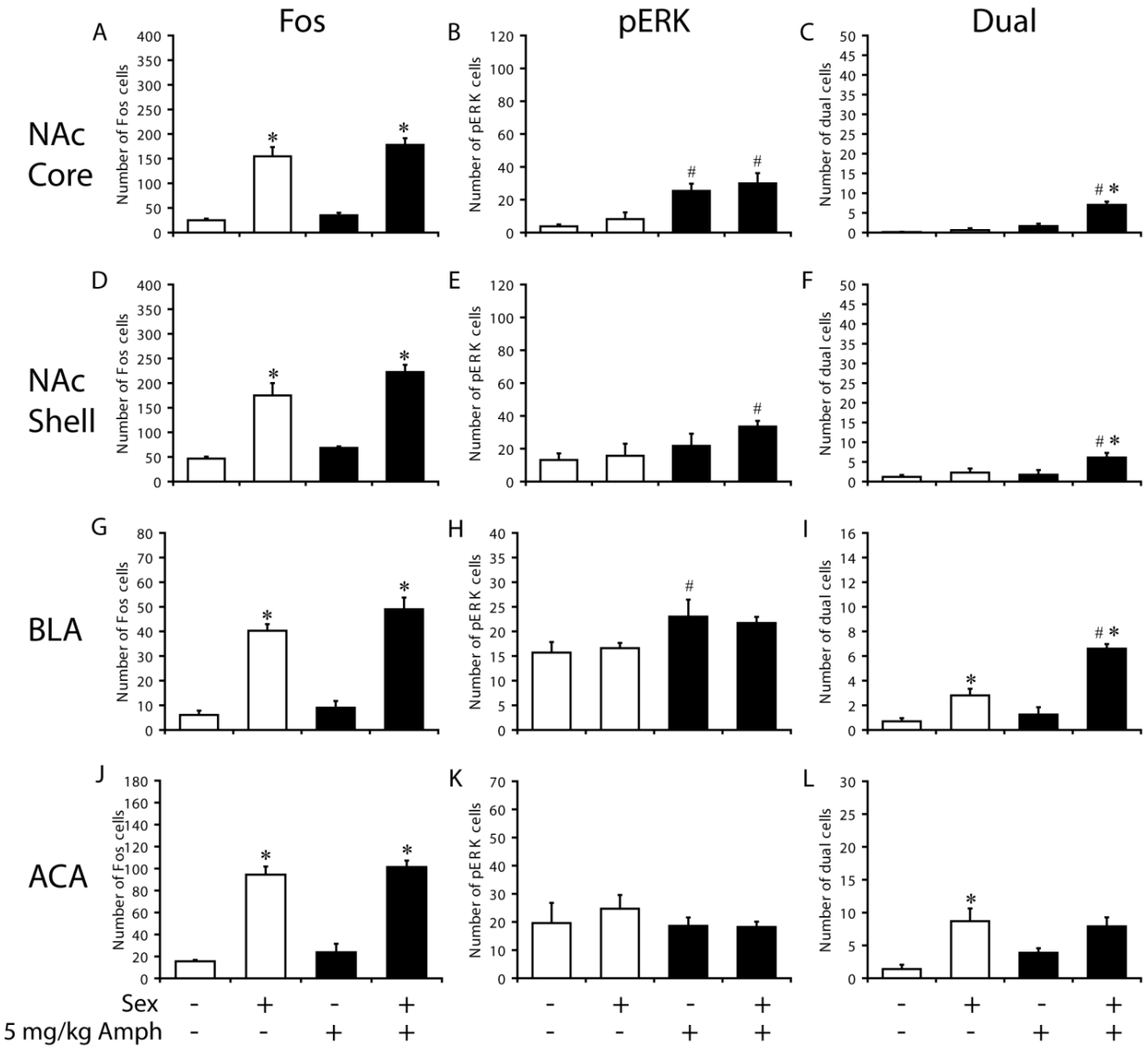

Figure 2.6 Sex-induced Fos and Amph-induced pERK expression in NAc, BLA, and ACA neurons 15 min following administration of 5 mg/kg Amph. Mean numbers ± SEM of Fos (A, D, G, and J), pERK (B, E, H, and K), and dual (C, F, I, and L) labeled cells in the NAc core (A-C) and shell (D-F), the BLA (G-I), and ACA (J-L). * indicate significant differences from unmated males of the same saline- or Meth-injected groups ($p<0.05$); # indicate significant differences from saline-injected groups of the same sex or no sex treatment ($p<0.05$

2.4 DISCUSSION

The current study demonstrates at a cellular level an overlap between neural activation by the natural reinforcer sexual behavior and the psychostimulant Meth. Therefore, these data show that not only do drugs act on the same brain regions that regulate natural reward, but in fact, drugs activate the same cells involved in the regulation of natural rewarding behavior. Specifically, it was shown here that sexual behavior and Meth co-activated a population of neurons in the NAc core and shell, BLA, and ACA region of the mPFC, identifying potential sites where Meth may influence sexual behavior.

The current finding that sexual behavior and administration of Meth activate overlapping populations of neurons in the NAc, BLA, and ACA is in contrast to findings from other studies showing that different populations of NAc neurons encode drug and natural reward. Specifically, electrophysiological studies that compared neural activation during self-administration of natural rewards (food and water) and intravenous cocaine have indicated that cocaine self-administration activated a differential, non-overlapping population of neurons that was generally not responsive during operant responding for water and food reinforcement (92%). Only 8% of accumbal neurons showed activation by both cocaine and natural reward (Carelli et al., 2000). In contrast, a majority (65%) of cell in the NAc showed activation by different natural rewards (food and water), even if one reinforcer was more palatable (sucrose) (Roop et al., 2002). Several factors may have contributed to the discrepancy with the current results. First, different technical approaches were used to investigate neural activity. The current study utilized a neuroanatomical method for detection of concurrent neural activation by two different

stimuli using dual fluorescencent immunocytochemisty for Fos and pERK, allowing for investigation of single cell activation over large spans of brain areas. Second, the current study investigated a different natural reward i.e. sexual behavior compared to previous studies, which used food and water in restricted rats (Carelli, 2000). Sexual behavior is highly rewarding and rats readily form CPP to copulation (Agmo and Berenfeld, 1990; Martinez and Paredes, 2001; Tenk, 2009). Although, diet restricted rats do form CPP for water (Agmo et al., 1993; Perks and Clifton, 1997) and food (Perks and Clifton, 1997), diet unrestricted rats preferably consume and form CPP for more palatable foods (Jarosz et al., 2006, 2007). Third, our studies included different drugs of abuse compared to previous studies, utilizing methamphetamine instead of cocaine. Drug experience may have also played a factor in our findings. The current studies utilized animals that were sexually experienced, but drug naïve. In contrast, previous electrophysiological studies used "well-trained" animals that received repeated exposures to cocaine (Carelli, 2002). It is possible that the drug-induced activation of neurons activated by sexual behavior may be altered in drug experienced rats. However, preliminary studies from our lab suggest that drug experience is unlikely to be a major factor in the current analysis as sexual behavior and Meth treatment in males chronically treated with Meth co-activated similar percentages of drug-activated neurons as reported in the current study (20.3 ± 2.5 % in NAc core and 27.8 ± 1.3 % in NAc shell; Frohmader and Coolen, unpublished observations). Finally, the current study investigated the "direct" action of drugs utilizing passive administration. Therefore, the current analysis does not reveal information regarding neural circuits involved in drug seeking or cues associated to drug reward, but rather reveals neural activity caused by the pharmacological action of the drug. In the

previous electrophysiological studies, NAc neural activity occurring within seconds of reinforced responses is not the result of the pharmacological action of cocaine, but is greatly dependent on associative factors within the self-administration paradigm (Carelli, 2000 , 2002). Specifically, NAc neural activity is influenced by response-independent presentations of stimuli associated with intravenous cocaine delivery as well as by instrumental contingencies (i.e., lever pressing) inherent in this behavioral paradigm (Carelli, 2000, 2002; Carelli and Ijames, 2001; Carelli and Wightman, 2004). In summary, our findings of co-activation by natural and drug reward may be specific for activation by sexual behavior and passively administered Meth.

Meth and sex activated overlapping populations of neurons in the NAc core and shell in a dose-dependent manner. The co-activated neurons in the NAc may mediate potential effects of Meth on the motivation and rewarding properties of sexual behavior as lesions of the NAc disrupt sexual behavior and reinforcement (Kippin et al., 2004; Liu et al., 1998). In addition, these neurons are potentially a locus for dose-dependent drug effects on mating, since the lower Meth dose (1 mg/kg) reduced the number of dual labeled cells by 50% compared to the higher dose of Meth (4 mg/kg). Although this study does not identify the chemical phenotype of co-activated neurons, previous studies have shown that drug-induced pERK and Fos expression in the NAc is dependent on both dopamine (DA) and glutamate receptors (Ferguson et al., 2003; Sun et al., 2008; Valjent et al., 2000; Valjent et al., 2005). Although it is not clear if mating-induced neural activation in the NAc is dependent on these receptors, this has been demonstrated in other brain regions, particularly in the medial preoptic area (Dominguez et al., 2007; Lumley

and Hull, 1999). Thus, Meth may act on neurons also activated during sexual behavior via activation of dopamine and glutamate receptors. The role of NAc glutamate in sexual behavior is currently unclear, but it is well established that DA plays a critical role in the motivation for sexual behavior (Hull et al., 2002; Hull et al., 2004; Pfaus, 2009). Microdialysis studies reported increases in NAc DA efflux during appetitive and consummatory phases of male sexual behavior (Fiorino and Phillips, 1999a; Lorrain et al., 1999) and mesolimbic DA efflux has been correlated to facilitation of the initiation and maintenance of rat sexual behavior (Pfaus and Everitt, 1995). Furthermore, DA manipulation studies show DA antagonists in the NAc inhibit sexual behavior, while agonists facilitate the initiation of sexual behavior (Everitt et al., 1989; Pfaus and Phillips, 1989). Thus, Meth may affect motivation for sexual behavior via activation of DA receptors.

In contrast to the NAc, the number of dual labeled cells in the BLA and ACA remained relatively unchanged regardless of the Meth dose. The BLA is critical for discrete associative learning and is strongly involved in conditioned reinforcement and reward evaluation during instrumental responding (Cardinal et al., 2002; Everitt et al., 1999; See, 2002). BLA lesioned rats display decreased lever pressing for conditioned stimuli paired with food (Everitt et al., 1989) or sexual reinforcement (Everitt, 1990; Everitt et al., 1989). In contrast, this manipulation does not affect the consummatory phase of feeding and sexual behavior (Cardinal et al., 2002). The BLA also plays a key role in memory of conditioned stimuli associated with drug stimuli (Grace and Rosenkranz, 2002; Laviolette and Grace, 2006). Lesions or pharmacological inactivations

of the BLA block the acquisition (Whitelaw et al., 1996) and expression (Grimm and See, 2000) of conditioned-cued cocaine reinstatement, while not affecting the process of drug administration. Furthermore, Amph infused directly into the BLA results in a potentiated drug reinstatement in the presence of the conditioned cues (See et al., 2003). Therefore, it is possible that psychostimulant-enhanced DA transmission in the BLA results in potentiated emotional salience and seeking (Ledford et al., 2003) of sexual reward , thus contributing to the enhanced sexual drive and desire reported by Meth abusers (Green and Halkitis, 2006; Semple et al., 2002).

In the ACA, neural activation of sex-activated neurons was dosage-independent and specific for Meth, as it was not observed with Amph. Although Meth and Amph have similar structural and pharmacological properties, Meth is a more potent psychostimulant than Amph with longer lasting effects (NIDA, 2006). Studies by Goodwin et al. (2009) showed that Meth generates a greater DA efflux and inhibits the clearance of locally applied DA more effectively in the rat NAc than Amph. However, it is not clear whether the different patterns of results are due to efficacy differences between the drugs or potency issues related to the doses employed and further investigation is required.

Co-activation by Meth and sex was not observed in other subregions of the mPFC (IL and PL). In the rat, the ACA has been extensively studied using appetitive tasks, supporting a role in stimulus–reinforcer associations (Cardinal et al., 2003; Everitt et al., 1999; See, 2002). There is ample evidence that the mPFC is involved in drug craving and relapse to drug-seeking and drug-taking behavior in both humans and rats (Capriles et al.,

2003; Childress et al., 1999; Grant et al., 1996; Kalivas and Volkow, 2005; McLaughlin and See, 2003; Shaham et al., 2003). In line with this, it has been proposed that mPFC dysfunctioning caused by repeated exposure to drugs of abuse might be responsible for reduced impulse control and increased drug-directed behavior as observed in many addicts (Jentsch and Taylor, 1999). Recent data from our laboratory demonstrated that mPFC lesions result in continued seeking of sexual behavior when this was associated with an aversive stimulus (Davis et al., 2010). Even though this study did not investigate the ACA, it supports the hypothesis that the mPFC (and the ACA specifically) mediates the effects of Meth on a loss of inhibitory control over sexual behavior as reported by Meth abusers (Salo et al., 2007).

In conclusion, together these studies form a critical first step towards a better understanding of how drugs of abuse act on neural pathways that normally mediate natural rewards. Moreover, these findings illustrate that in contrast to the current belief that drugs of abuse do not activate the same cells in the mesolimbic system as natural reward, Meth, and to a lesser extent Amph, activate the same cells as sexual behavior. In turn, these co-activated neural populations may influence seeking of natural reward following drug exposure. Finally, the results of this study may significantly contribute to our understanding of the basis of addiction in general. Comparisons of the similarities and differences, as well alterations in neural activation of the mesolimbic system elicited by sexual behavior versus drugs of abuse may lead to a better understanding of substance abuse and associated alterations in natural reward.

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CHAPTER 3:

Effects of methamphetamine on sexual performance and compulsive sex behavior in male rats[1]

[1] Research article published in Psychopharmacology (2010) 212, 93-104

3.1 INTRODUCTION

It is well recognized that psychostimulants, opiates, and alcohol impact sexual function, performance, and arousal in both men and women (Frohmader et al., 2010a; Peugh and Belenko, 2001; Pfaus, 2009; Rawson et al., 2002). The prevalence of sexual health related diseases within addict populations has raised awareness of the effects these drugs have on sexual behavior, as chronic drug use is associated with unsafe sexual practices or sexual risk behaviors, resulting in increased rates of sexually transmitted infections including Human Immunodeficiency Virus (HIV) (Crowe and George, 1989; Fisher et al., 2011; Peugh and Belenko, 2001; Raj et al., 2007; Rawson et al., 2002; Sánchez et al., 2002). The effect of drugs of abuse on sexual behavior is particularly well documented for the psychostimulant methamphetamine (Meth). Meth is one of the most abused illicit drugs in the world (Ellkashef et al., 2008; NIDA, 2006; SAMHSA, 2008). Meth users report heightened sexual desire and arousal and enhanced sexual pleasure (Green and Halkitis, 2006; Schilder et al., 2005; Semple et al., 2002). However, chronic Meth abuse can also lead to decreased sexual function as it results in the inability to reach full erection and delays ejaculation and orgasm (Bell and Trethrowan, 1961; Buffum, 1988; Frosch et al., 1996; Peugh and Belenko, 2001). Furthermore, Meth abuse is commonly associated with loss of inhibitory control of sex behavior or sexually compulsive behavior (Green and Halkitis, 2006; Halkitis et al., 2001; Mckirnan et al., 2001; Rawson et al., 2002) and increased prevalence of HIV (Frosch et al., 1996; Halkitis et al., 2001; Parsons and Halkitis, 2002). Users often report having numerous sexual partners and are less likely to use protection than other drug abusers (Frosch et al., 1996; Somlai et al., 2003; Springer et al., 2007). Unfortunately, human reports demonstrating Meth use as a

predictor of sexual risk behavior are confounded by several factors. These studies are based on self-reports from chronic Meth users lacking a reliable instrument to measure the relationship between Meth use and sexual function. Moreover, subjects have variable drug histories, including differences in numbers of exposures to one or many drugs as well as rate of administration (daily, weekly, etc). Therefore, an investigation into Meth-induced changes in sexual behavior under controlled experimental settings using an animal model is required for understanding the complex connection between Meth and sexual behavior.

Thus far, few behavioral studies have investigated the effects of Meth on sexual performance and motivation, and utilized different animal models and Meth dosages. Male Japanese quails chronically treated with Meth followed by a withdrawal period showed reduced sexual motivation, but did not display altered sexual performance (Bolin and Akins, 2009), while female rats sub-chronically treated with Meth showed increased display of proceptive and sexually receptive behaviors (Holder et al., 2010). However, there are no studies investigating the effects of acute or chronic administration of Meth on sexual performance and motivation in male rats. Furthermore, there are no studies that have attempted to investigate effects of Meth on sexual risk behaviors as observed in humans in an animal model. Therefore, the goal of the current study was to investigate the effects of acute Meth administration on sexual motivation and performance in male rats with specific focus on maladaptive sex-seeking behaviors.

3.2 MATERIALS AND METHODS

3.2.1 Subjects

Adult male Sprague-Dawley rats (210-225 g) obtained from Charles River Laboratories (Montreal, QC, Canada) were dual-housed in standard Plexiglas cages (home cages). The animal room was maintained at a 12/12 h reversed light/dark cycle (lights off at 10.00 h) with food and water available *ad libitum*. Experiments were performed during the first half of the dark cycle under red illumination. Females used for sexual behavior were bilaterally ovariectomized and implanted with a subcutaneous capsule containing 5% estradiol benzoate (EB) and 95% cholesterol. Females were administered 0.5 mg progesterone in 0.1 ml sesame oil (s.c.) 4 h prior to testing to induce sexual receptivity. All experimental procedures were authorized by the Animal Care Committee at the University of Western Ontario and conform to the guidelines outlined by the Canadian Council on Animal Care.

3.2.2 Experimental designs

3.2.2A Experiment 1

To investigate the effects of an acute dose of Meth on sexual performance, 32 sexually naïve males were used. Males were administered 1 ml/kg of saline (s.c.) daily during three days prior to behavioral testing, and placed in Plexiglas Locomotor Activity Chambers (LAC; 40.5 x 40.5 cm; San Diego Instruments) equipped with 16 x 16 photobeam arrays for 30 min. Animals were randomly distributed into four experimental groups (n=8 each) and received either 1, 2, or 4 mg/kg of Meth or saline (1 ml/kg; s.c.).

Immediately following injection, males were placed in the LAC and locomotor activity was recorded for 30 min, after which a receptive female was introduced into the chamber and males were allowed to mate for 1 h or 1 ejaculation (E), whichever occurred first. Previous studies have demonstrated that peak plasma concentration of Meth is reached 10 min following systemic drug injection and it is completely cleared from the system after 2 hrs (Melega et al., 1995; Riviere et al., 1999). During the mating session, standard parameters for sexual behavior were observed and recorded, including latencies to mount (time from introduction of female to first mount) and intromission (time from introduction of female to first intromission), which are indicative of sexual motivation (Hull et al., 2002), as well as latency to ejaculation (time from first intromission to ejaculation), numbers of mounts and intromissions prior to ejaculation, and post-ejaculatory interval, which are measures of sexual performance (Hull et al., 2002; Pfaus, 2009). If animals did not display mounts, intromissions, or ejaculation, latencies were analyzed in two ways: 1. Latencies of 3600 seconds (end of test) were assigned or 2. Animals were excluded from the data analysis.

One week later, males gained sexual experience in mating cages (60 x 45 x 50 cm) containing clean bedding during four twice-weekly mating sessions of 30 min or until display of 1 ejaculation. Three days after the last mating session, animals were again distributed into four experimental groups matched for dosing history (each experimental group contained two animals from each of the previous dosage groups). Sexually experienced males received 1, 2, or 4 mg/kg of Meth or saline (1 ml/kg; s.c.) and were placed in the LAC. Locomotor activity was recorded for 30 min after which a receptive

female was introduced. Males were allowed to mate for 1 h or 1 ejaculation. Parameters for sexual behavior were recorded and calculated as described above.

To determine the effects of acute Meth on sexual behavior in sexually naïve and experienced male groups means and SEMs were calculated and compared for each parameter of sexual behavior using a two-way ANOVA (factors: *dose, experience*). Post hoc comparisons were conducted using Holm-Sidak tests, at a significance level of 0.05. Percentages of animals displaying mounts, intromissions, and ejaculation were analyzed using Pearson chi-Square analysis at a significance level of 0.05.

In addition, mating sessions in sexually experienced males were videotaped for 1 h for analysis of investigative behavior of the receptive female partners by saline or Meth-injected males. An observer blind to treatment, recorded parameters of investigative behavior during the initial five minutes of the mating test including the number and duration of each investigative approach of the body and anogenital area of the female, as well as number of attempted mounts. Group mean and SEMs for each parameter were calculated and compared between groups using a one-way ANOVA (factor: *dose*) and Holm-Sidak tests at a significance level of 0.05.

Locomotor activity was analyzed using San Diego Instruments Pas-reporter analysis software as distance traveled within five-minute intervals. Group means and SEMs for each experimental dose during both sexually naïve and experienced test days were calculated and compared using one-way ANOVA (factor: *dose*) and Holm-Sidak

post hoc comparisons, non-parametric Kruskal-Wallis analysis and Dunn's post hoc tests were used when appropriate. In addition, Meth-induced locomotor activity was compared between sexually naïve and experienced males to test for a sensitizing effect of sexual experience. Meth-induced activity was compared between naïve and experienced animals using unpaired t-tests, during the last 10 minutes of the test, as this is the time of greatest drug-induced activity and sex experience-induced sensitization has previously been shown during this time for D-amphetamine (Pitchers et al., 2010). A significance level of 0.05 was applied for all comparisons.

3.2.2B Experiment 2

Investigative behavior

To examine the effects of an acute administration of Meth on investigative behavior of a non-sexual partner 20 sexually experienced males were handled and injected with saline and distributed into four experimental groups matched for prior sexual performance (n=5 each). Experimental groups included males treated with either Meth (1 mg/ml/kg; s.c.) or saline (1 ml/kg). This Meth dose was chosen as the previous experiment showed that it does not inhibit sexual performance (see Results). Thirty minutes following injection, males were exposed to either a non-receptive ovariectomized female or a young, smaller male as a stimulus partner in a clean test cage. The use of young small males assured a lack of aggressive behavior by experimental males. Sessions were videotaped for 30 minutes and investigative behavior was recorded as previously described. Group mean and SEMs for each parameter of investigative behavior were calculated and compared

between groups using a two-way ANOVA (factors: *treatment*, *stimulus partner*) and Holm-Sidak tests at a significance level of 0.05.

Conditioned copulation aversion

Two weeks following the study of investigative behavior described above, animals used in experiment 2.1 and an additional 20 males (treated identically with Meth and saline and exposure to stimulus female or male) were subjected to a conditioned copulation aversion paradigm. Males were subdivided into four experimental groups (n=10 each) according to pretreatment (1 mg/kg of either Meth or saline (s.c.)) and conditioning (lithium chloride (LiCl)-paired or -unpaired). The conditioned aversion paradigm consisted of nine consecutive two-day conditioning trials. During the first day, all males were placed in a test cage for a 10 min habituation period after which a receptive female was introduced. Stimulus females were scented by swabbing almond oil on the neck and base of the tail prior to mating as olfactory cues have been shown to facilitate male approach behavior and to strengthen conditioning (Agmo, 2002; Lawrence and Kiefer, 1987). Males were allowed to mate for 30 min or until one ejaculation. If intromissions did not occur within 15 min mating was terminated. One min following ejaculation or trial termination males were given a 63.6 mg/kg intraperitoneal (i.p.) injection of LiCl administered at a volume of 20 ml/kg to induce visceral illness (LiCl-paired) or saline (LiCl-unpaired). LiCl or saline were administered regardless of whether mating occurred. The following day, unpaired males received a 20 ml/kg injection of LiCl while LiCl-paired males received saline. Upon completion of the ninth conditioning trial, the ability of Meth to disrupt conditioned aversion to copulation was examined. Males were

administered either 1 mg/kg (s.c.) Meth (19 LiCl-paired and 10 LiCl-unpaired) or saline (10 LiCl-unpaired) and were placed in the test cage. After 45 min an almond-scented receptive female was introduced and males were allowed to mate for 30 min or until one ejaculation. Parameters for sexual behavior were recorded during conditioning trials and final test day as described above.

Group means and SEMs for each parameter of sexual behavior as well as percentages of males that displayed mounts, intromissions, or ejaculation during each conditioning day were calculated. Statistical differences in sexual performance were determined for each conditioning day using a two-way ANOVA (factors: *Meth/Saline pretreatment, conditioning*). Pearson chi-square analysis was used to compare differences in the percentages of males that displayed mounts, intromissions, or ejaculation on each conditioning day. To determine the ability of Meth to override conditioning, pairwise comparisons within each experimental group were made between the ninth conditioning day and the test day for each mating parameter. Differences in the percentage of males that displayed mounts, intromissions, or ejaculation between the ninth conditioning day and the test day were compared using McNemar's chi-square analysis at a significance level of 0.05. Only animals that displayed a particular behavior were included in the analysis.

3.3 RESULTS

3.3.1 Effects of methamphetamine on sexual behavior: Experiment 1

3.3.1A Sexual behavior

Meth significantly affected the percentages of males that displayed sexual behavior. Meth administration in sexually naïve males at 2 and 4 mg/kg decreased percentages of males that displayed mounts, intromissions (M and I; p=0.043-0.001), and ejaculation (E; p=0.005-<0.001) (Figure 3.1A). This effect was attenuated by sexual experience as only 4 mg/kg, but not 2 mg/kg resulted in decreased percentages of males that displayed mounts, intromissions, or ejaculation (p=0.043-0.005; Figure 3.1B). Moreover, Meth significantly affected all parameters of sexual behavior ($F(3,58)=39.51-49.24$, $p<0.001$). Males injected with 2 or 4 mg/kg Meth showed increased latencies to mount (ML), intromit (IL), and ejaculate (EL) compared to saline controls. Sexual experience did not attenuate these effects of Meth. The effects of Meth on latencies to initiate or consummate behavior were evident both when all animals were included in the analysis (and latencies were assigned to males that did not display behavior; p=<0.001; Figure 3.1C and D) or when only latencies of males that displayed the particular parameter of sexual behavior were included in the analysis (see Table 3.1). Finally, 1 mg/kg Meth dose had no effect on expression of sexual behavior or percentages of males that mated.

Meth did not appear to affect erectile function, as it did not alter numbers of mounts and intromissions or copulation efficiency, except for sexually naïve males injected with 4 mg/kg Meth which displayed significantly decreased numbers of

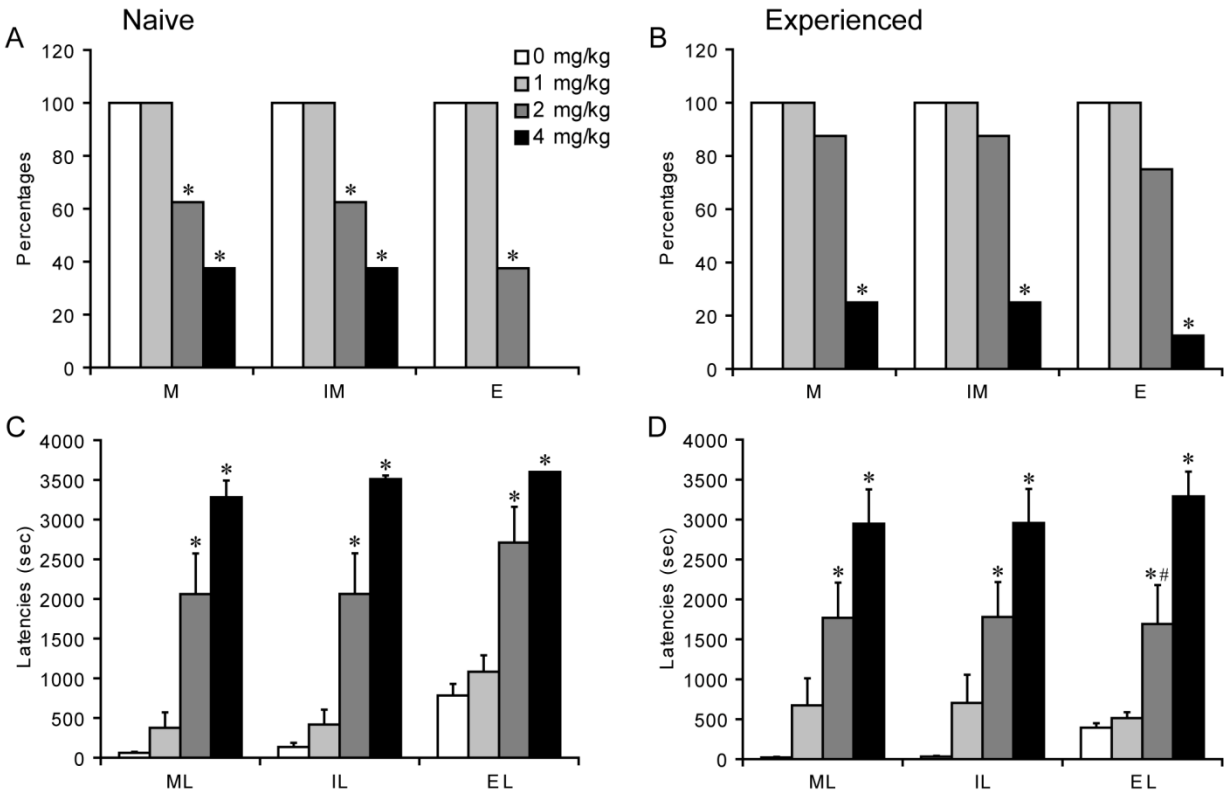


Figure 3.1 Effects of Meth on sexual performance. Mean percentages of mounts (M), intromissions (IM), and ejaculations (E) and mean number ± SEM latencies to mount (ML), intromit (IL), and ejaculate (EL) following administration of 0, 1, 2, or 4 mg/kg Meth in sexually naïve (A and C) and experienced (B and D) males rats. * indicate significant differences from saline-injected males; # indicates significant differences within experience for the same dose ($p<0.05$).

Sexual behavior	Meth dose (mg/kg)			
	0	1	2	4
Naive				
ML	62.7 ± 10.1	376.4 ± 171.8	1139.0 ± 330.1[a]	2748.3 ± 346.2[a]
IL	136.3 ± 45.5	419.4 ± 163.8	1142.0 ± 331.6[a]	3361.3 ± 6.7[a]
EL	784.2 ± 130.3	1084.1 ± 181.5	1223.7 ± 261.6	———
# Mounts	11.1 ± 4.3	18.9 ± 5.8	22.6 ± 7.4	2.7 ± 1.5
# Intromissions	13.6 ± 1.2	15.1 ± 1.7	8.6 ± 2.9	2.3 ± 0.6[a]
Copulation efficiency	0.70 ± 0.09	0.52 ± 0.06	0.39 ± 0.16	0.62 ± 0.16
Experienced				
ML	22.4 ± 6.3	673.9 ± 337.6	1506.4 ± 411.4[a]	991.5 ± 291.5
IL	32.2 ± 9.3	702.8 ± 354.3	1520.3 ± 406.3[a]	1027.5 ± 327.5
EL	392.8 ± 57.7	513.8 ± 73.5	1057.5 ± 345.9	1119.0 ± 0.000
# Mounts	6.3 ± 1.1	10.1 ± 1.9	14.7 ± 3.7	3.5 ± 0.5
# Intromissions	10.4 ± 10.1	11.3 ± 1.1	11.0 ± 1.8	6.5 ± 1.5
Copulation efficiency	0.70 ± 0.06	0.55 ± 0.03	0.52 ± 0.10	0.65 ± 0.02

Table 3.1 Effects of Meth on sexual performance in male rats displaying sexual behavior. Mean ± SEM latencies to mount (ML), intromit (IL), and ejaculate (EL) and numbers of mounts and intromissions in sexually naïve and experienced males rats that mated following administration of 0, 1, 2, or 4 mg/kg Meth. Latencies are based on animals that displayed the particular measure of behavior, and numbers of mounts and intromissions are either prior to ejaculation, or during the entire test period in animals that did not ejaculate. [a] indicate significant differences from saline-injected males ($p<0.05$).

intromissions compared to controls (p=0.003; Table 3.1). Meth also did not significantly alter investigation of the receptive females by Meth-treated sexually experienced males, compared to saline-injected controls, at any dosage, as there were no differences in time spent investigating the body or anogenital regions of the receptive female partners (Table 3.2).

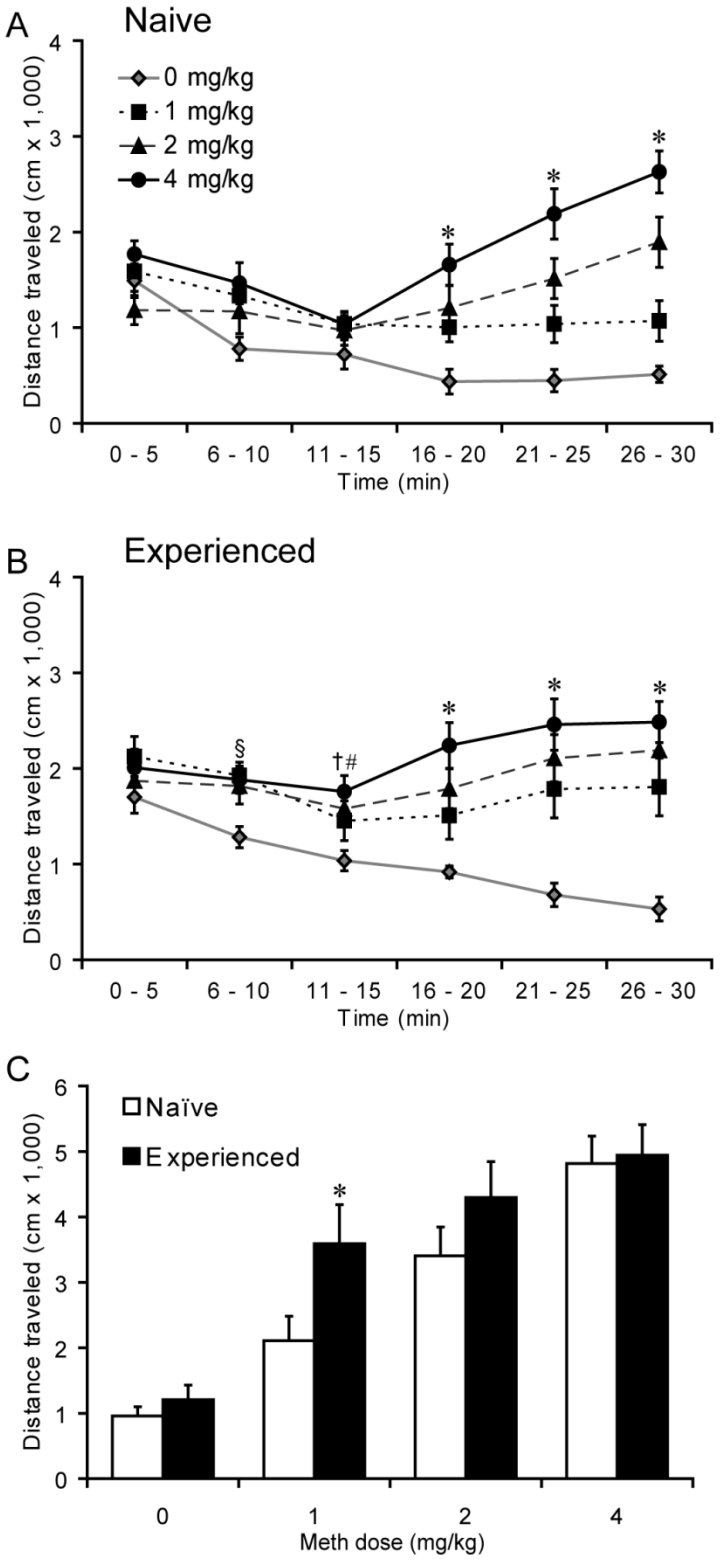
3.3.1B Locomotor activity

Meth at all dosages increased locomotor activity in both sexually naïve and experienced animals, but only in the second 15 minutes of the test ($F(3,28)=3.96$-23.13; $p=0.018$-<0.001). In naïve males each dosage of Meth treatment resulted in significantly greater locomotor activity when compared to controls 16-20 min ($p=0.031$, $p=0.006$, $p<0.001$; 1, 2, and 4 mg/kg, respectively), 21-25 min ($p=0.039$, $p=0.001$, $p<0.001$; 1, 2, and 4 mg/kg, respectively), and 26-30 ($p=0.049$, $p<0.001$, $p<0.001$; 1, 2, and 4 mg/kg, respectively) min following administration (Figure 3.2A). These effects were paralleled in sexually experienced males following each Meth treatment (Figure 3.2B). Meth treatment resulted in significantly greater locomotor activity when compared to controls 6-10 min ($p=0.013$; 1 mg/kg), 11-15 min ($p=0.024$, $p=0.003$; 2 and 4 mg/kg, respectively), 16-20 min ($p=0.038$, $p=0.004$, $p<0.001$; 1, 2, and 4 mg/kg, respectively), 21-25 min ($p=0.002$, $p<0.001$, $p<0.001$; 1, 2, and 4 mg/kg, respectively), and 26-30 min ($p=0.001$, $p<0.001$, $p<0.001$; 1, 2, and 4 mg/kg, respectively) following administration.

Investigative behavior	Meth dose (mg/kg)			
	0	**1**	**2**	**4**
Body investigation				
Number	8.7 ± 2.0	18.0 ± 5.2	20.4 ± 4.1	12.7 ± 2.2
Total duration	27.3 ± 4.9	55.8 ± 19.6	47.4 ± 16.8	26.1 ± 6.7
Anogenital investigation				
Number	6.7 ± 2.6	7.0 ± 2.4	3.2 ± 1.6	6.4 ± 4.1
Total duration	20.5 ± 8.8	23.8 ± 8.6	8.6 ± 5.0	12.3 ± 8.1
Attempted mounts				
Number	2.2 ± 0.9	4.0 ± 1.1	0.8 ± 0.8	2.7 ± 2.7

Table 3.2 Effects of Meth on investigative behavior of a sexually receptive female. Mean ± SEM total duration of body and anogenital investigation, numbers of body and anogenital investigations, and attempted mounts of a receptive female by males injected with 0, 1, 2, or 4 mg/kg Meth.

Figure 3.2 Effects of Meth on locomotor activity. Mean ± SEM distance traveled by sexually naïve (A) and experienced (B) males administered 0, 1, 2, or 4 mg/kg of Meth. * indicate significant differences from control for all treatment groups ($p<0.05$); # indicates significant differences between 4 mg/kg Meth and control only ($p<0.05$); † indicates significant differences between 2 mg/kg Meth and control only ($p<0.05$). § indicates significant differences between 1 mg/kg Meth and control only ($p<0.05$). Sexual experience-induced sensitized locomotor response to Meth. Mean ± SEM distance traveled by sexually naïve and experienced males administered 0, 1, 2, or 4 mg/kg Meth (C) during the last 10 min of locomotor activity recordings. * indicates significant difference from sexually naïve males of the same treatment group ($p<0.05$).


A
Naive
0 mg/kg
1 mg/kg
2 mg/kg
4 mg/kg
Distance traveled (cm x 1,000)
0 - 5
6 - 10
11 - 15
16 - 20
21 - 25
26 - 30
Time (min)
B
Experienced
§
†#
C
Naïve
Experienced
Meth dose (mg/kg)

Sexual experience resulted in an enhanced (sensitized) locomotor response to Meth, as sexually experienced males displayed significantly greater locomotor activity to 1 mg/kg Meth, 21-30 minutes following drug administration ($p=0.05$; Figure 3.2C). This effect of experience was not observed with 2 and 4 mg/kg Meth or saline administration.

3.3.2 Effects of methamphetamine on sexual inhibition: Experiment 2

3.3.2A Investigative behavior

Since in the previous experiment 1 mg/kg Meth did not affect sexual motivation or performance, this dose was used for the next set of experiments testing the effects of Meth on sexual risk behaviors. First, the effect of Meth on interactions with non-sexual partners was examined. Meth-injected males displayed a more persistent investigation of the non-receptive female partner as they spent a significantly greater amount of time investigating the bodies of non-receptive females ($p=0.05$) and displayed increased investigative approaches compared to saline-injected males ($p=0.006$; Figure 3.3A). Furthermore, Meth-injected males displayed significantly fewer numbers of attempted mounts of the female partners compared to saline-injected males ($p=0.024$; Figure 3.3E). There were no significant differences in any of the recorded parameters for anogenital investigation of the stimulus partners between Meth and saline-injected groups (Figure 3.3C and D). The only parameter of investigative behavior towards the male stimulus partner significantly altered by Meth compared to saline-treated controls was the duration of each body investigation ($p=0.028$; Figure 3.3B).

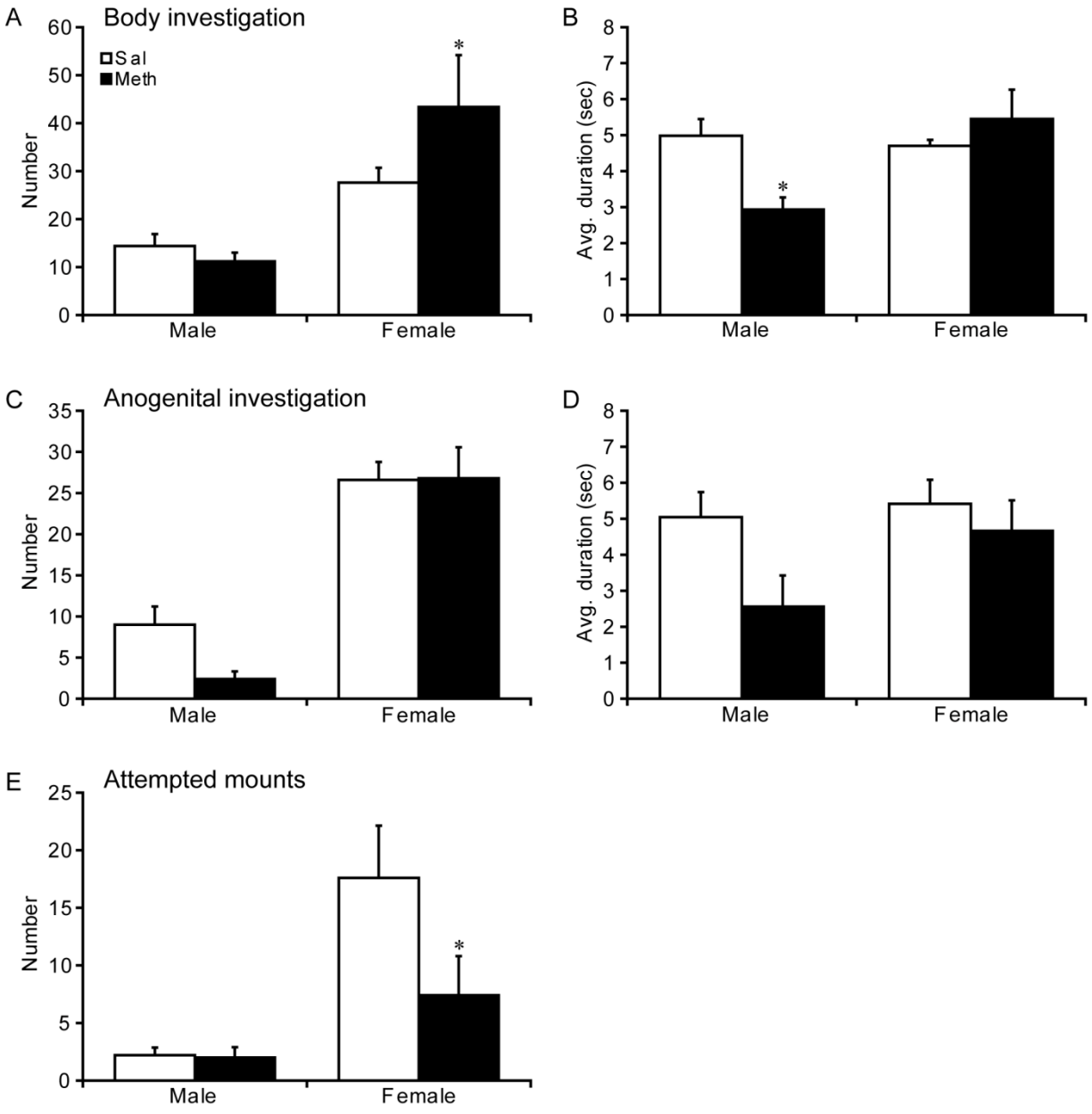


Figure 3.3 Effects of Meth on investigative behavior of a non-sexual stimulus partner. Mean ± SEM duration of each body (A) and anogenital (C) investigations, number of body (B) and anogenital (D) investigations, and attempted mounts (E) of a younger male or a non-receptive female stimulus partner by either saline- or Meth-injected males. * indicate significant difference from saline-injected males ($p<0.05$).

3.3.2B Conditioned copulation aversion

Conditioned copulation aversion induced by LiCl significantly inhibited sexual behavior in saline-pretreated males, while saline pairing had no effect on mating. Specifically, decreased percentages of LiCl-paired males that mounted and intromitted compared to unpaired were first evident on the fifth conditioning day ($p=0.013$, M and I) and persisted through conditioning days 6 ($p=0.013$, M and I), 7 ($p=0.004$, M and I), 8 ($p=0.038$, M and I), and 9 ($p<0.001$, M and I) (Figure 3.4A). A significant difference in the percentage of males that ejaculated was first evident on the fourth conditioning day ($p=0.038$) and persisted through conditioning days 5 ($p=0.001$), 6 ($p=0.024$), 7 ($p<0.001$), 8 ($p=0.024$), and 9 ($p<0.001$) (Figure 3.4B). Meth pretreatment affected the conditioned aversion as Meth-pretreated males paired with LiCl required more conditioning trials to reach significant inhibition of sexual behavior than saline-pretreated males. Meth pretreatment did not alter percentages of males that displayed mounts or intromissions during the first trial of conditioning, confirming that pretreatment with 1 mg/kg Meth did not alter sexual motivation or performance. However, Meth pretreatment increased the number of conditioning trials needed for significant inhibition of sexual behavior and percentages of males that displayed mounts or intromissions were not significantly decreased compared to unpaired Meth-pretreated males until the eighth ($p=0.013$, M and I) and ninth ($p=0.013$, M and I) conditioning day (Figure 3.4A). A significant difference in the percentage of males that ejaculated between LiCl-paired and -unpaired males within the Meth pretreatment group was evident on conditioning days 5 ($p=0.033$), 6 ($p=0.013$), 8 ($p=0.005$) and 9 ($p<0.001$) (Figure 3.4B). Thus, Meth pretreatment two weeks prior to onset of the conditioning paradigm affected the acquisition or expression of conditioned sex aversion.

During the final test, Meth administration increased the percentages of males displaying mounts, intromissions, and ejaculation by 20% in both saline- and Meth-pretreated LiCl-paired males. This increase in percentages failed to reach statistical significance when compared to the percentages during the final conditioning trial. However, the Meth-induced increases in percentages of LiCl-paired males that mounted or intromitted were sufficient to eliminate the statistically significant differences in Meth-pretreated males when compared to unpaired controls (either Meth- or saline-pretreated) during the final test. In contrast, significant differences of LiCl-paired males that mounted or intromitted persisted in saline-pretreated males ($p=0.007$; Figure 3.4A). Percentages of males that ejaculated following Meth administration during the final test remained significantly reduced in either Meth- ($p=0.005$) or saline- ($p=0.007$) pretreated LiCl-paired males compared to unpaired males (Figure 3.4B).

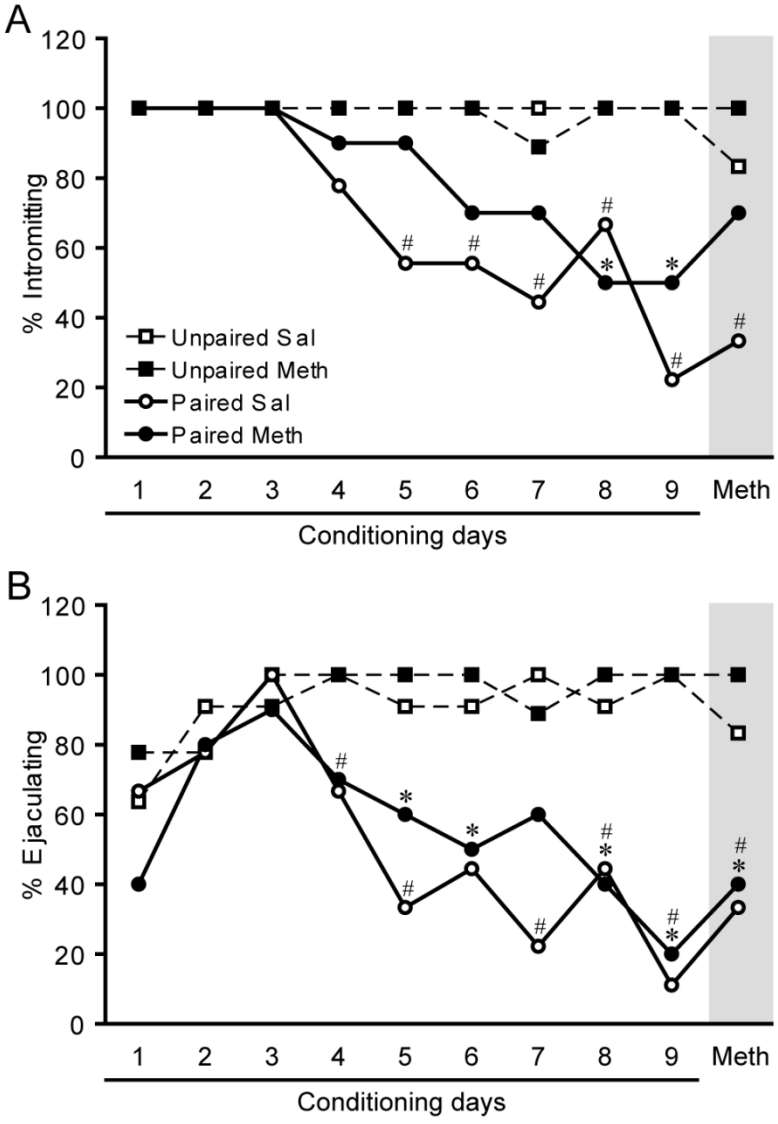


Figure 3.4 Effects of Meth on inhibition of maladaptive sex-seeking behavior. Percentages of males intromitting (A) and ejaculating (B) during aversive conditioning to copulation. Four groups were included: LiCl-unpaired pretreated with saline, LiCl-unpaired pretreated with Meth, LiCl-paired pretreated with saline, and LiCl-paired pretreated with Meth. During the final test, all males were treated with 1 mg/kg Meth. * indicate significant difference from Meth-pretreated unpaired males ($p<0.05$); # indicate significant difference from saline-pretreated unpaired males ($p<0.05$).

3.4 DISCUSSION

The current study examined the effects of acute Meth on sexual behavior and seeking of maladaptive sexual behavior in male rats. Meth administration 30 minutes prior to mating inhibited sexual motivation and performance. Moreover, Meth had short- and long-term effects on conditioned aversion for sex behavior. Meth treatment two weeks prior to the conditioned avoidance paradigm delayed the acquisition of inhibited sexual responses and Meth administration following conditioning resulted in loss of expression of inhibited sexual behavior.

The current findings demonstrated that acute doses of Meth increased the latencies to mount, intromit, and ejaculate and reduced the percentages of animals that initiated sexual behavior or completed copulation. Hence, Meth impaired sexual motivation as well as performance. However, number of mounts and intromissions were not affected, suggesting that Meth did not disrupt erectile function. Previous studies examining the effects of acute psychostimulant exposure on sexual behavior have been contradictory and either report facilitation of sexual behavior (Bignami 1966; Butcher et al. 1969) or no effect (Agmo and Fernandez, 1989; Agmo and Villalpando, 1995). Specifically, acute administration of D-amphetamine (Amph; i.p. 0.5 and 1 mg/kg) or amfonelic acid (0.25 and 0.5 mg/kg; i.p.) caused facilitation of sexual performance in sexually naïve male rats (Agmo and Berenfeld, 1990), but did not affect sexual motivation (Agmo, 2003). Moreover, amfonelic acid administration resulted in significantly increased frequency of sniffing and rearing behavior while behaviors associated with sexual behavior (female pursuit and mounting attempts) were not altered

(Agmo and Berenfeld, 1990). Therefore, Agmo and colleagues suggest that enhanced dopaminergic neurotransmission induced by psychostimulants may indirectly stimulate sexual behavior in male rats by increasing general arousal. It is unlikely that the effects of Meth on sexual behavior are caused by effects on locomotor activity, since of the dosages of Meth used in this study did not greatly affect locomotion. Effects of the higher dosages of Meth on sexual performance and motivation may have been influenced by effects on behavioral arousal and locomotor activity. However, the current data on effects of Meth on sexual performance differ from the findings with Amph and show decreased sexual performance with the higher dosages. In contrast, a previous study in male Japanese Quails failed to detect effects of Meth on sexual performance. Chronic administration of Meth (1 or 3 mg/kg daily for 10 consecutive days) followed by a 10 day withdrawal period reduced sexual motivation in male Japanese quails as measured by a one-arm runway test with visual access to a female (Bolin and Akins, 2009), but did not affect parameters of sexual performance. Hence, these effects of Meth do not appear to sustain in the absence of drug.

Human studies have indicated that one of the most prominent effects of drugs of abuse on sexual behavior is the increase in risk taking behaviors related to sexual activity. In male rats, sexual behavior is a highly rewarding behavior (Pfaus et al., 2001). Male rats will form conditioned place preference to copulation (Agmo and Berenfeld, 1990; Martinez and Paredes, 2001; Tenk et al., 2009) and complete operant responses to gain access to a receptive female (Everitt et al., 1987; Everitt and Stacey, 1987). However, sexual behavior does not generally lead to compulsive seeking of sex as male rats learn to

suppress mating when it is followed by an aversive stimulus. Therefore, the effects of Meth on maladaptive seeking of sex was tested using a reversal learning paradigm to establish copulation aversion by pairing mating with LiCl injections, resulting in inhibition of the initiation of sexual behavior (Agmo, 2002; Davis et al., 2010; Peters, 1983; Peters et al., 1989). The present data showed that a single low dose Meth treatment two weeks prior to onset of the conditioning paradigm, delayed acquisition of conditioned aversion. Moreover, Meth treatment following acquisition of conditioned sex aversion partially restored percentages of animals that initiated behavior. Hence, these findings suggest that Meth enhanced seeking of maladaptive sexual behavior following learned inhibition of mating. Similar findings have been reported for alcohol (Pfaus and Pinel, 1989; Pinel et al., 1992). Although, low and high doses of alcohol were found to inhibit sexual performance, Pfaus and colleagues (Pfaus and Pinel, 1989) tested whether alcohol facilitates sexual behavior by releasing it from inhibitory control. Male rats were first trained to inhibit mating when presented with a sexually non-receptive female, while maintaining normal sexual behavior with receptive females. Next, the ability of alcohol to override the learned inhibition of sexual behavior with non-receptive females was tested. Indeed, alcohol administration resulted in significantly increased percentages of males that displayed copulatory behaviors when exposed to non-receptive females, despite uncooperative behaviors of the females. In summary, alcohol disrupted learned inhibition of sexual behavior, even though sexual performance was impaired (Pfaus and Pinel, 1989).

An alternate explanation for the present findings is that Meth impaired learning, or reversal learning in particular. There is ample evidence that chronic use of Meth use leads to cognitive impairments and brain abnormalities in humans. Meth users have been found to perform poorly during visual and verbal memory tests, and show impaired attention and motor performance (Kalechstein et al., 2003; Ornstein et al., 2000; Simon et al., 2001). In addition, attenuated cognitive processes have been correlated to dopamine transporter reductions (Volkow et al., 2001), decreased cortical activation (Paulus et al., 2003), and structural brain abnormalities (Thompson et al., 2004). Similarly, it has been documented that chronic meth administration results in behavioral and neurobiological impairments in rats. Meth treated rats display learning deficits as reflected by deficient performance during radial maze (Chapman et al., 2001), Morris water maze (Friedman et al., 1998), and object recognition tasks (Schröder et al., 2003), as well as poor motor performance in avoidance and balance beam paradigms (Walsh and Wagner, 1992). Furthermore, Meth administration results in the degeneration of the dopaminergic and serotonergic systems of the brain (Chapman et al., 2001; Walsh and Wagner, 1992). Although the current study did not test whether Meth pretreatment impaired learning, it is unlikely that the Meth dose administered in the current study (one injection of 1 mg/kg Meth, s.c.) prior to sex aversion conditioning resulted in neurotoxicity and subsequent memory impairments. Single day Meth bingeing paradigms have established that four injections of 1 mg/kg Meth administered every two hours do not impair memory retention for familiar objects nor causes significant neurotoxicity of the dopaminergic and serotonergic circuitry (Marshall et al., 2007). Another alternate explanation for the delayed acquisition of conditioned aversion reported here is a loss of sensitivity for LiCl

following Meth treatment. However, since a single low dose of Meth was administered two weeks prior to conditioning, this appears unlikely. Qualitatively, animals displayed equal signs of visceral illness and discomfort, independent of the Meth or saline pretreatment. Although, Meth-induced memory impairments and changes in sensitivity to LiCl are unlikely factors to result in maladaptive sexual behavior reported in the current study, they cannot be ruled out completely and will be addressed in future studies.

This study does not address the mechanism by which Meth causes maladaptive sex seeking behavior. However, a recent neuroanatomical study from our laboratory has made progress in identifying potential sites where Meth may act to alter sexual behavior (Frohmader et al., 2010b). This study examined neural activation induced by sexual behavior or Meth using neural activity markers such as Fos and phosphorylation of MAP Kinase, respectively. Meth was shown to activate mating-activated neurons in the nucleus accumbens core and shell subregions, basolateral amygdala, and the anterior cingulate area of the medial prefrontal cortex (mPFC; (Frohmader et al., 2010b). Inability to alter behavior once it leads to negative outcomes is commonly associated symptom with a variety of psychiatric disorders (Dalley et al., 2004; Everitt and Robbins, 2005; Graybiel and Rauch, 2000; Reuter et al., 2005; Robbins and Everitt, 2002). The mPFC is of particular interest as it is well recognized for its role in addictive behaviors (Kalivas et al., 2005; Kalivas and Volkow, 2005). Moreover, hypoactivity of the mPFC has been correlated with several psychiatric conditions (Graybiel and Rauch, 2000; London et al., 2005; Taylor et al., 2002) suggesting that compulsive sexual behavior may be related with other disorders. Moreover, high incidence of compulsive sexual behavior overlaps

with other psychiatric disorders including drug addiction, anxiety, and mood disorders (Bancroft, 2008). In line with this, our laboratory recently showed that mPFC lesions result in maladaptive sex behavior utilizing the same conditioned aversion paradigm used in the current study (Davis et al., 2010). Therefore, these brain regions may be associated with the effects of Meth on maladaptive sex seeking and mPFC dysfunction caused by exposure to drugs of abuse is hypothesized to be responsible for reduced impulse control (Brewer and Potenza, 2008; Fineberg et al., 2009) and increased sex-directed behavior as observed in many addicts (Bancroft, 2008; Jentsch and Taylor, 1999).

In conclusion, together these studies form a critical step towards a better understanding of Meth effects on sexual motivation and performance and maladaptive seeking of sexual behavior. Furthermore, the results in male rats parallel those reported in human addicts, suggesting that this animal model can be used to further investigate the cellular mechanisms by which Meth alters sex behavior and potentially contribute to future treatment of Meth addiction.

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CHAPTER 4:

Concurrent exposure to methamphetamine and sexual behavior enhances subsequent drug reward and causes compulsive sexual behavior in male rats [1]

[1] Research article pushed in the Journal of Neuroscience (2011, in press)

4.1 INTRODUCTION

Sexual health related diseases within addict populations have raised awareness on the effects of drugs of abuse on sexual behavior, as chronic drug use is associated with unsafe sexual practices resulting in increased prevalence of sexually transmitted infections including Human Immunodeficiency Virus (HIV) (Crowe and George, 1989; Fisher et al., 2011; Peugh and Belenko, 2001; Raj et al., 2007; Sánchez et al., 2002). These effects of drugs on sexual behavior are well documented for the psychostimulant methamphetamine (Meth). Meth users often report heightened sexual desire, arousal, and pleasure and identify these factors as primary motivation for drug use (Green and Halkitis, 2006; Schilder et al., 2005; Semple et al., 2002). Moreover, Meth abuse is commonly associated with loss of inhibitory control of sex behavior or sexually compulsive behavior (Green and Halkitis, 2006; Halkitis et al., 2001; Mckirnan et al., 2001; Rawson et al., 2002) and increased prevalence of HIV (Frosch et al., 1996; Halkitis et al., 2001; Parsons and Halkitis, 2002).

Human reports demonstrating Meth use as a predictor of sexual risk behaviors are based on self-reports from chronic Meth users that lack a reliable measurement for the relationship between Meth use and sexual behavior (Frohmader et al., 2010b). Thus, an investigation into Meth-induced changes in sexual behavior under controlled experimental settings using an animal model is required for understanding the complex association between Meth and sexual behavior.

Recently, our laboratory has examined the effects of acute Meth on compulsive sex-seeking in male rats (Frohmader et al., 2010a). These studies utilized a conditioned sex aversion paradigm in which male rats learned to associate mating with subsequent visceral illness (Agmo, 2002; Peters, 1983). Once this association between mating and the aversive stimulus was established, animals would not initiate mating behavior (Davis et al., 2010; Frohmader et al., 2010a). Meth pretreatment of a single injection weeks prior to conditioning disrupted the acquisition of inhibited sexual responses (Frohmader et al., 2010a). Thus, Meth-pretreated male rats were seeking sex even though mating was associated with an aversive stimulus; hence this was termed maladaptive or compulsive mating.

As previous studies tested the effects of an acute drug injection and research investigating the effects of repeated Meth on male rat sexual behavior is limited, the main goal of the current study was to investigate the effects of repeated Meth administration on different aspects of sexual behavior including performance, compulsive seeking of sex, and reward. First, the effects of Meth on mating were tested following drug administration and after periods of drug abstinence to distinguish between short and long term effects of Meth on sexual function. Next, the effects of repeated Meth administration on maladaptive sex behavior were investigated implementing the conditioned sex aversion paradigm. In addition, it was determined whether learned associations between repeated Meth exposure and sexual behavior were essential for the effects of Meth on maladaptive sex behavior. Finally, it was tested if repeated Meth

exposure results in enhanced reward for Meth and/or mating as determined by conditioned place preference (CPP) paradigms.

4.2 MATERIALS AND METHODS

4.2.1 Subjects

Adult male Sprague-Dawley rats (210-225 grams) were obtained from Charles River Laboratories (Montreal, QC, Canada; Wilmington, MA, USA) and housed in same-sex pairs in standard Plexiglas cages (home cages) containing pieces of PVC pipe for environmental enrichment. Animals were housed in a room maintained at a 12/12 h reversed light/dark cycle (lights off at 11.00 h) with food and water available *ad libitum*. All testing was performed during the dark cycle under red illumination. Stimulus females (Charles River Laboratories; 200-225 grams) used for sexual behavior were bilaterally ovariectomized and received a subcutaneous implant containing 5% estradiol benzoate (EB) and 95% cholesterol. To induce sexual receptivity, females were administered 0.5 mg progesterone in 0.1 ml sesame oil (s.c.) 4 h prior to sexual behavior. Experimental procedures were approved by the Animal Care Committee at the University of Western Ontario and the University of Michigan Committee on Animal Care and Use and were in agreement with guidelines outlined by the Canadian Council on Animal Care and National Institutes of Health.

4.2.2 Experimental designs

4.2.2A Sexual behavior

The current experiment investigated the effects of repeated Meth on sexual performance and motivation immediately following drug injection and following drug abstinence periods. Thirty three male rats gained sexual experience in separate test cages (mating arenas; 60 x 45 x 50 cm) containing clean bedding during five twice-weekly mating sessions. During each mating session, males were allowed to mate with a receptive female until the display of 1 ejaculation or for 1 hr, whichever occurred first. One week following the last mating session, males were habituated to experimental procedures and received a subcutaneous (s.c.) injection of 1 ml/kg of saline for three consecutive days. Following each injection animals were placed in Plexiglas Locomotor Activity Chambers (40.5 by 40.5 cm; Med Associates, St. Albans, VT, USA) equipped with 16x16 photobeam arrays and locomotor activity was recorded for 30 min. In addition to recording ambulatory behavior following treatment injection, placing males in the locomotor chambers provided a drug-associated environment distinct from that of mating behavior. Next, males received a daily injection of 1 or 2 mg/ml/kg Meth or vehicle (saline; 1 ml/kg) (n=11 each) for 7 consecutive days. Following each injection, males were placed in the locomotor activity chambers and locomotor activity was recorded for 30 min, after which they returned to their holding cages. On the last day of Meth administration, males were removed from the locomotor activity chamber after 30 minutes, and placed in the mating arena to test for effects of Meth on sexual behavior.

Animals were tested again for sexual behavior in the mating arenas following one day or one week of drug abstinence.

During the mating sessions, standard parameters for sexual behavior were observed and recorded, including latencies to mount (time from introduction of female to first mount) and intromission (time from introduction of female to first intromission), which are indicative of sexual motivation (Hull et al., 2002), as well as latency to ejaculation (time from first intromission to ejaculation), numbers of mounts and intromissions prior to ejaculation, and post-ejaculatory interval, which are measures of sexual performance (Hull et al., 2002; Pfaus, 2009). Differences between groups were determined for each parameter of sexual behavior using non-parametric Kruskal-Wallis analysis and Dunn's post hoc comparisons, at significance levels of 0.05.

4.2.2B Locomotor activity

Locomotor activity following each Meth injection was analyzed using Med Associates analysis software as distance traveled within five-minute intervals. Group differences were examined using non-parametric Kruskal-Wallis analysis and Dunn's post hoc comparisons. In addition, to examine Meth-induced locomotor sensitization, Meth-induced activity during the last 10 minutes of the test was compared between the first and seventh injection day within each drug dose treatment group using paired *t* tests. A significance level of 0.05 was applied to all comparisons.

4.2.2C Conditioned sex aversion

Experiment 1: First, 50 male rats were habituated to saline injections for three consecutive days and males gained sexual experience during three mating sessions. Prior to each mating session, animals were injected with either 1 mg/kg (s.c.) Meth or saline (1 ml/kg), placed in mating arenas and 30 min later were allowed to mate with a receptive female until ejaculation or 1 hr. Parameters for sexual behavior were recorded and analyzed as described above. Two weeks later, animals were subjected to a conditioned sex aversion paradigm. Males were subdivided into four experimental groups according to pretreatment (Meth or saline) and conditioning (lithium chloride (LiCl)-paired or -unpaired): saline unpaired (n=12), Meth unpaired (n=12), saline paired (n=13), and Meth paired (n=13). The conditioned aversion paradigm consisted of eight consecutive two-day conditioning trials. During the first day, all males were placed in the mating arena for a 10 min habituation period after which a receptive female was introduced. Females were scented by swabbing almond oil on the neck and base of the tail prior to mating as olfactory cues have been shown to facilitate male approach behavior and to strengthen conditioning (Agmo, 2002; Lawrence and Kiefer, 1987). Males were allowed to mate for 30 min or until 1 ejaculation. If intromissions did not occur within the first 15 min mating was terminated. One minute following ejaculation or trial termination males were given a 127.2 mg/kg intraperitoneal (i.p.) injection of LiCl administered at a volume of 10 ml/kg (LiCl for paired males) or saline (unpaired males). LiCl or saline were administered regardless of whether mating occurred. The following day, unpaired males received a 10 ml/kg injection of LiCl while paired males received saline. Animals were returned to the home cage after injections.

Experiment 2: To test if the effects of Meth pretreatment on conditioned sex aversion were dependent on the simultaneous exposure to Meth and mating or due to Meth alone, an additional experiment was conducted. Male rats (n=20) received sexual experience during five mating sessions but without Meth or saline treatments (n=10 each). Instead, one week after sexual experience they received 7 once-daily injections of either Meth (1 mg/kg; s.c.) or saline and two weeks later were subjected to the conditioned sex aversion paradigm described above.

For both experiments and during each conditioning trial parameters for sexual behavior were analyzed and group differences were determined for each conditioning trial using a two-way ANOVA (factors: *Meth/Saline pretreatment, conditioning*). Pearson chi-square analysis was used to compare differences between groups in the percentages of males that displayed mounts, intromissions, or ejaculation within each conditioning trail.

4.2.2D Conditioned place preference

To test if Meth pretreatment affected reward for Meth or sexual behavior, CPP experiments were conducted. A three-compartment apparatus (Med Associates, St. Albans, VT, USA) containing two larger outer chambers (28 × 22 × 21 cm) with distinguishable visual and tactile cues, separated by a small central compartment (13 × 12 × 21 cm) was used for all CPP experiments. Doors on both sides of the central compartment separated the chambers, and could be raised to allow the animals' free movement throughout the apparatus, or lowered to confine them to a particular area. The

apparatus was equipped with photo-beams to measure the time spent in each chamber. On the first day, a 15 min pretest was conducted to determine each animal's initial chamber preference, whereby each animal was allowed to roam freely between chambers of the CPP apparatus. No significant preferences for either chamber were detected between experimental groups. Animals were excluded from the study if they displayed an extended preference for a specific chamber (a difference over 120 seconds; shown by less than 10% of the subjects). Conditioning was conducted during days two and three. During conditioning, the initially un-preferred chamber (paired chamber) was paired with a reward manipulation for 30 min. The initially preferred chamber (unpaired chamber) was paired with a control manipulation. The order in which the animals were exposed to the paired and unpaired chambers was counterbalanced within each experimental group. A posttest that was procedurally identical to the pretest was conducted on the fourth and final day.

Experiment 1: First, 50 male rats were habituated to saline injections for three consecutive days and males gained sexual experience during three mating sessions. During each mating session, animals were injected with either 1 mg/kg (s.c.) Meth or saline (1 ml/kg), placed in test cages and 30 min later were allowed to mate with a receptive female until ejaculation or 1 hr. Parameters for sexual behavior were recorded and analyzed as described above. One week later, animals were distributed into four experimental groups matched for drug treatment and sexual performance for CPP testing. During conditioning, males were injected with either Meth or saline (matching the prior drug treatment) and 30 min later were allowed to mate until ejaculation. One min

following ejaculation the animal was placed into the paired chamber. The unpaired chamber was associated with either an injection of Meth or saline, or mating alone. Following the posttest, a preference score (the percentage of time spent in the paired chamber during the pretest and posttest; calculated as time spent on paired chamber divided by time in paired + unpaired chamber x 100) and CPP score (experiment 1; difference in time spent in the paired chamber during the posttest minus the pretest) were calculated for each subject. Preference scores were compared within experimental groups using paired *t* tests and CPP scores were compared between experimental groups using a one-way ANOVA and Fisher's LSD for *post hoc* comparisons, all with 95% confidence levels.

Experiment 2: To test if effects of Meth pretreatment on CPP for Meth or mating where dependent on the simultaneous exposure to Meth and mating or due to Meth alone, an additional experiment was conducted. Male rats received Meth (1 mg/kg) and mating simultaneously for 4 consecutive days (n=10). Two control groups remained sexually naïve and received either Meth or saline (n=10 each). One week later, CPP for Meth was conducted. All males received an injection of Meth in the paired chamber and a saline injection was associated with the unpaired chamber. Preference scores were calculated and compared within experimental groups using paired *t* tests with significance level of 0.05.

Experiment 3: To test if simultaneous exposure to Meth and mating is critical for altered sexual reward, a mating CPP study was conducted. Male rats received either Meth (1

mg/kg) or saline simultaneously with mating for 4 consecutive days (n=10 each). One week later, CPP for sexual behavior was tested. All males were placed in the paired chamber following mating and no mating was associated with the unpaired chamber. Preference scores were calculated and compared within experimental groups using paired *t* tests with significance level of 0.05.

4.2.2E Conditioned place aversion

To test whether Meth exposure alters sensitivity to LiCl-induced illness a conditioned place aversion (CPA) experiment was conducted. CPA testing was conducted during the first half of the dark period using the same apparatus as used for CPP experiments described above. For three consecutive days, male rats gained sexual experience concurrently with Meth (1 mg/kg) or saline (n=10 each). One week later, all males received a LiCl injection (10 ml/kg; i.p.) paired with the initially preferred chamber, while an equivalent dose of saline was associated with the initially un-preferred chamber. Following the posttest, mean preference score (the percentage of time spent in the paired chamber during the pretest and posttest; calculated as time spent on paired chamber divided by time in paired + unpaired chamber x 100) and CPA score (difference in time spent in the paired chamber during the posttest minus the pretest) were calculated for each subject. Preference scores were compared within experimental groups using paired *t* tests, while CPA scores were compared between experimental groups using unpaired *t* tests, all with significance level of 0.05.

4.3 RESULTS

4.3.1 Sexual behavior

Meth significantly affected initiation of sexual behavior when mating was tested 30 minutes following the last drug injection. This effect was dose dependent as 2 mg/kg, but not 1 mg/kg Meth significantly increased mount and intromission latencies ($p=0.001$ and 0.002 respectively) compared to saline controls (Figure 4.1A). Meth did not affect the percentages of males that initiated behavior, and 100% of males mated in all 3 treatment groups. Meth did not have longer term effects on initiation of sexual behavior as Meth-pretreated males did not display altered mating behavior compared to saline-pretreated controls when mating was tested during drug abstinence days 1 and 7 (Figure 4.1B and C). Finally, Meth did not affect sexual performance at any time as there were no effects on latencies to ejaculation (Figure 4.1), numbers of mounts and intromissions (data not shown). Thus, repeated Meth impaired initiation of mating when tested shortly after administration, but did not have long term effects on sexual motivation or performance.

4.3.2 Locomotor activity

Meth at either 1 or 2 mg/kg doses increased locomotor activity compared to controls ($p<0.001$, 1 and 2 mg/kg; Figure 4.2A and B). Repeated Meth administration resulted in a sensitized locomotor response as males that were administered 1 mg/kg Meth displayed a significantly greater locomotor activity following the last drug injection compared to the first injection ($p=0.042$; Figure 4.2C). In contrast, 2 mg/kg Meth resulted in significantly

decreased locomotor activity on the last day compared to the first day ($p=0.009$; Figure 4.2C), which may be indicative of increases in stereotypic behaviors.

4.3.3 Conditioned sex aversion

4.3.3A Sexual behavior

During the Meth pretreatment phase of the experiment, sexual behavior was unaffected by 1 mg/kg Meth treatment during each of the 3 subsequent sessions, compared to saline-pretreated males (Table 4.1). These results confirm lack of effects of this dose of Meth on sexual behavior, even when administered in the same environment. Moreover, Meth pretreatment did not alter sexual behavior during the first day of the conditioning paradigm (prior to LiCl pairing; Table 4.1) or during any of the conditioning days in the LiCl-unpaired groups. These results confirm that Meth did not have long term effects on sex behavior.

4.3.3B Compulsive sexual behaviour

Experiment 1: In contrast, repeated Meth treatment did enhance compulsive sex seeking. In control, saline-pretreated animals, conditioned sex aversion significantly inhibited sexual behavior. Specifically, decreased percentages of LiCl-paired males that mounted and intromitted compared to unpaired saline-pretreated males were first evident on the sixth ($p=0.039$) conditioning trial and persisted through conditioning trials 7 ($p=0.005$; data not shown) and 8 ($p<0.001$) (Figure 4.3B). A significant difference in the percentage of males that ejaculated was first evident on the fourth ($p=0.041$) conditioning trial and persisted throughout conditioning ($p<0.001$) (Figure 4.3C). However, Meth pretreatment did affect conditioned sex aversion as Meth-pretreated males paired with LiCl did not

reach significant inhibition of sexual behavior until the last conditioning trial compared to unpaired Meth-pretreated males. Specifically, the percentages of Meth-pretreated LiCl-paired males displaying intromissions and ejaculation were significantly decreased only during conditioning trial 8 ($p=0.03$ and $p=0.011$, respectively). Thus, Meth pretreatment two weeks prior to the onset of conditioning resulted in maladaptive or compulsive sex-seeking behavior.

Experiment 2: The effects of Meth pretreatment on conditioned sex aversion were dependent on concurrent Meth and mating experience. Specifically, conditioned sex aversion was not affected in sexually experienced males that received Meth pretreatment and mating experience at different times (non-concurrent). Percentages of Meth-pretreated LiCl-paired males that displayed mounts and ejaculations were not different from saline-pretreated paired males (Figure 4.4). These data suggest that the initial association between Meth and sexual experience was a contributing factor to the effects of Meth on compulsive sex behavior.

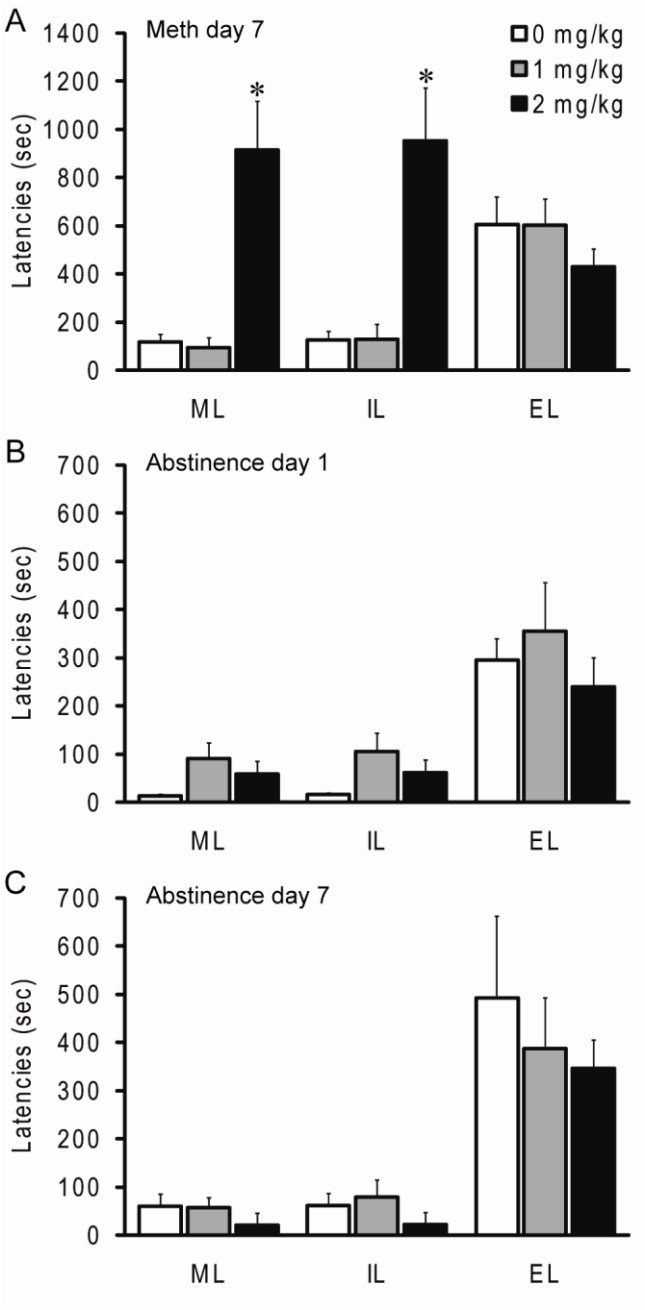
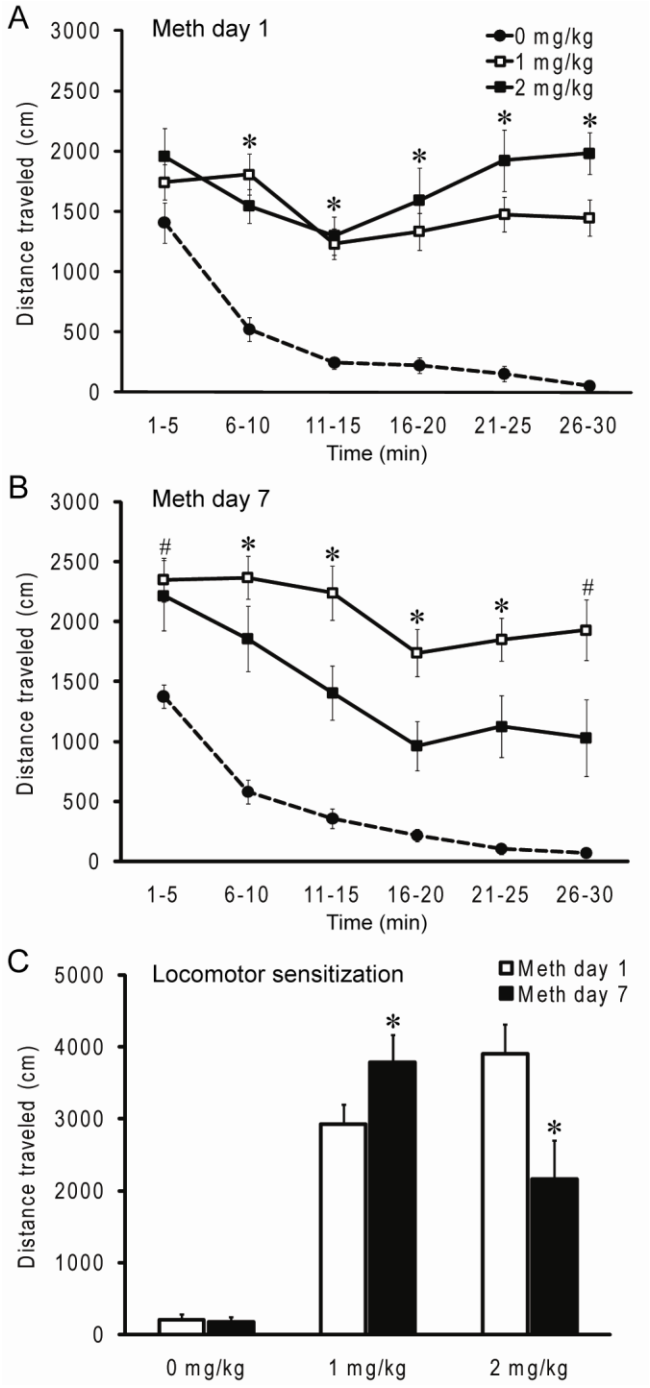


Figure 4.1 Effects of repeated Meth on sexual performance. Latencies to mount (ML), intromission (IL), and ejaculation (EL) following administration of 0, 1, or 2 mg/kg Meth 30 min following the seventh and last drug injection (A), drug abstinence days 1 (B) and 7 (C). Data are presented as mean ± SEM. * indicate significant differences from saline injected males ($p<0.05$).

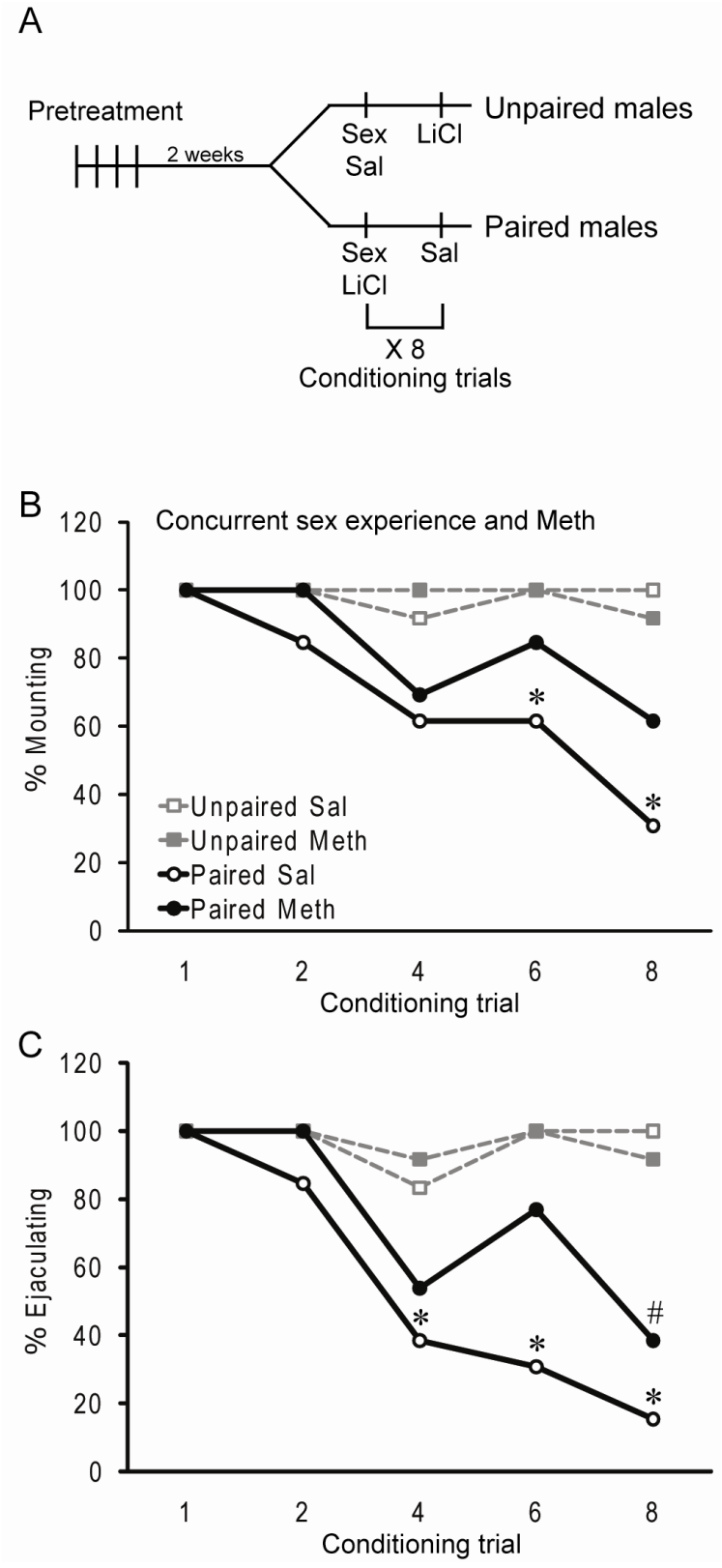
Figure 4.2 Effects of repeated Meth on locomotor activity. Distance traveled by males administered 0, 1, or 2 mg/kg of Meth following the first (A) and last (B) Meth injection. Data are presented as mean ± SEM. * indicate significant differences from control for all treatment groups ($p<0.05$); # indicate significant differences between 1 mg/kg Meth and control only ($p<0.05$). Meth-induced sensitized locomotor response. Distance traveled by males administered 0, 1, or 2 mg/kg Meth following the first and last Meth injection during the last 10 min of locomotor activity recordings (C). Data are presented as mean ± SEM. * indicates significant difference from sexually naïve males of the same treatment group ($p<0.05$).


A
Meth day 1
0 mg/kg
1 mg/kg
2 mg/kg
Distance traveled (cm)
3000
2500
2000
1500
1000
500
0
1-5
6-10
11-15
16-20
21-25
26-30
Time (min)
B
Meth day 7
Distance traveled (cm)
Time (min)
C
Locomotor sensitization
Meth day 1
Meth day 7
Distance traveled (cm)
5000
4000
3000
2000
1000
0
0 mg/kg
1 mg/kg
2 mg/kg

Pretreament	Mating Session		Conditioning Day
	1	3	1
Sex+Saline			
ML	121.8 ± 23.3	211.3 ± 123.6	56.2 ± 26.4
IL	132.8 ± 22.7	260.4 ± 123.3	89.3 ± 32.4
Sex+Meth			
ML	156.4 ± 47.9	320.3 ± 162.4	55.2 ± 10.8
IL	168.1 ± 47.1	322.8 ± 161.9	60.5 ± 11.8

Table 4.1 Overview of sexual behavior during mating sessions 1 and 3 (30 minutes following saline or Meth administration) and during the first day of the conditioned sex aversion paradigm (experiment 1; 2 weeks following pretreatment of mating concurrent with saline or Meth). Latencies to mount (ML) and intromission (IL) are presented as mean ± SEM.

Figure 4.3 Effects of concurrent mating (sex) and Meth pretreatment on conditioned sex aversion (experiment 1). (A) Experimental groups included: Saline- or Meth-pretreated males that received LiCl following mating (Paired males) and Saline- or Meth-pretreated males that received saline following mating (Unpaired males). During the second day of each conditioning trial, paired males received Sal and unpaired males received LiCl. Percentage of males mounting (B) and ejaculating (C) during conditioned sex aversion following Meth pretreatment administered simultaneously with sexual experience. * indicate significant difference from saline-pretreated unpaired males ($p<0.05$); # indicate significant difference from Meth-pretreated unpaired males ($p<0.05$).


A
Pretreatment
2 weeks
Sex
LiCl
Sal
Unpaired males
Sex
Sal
LiCl
Paired males
X 8
Conditioning trials
B
Concurrent sex experience and Meth
% Mounting
120
100
80
60
40
20
0
Unpaired Sal
Unpaired Meth
Paired Sal
Paired Meth
1
2
4
6
8
Conditioning trial
C
% Ejaculating
120
100
80
60
40
20
0
#
1
2
4
6
8
Conditioning trial

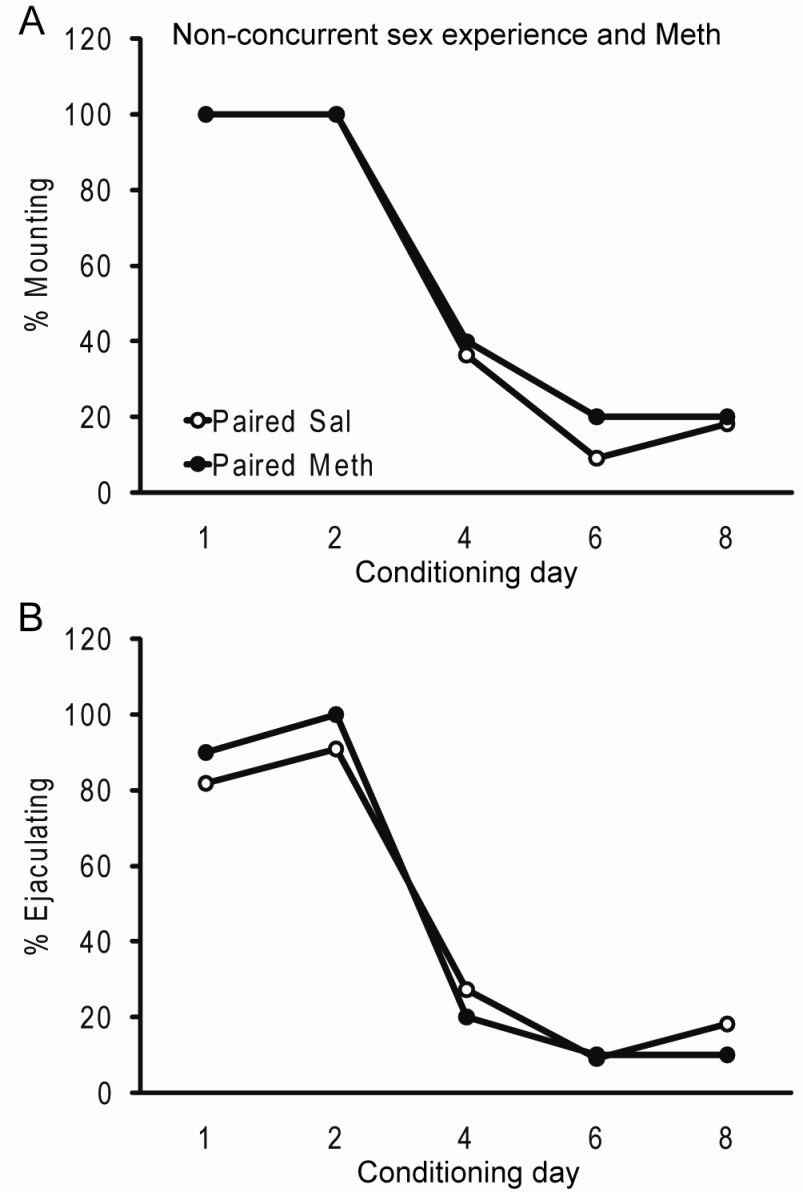


Figure 4.4 Effects of non-concurrent mating (sex) and Meth pretreatment on conditioned sex aversion (experiment 2). Percentage of males mounting (A) and ejaculating (B) during LiCl conditioned sex aversion following Meth pretreatment not associated with sexual experience. Two groups were included: LiCl-paired pretreated with saline and LiCl-paired pretreated with Meth.

4.3.4 Conditioned place aversion

Additional control experiments revealed that the inability to inhibit mating following Meth pretreatment is not due to a blunted sensitivity to LiCl-induced visceral illness as all males formed an aversion to the chamber associated with a single dose of LiCl. Specifically, both saline- and Meth-pretreated males spent significantly less time in the LiCl-paired chamber during the posttest compared to the pretest ($p=0.037$ and $p=0.045$ respectively; Figure 4.5A). Moreover, the difference of time spent in the LiCl-paired chamber following the posttest versus pretest was identical in Meth- and saline-pretreated groups (Figure 4.5B).

4.3.5 Conditioned place preference

Experiment 1: Self-report studies reveal that Meth use enhances sexual pleasure and is primary motivation for drug use (Green and Halkitis, 2006; Schilder et al., 2005; Semple et al., 2002). This Meth-induced enhancement of sexual pleasure has not been tested in the rodent model. Therefore, CPP paradigm was used to test whether sexual behavior with Meth is more rewarding than either mating or Meth administration alone. In agreement with previous studies (Agmo and Berenfeld, 1990; Pfaus and Phillips, 1991; Tenk et al., 2009), mating in saline-pretreated control males resulted in CPP as males spent more time in the Sex+Saline-paired chamber than the saline-paired chamber during the posttest ($p=0.001$; Figure 4.6C and D). In addition, control males did not form a preference for the Sex+Saline-paired chamber over the sex-paired chamber, demonstrating that a saline injection prior to mating did not affect sexual reward (Figure 4.6C and D). Results showed that Meth increased CPP for sex compared to either mating

or Meth alone. Males spent more time during the posttest in the Sex+Meth-paired chamber than the sex-paired chamber ($p<0.001$; Figure 4.6C) and the Meth-paired chamber ($p=0.02$; Figure 4.6C), or compared to the control group ($p=0.002$ and 0.05 respectively; Figure 4.6D). Therefore, sexual behavior concurrent with Meth appears to be more rewarding than sexual behavior or Meth alone in animals that were pretreated with sexual behavior and Meth concurrently.

Experiment 2: Next, it was determined if concurrent pretreatment of Meth and sex influenced CPP for Meth alone compared to saline treatment in the unpaired chamber. Indeed, Meth-pretreated males that mated concurrently with each drug injection formed a preference for the Meth-paired chamber ($p=0.01$; Figure 4.7). In contrast, males that received repeated saline or Meth injections without the context of mating did not show increased preference for the Meth-paired chamber during the post test.

Experiment 3: Finally, it was tested if concurrent Meth and mating pretreatment affected CPP for mating alone. Males pretreated with Meth and mating did not form a preference for sexual behavior, evidenced by a lack of increased time spent in the sex-paired chamber. In contrast, males that were treated with saline and mating did form a preference for the sex-paired chamber ($p=0.003$; Figure 4.8). Together, these data suggest that the association between Meth and mating results in increased incentive salience for Meth in the absence of mating and for mating concurrently with Meth, but reduced incentive salience for mating in the absence of the drug.

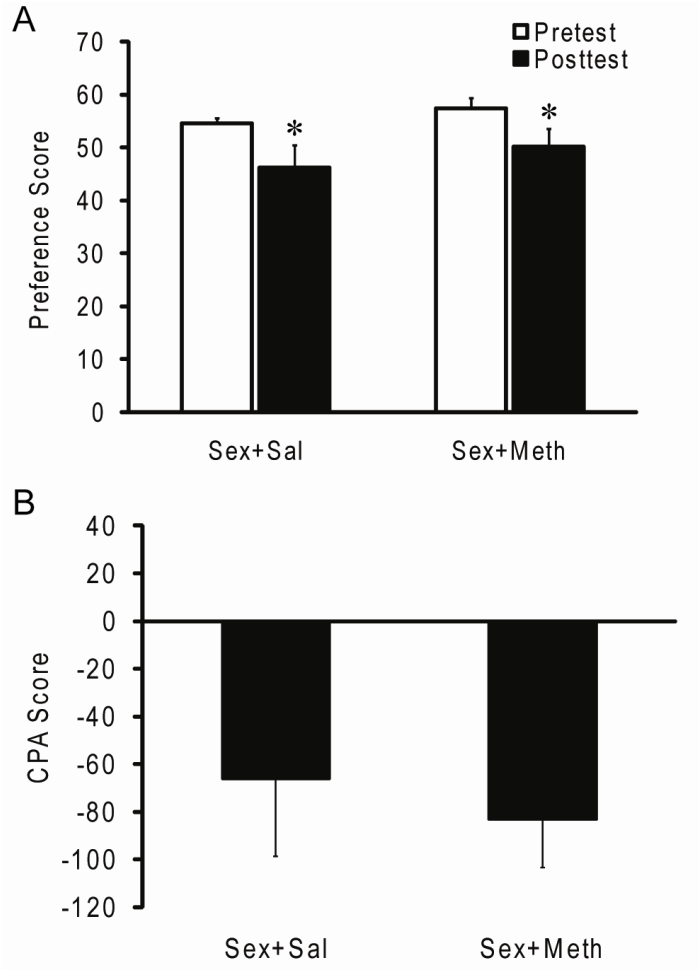
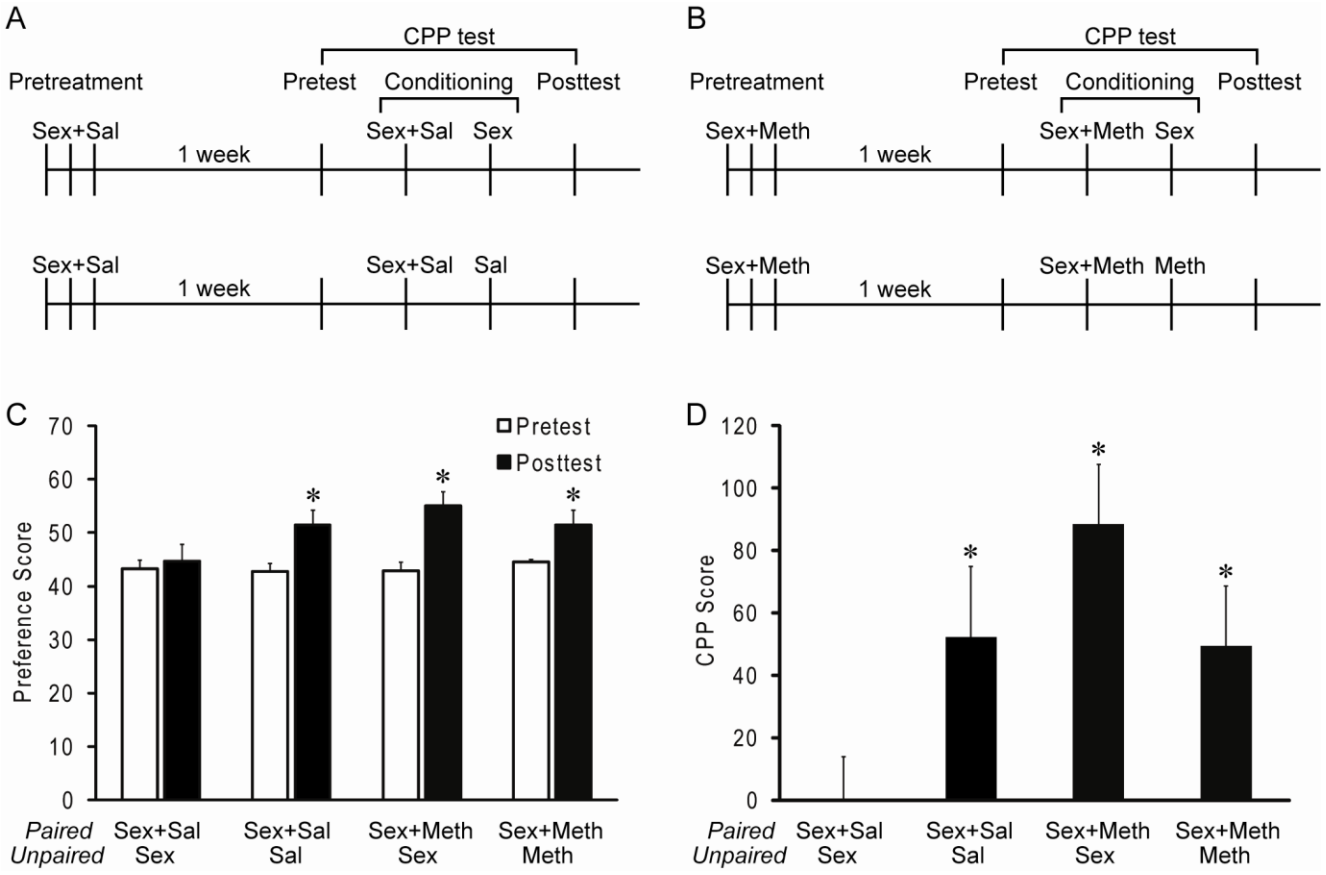


Figure 4.5 Effects of concurrent mating (sex) and Meth pretreatment on LiCl-induced CPA. Preference score (A; time spent on paired chamber divided by time in paired + unpaired chamber x 100) and CPA score (B; difference in time spent in the paired chamber during the posttest minus to pretest) in mated males pretreated with saline (Sex+Saline) or Meth (Sex+Meth). Data are presented as mean ± SEM. * indicate significant differences from pretest within the same experimental group ($p<0.05$).

Figure 4.6 Effects of concurrent mating (sex) and Meth pretreatment on mating- and Meth-induced CPP (experiment 1). Four groups were included. Two groups received sex+saline pretreatment and the following treatment in the paired/unpaired chamber: Sex+Sal/Sex, Sex+Sal/Sal (A). The first group served as a negative control as saline is not expected to alter CPP for sex. The second group served as a positive control as sex is expected to cause CPP. The other two groups received sex+Meth pretreatment and the following in the paired/unpaired chambers: Sex+Meth/Sex or Sex+Meth/Meth (B). The order in which the animals were exposed to the paired and unpaired chambers was counterbalanced within each experimental group. Preference score (C; time spent on paired chamber divided by time in paired + unpaired chamber x 100). Data are presented as mean ± SEM. * indicate significant differences from pretest within the same experimental group ($p<0.05$). CPP score (D; difference in time spent in the paired chamber during the posttest minus the pretest). Data are presented as mean ± SEM. * indicate significant differences from the Sex+Sal/Sex group ($p<0.05$).


A
CPP test
Pretreatment
Pretest
Conditioning
Posttest
Sex+Sal
1 week
Sex+Sal
Sex
Sex+Sal
1 week
Sex+Sal
Sal
B
CPP test
Pretreatment
Pretest
Conditioning
Posttest
Sex+Meth
1 week
Sex+Meth
Sex
Sex+Meth
1 week
Sex+Meth
Meth
C
Pretest
Posttest
Preference Score
70
60
50
40
30
20
10
0
Paired
Unpaired
Sex+Sal
Sex
Sex+Sal
Sal
Sex+Meth
Sex
Sex+Meth
Meth
D
CPP Score
120
100
80
60
40
20
0
Paired
Unpaired
Sex+Sal
Sex
Sex+Sal
Sal
Sex+Meth
Sex
Sex+Meth
Meth

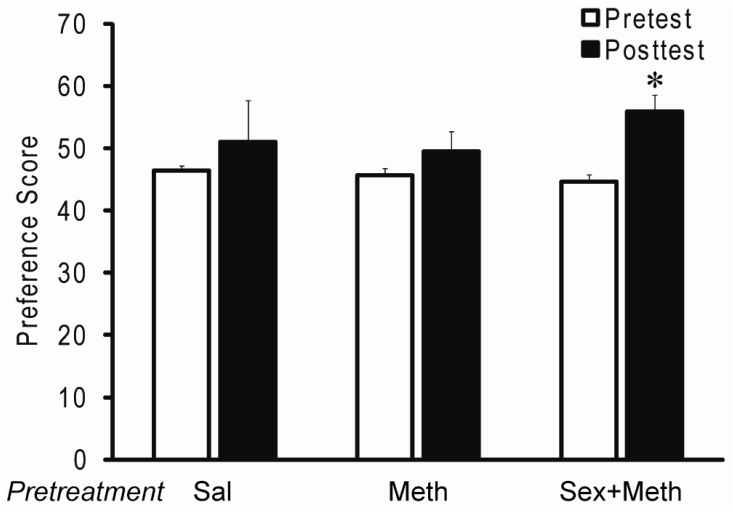


Figure 4.7 Effects of concurrent mating (sex) and Meth pretreatment on Meth-induced CPP (experiment 2). Preference score (time spent on paired chamber divided by time in paired + unpaired chamber x 100) in males pretreated with Saline, Meth, or Sex+Meth. Data are presented as mean ± SEM. * indicates significant difference from pretest within the same experimental group ($p<0.05$).

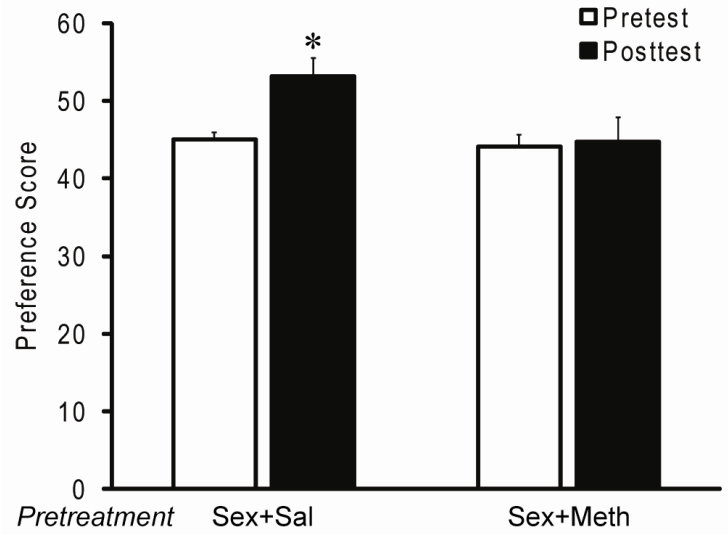


Figure 4.8 Effects of concurrent mating (sex) and Meth pretreatment on mating-induced CPP (experiment 3). Preference score (time spent on paired chamber divided by time in paired + unpaired chamber x 100) in males pretreated with Sex+Saline or Sex+Meth. Data are presented as mean ± SEM.

4.4 DISCUSSION

The current study tested the effects of repeated Meth on sexual behavior with specific focus on sexual performance, maladaptive or compulsive sex-seeking, and sex and/or Meth reward. The main finding of this study was that Meth pretreatment did not affect expression of sexual behavior, but caused compulsive sexual behavior weeks following pretreatment. This effect on compulsive sex behavior was dependent on the concurrent experience with Meth and mating. Furthermore, concurrent Meth and mating pretreatments enhanced Meth reward, but reduced sex reward. Together, these studies show that an association between Meth and mating is critical for the development or expression of compulsive sex behavior and changes in sex and drug reward.

Meth pretreatment, when concurrent with mating, had long term effects on the ability to inhibit sexual behavior in the conditioned sex aversion paradigm. This effect can not readily be explained by a deficit in learning or memory, since Meth-pretreated males did not show any evidence of impaired learning during CPP or LiCl-induced sex aversion paradigms. In addition, it is unlikely that repeated administration of the low dose of Meth caused cognitive impairments and neurotoxicity as following chronic exposure to high doses of Meth in rats (Chapman et al., 2001; Friedman et al., 1998; Schröder et al., 2003; Walsh and Wagner, 1992) and humans (Kalechstein et al., 2003; Ornstein et al., 2000; Simon et al., 2001). Single day Meth bingeing paradigms using the same dose as the current study did not impair object recognition learning nor resulted in neurotoxicity (Marshall et al., 2007). Another alternate explanation for the impaired acquisition or expression of conditioned sex aversion is a loss of sensitivity for LiCl. However, animals

were equally capable of acquiring a conditioned aversion to a chamber previously paired with LiCl. Hence, Meth-pretreated males did not have impaired associative memory nor reduced sensitivity to LiCl or LiCl-induced illness. It appears that Meth pretreatment caused maladaptive or compulsive sex seeking, despite learned negative consequences, which is in line with human reports (Frosch et al., 1996; Green and Halkitis, 2006; Halkitis et al., 2001; Mckirnan et al., 2001; Rawson et al., 2002; Somlai et al., 2003; Springer et al., 2007).

Moreover, the effect of Meth and sex pretreatment on reduced inhibition of maladaptive sex behavior is not readily explained by an enhanced reward associated with mating. In contrast, in animals that received concurrent Meth and mating experience, reward seeking associated with mating was reduced. Therefore, another explanation must be proposed for the effects of concurrent Meth and sex pretreatment on the expression of maladaptive sexual behavior. A recent neuroanatomical study from our laboratory identified brain areas where Meth may mediate effects on sexual behavior (Frohmader et al., 2010c). Here, neural activation induced by mating or Meth were examined using neural activity markers such as Fos and phosphorylation of MAP Kinase, respectively. Meth and mating co-activated neurons in the nucleus accumbens, basolateral amygdala, and the anterior cingulate area of the medial prefrontal cortex (Frohmader et al., 2010c) and in the orbitofrontal cortex (Frohmader and Coolen, 2010). The prefrontal and orbitofrontal cortices are of particular interest as they contribute to addictive behaviors (Kalivas et al., 2005; Kalivas and Volkow, 2005; Lasseter et al., 2010; Winstanley et al., 2010). Moreover, hypoactivity of these brain areas has been correlated with several

psychiatric conditions associated with loss of inhibitory control (Graybiel and Rauch, 2000; London et al., 2005; Taylor et al., 2002). These lines of evidence suggest that Meth may act in these frontal cortices to cause long term alterations that in turn mediate compulsive sexual behavior. In line with this, high incidence of compulsive sexual behavior has been shown to overlap with other psychiatric disorders including drug addiction, anxiety, and mood disorders (Bancroft, 2008). And drug-induced dysfunction of the medial prefrontal and orbitofrontal cortices are hypothesized to be responsible for reduced impulse control (Brewer and Potenza, 2008; Fineberg et al., 2009) and increased sex-directed behavior as observed in many addicts (Bancroft, 2008; Jentsch and Taylor, 1999). In agreement, lesions of the medial prefrontal cortex in male rats resulted in compulsive sex-seeking behavior in the conditioned sex aversion paradigm utilized in the current studies (Davis et al., 2010).

Previous research has shown that repeated administration to psychostimulants or opiates enhance drug-induced reward as measured by CPP (Lett, 1989; Shippenberg and Heidbreder, 1995; Shippenberg et al., 1996). In addition, sexual experience caused subsequent sensitization of D-amphetamine reward (Pitchers et al., 2010). In the current study, the effects of Meth and/or sexual experience on Meth CPP was tested under conditions that were not expected to result in drug CPP: low dose of Meth, a single conditioning trial, and testing during the dark phase of the day at times of lowest CPP (Webb et al., 2009a,b). The sensitizing regimens of repeated Meth or of sexual experience used in the current study did not cause enhanced Meth CPP. However, Meth pretreatment concurrent with mating did enhance Meth reward, indicating that this

association between Meth and sex caused an enhancement in reward seeking for Meth. These results appear in agreement with human reports of increased Meth seeking identifying sexual pleasure during Meth taking as a primary drive for drug use (Green and Halkitis, 2006; Schilder et al., 2005; Semple et al., 2002). It is currently unclear which components of sexual behavior are critical for the association between Meth and sex. In the current study, all males mated to ejaculation. However, our previous findings suggest that social interactions may be sufficient to induce maladaptive sex-seeking behavior (Frohmader et al., 2010a).

Neural substrates that may mediate the enhancing effects of concurrent Meth and sex pretreatment on Meth reward include the nucleus accumbens and basolateral amygdala. Long-lasting changes in dendritic spine density and morphology in the accumbens result from repeated drug administration (Brown and Kolb, 2001; Li et al., 2003; Robinson et al., 2002; Robinson and Kolb, 2004) or sexual experience (Meisel and Mullins, 2006; Pitchers et al., 2010), and are hypothesized to mediate drug-induced locomotor and reward sensitization (Li et al., 2004; Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000). The basolateral amygdala is critical for memory of conditioned stimuli associated with drug stimuli (Grace and Rosenkranz, 2002; Laviolette and Grace, 2006) and involved in reward sensitization and reinforcement (Cardinal et al., 2002; Everitt et al., 1999; See, 2002). Lesions or inactivations of the basolateral amygdala block the acquisition (Whitelaw et al., 1996) and expression (Grimm and See, 2000) of conditioned-cued cocaine reinstatement. Moreover, basolateral amygdala lesions result in reduced responding for conditioned stimuli paired with food (Everitt et al., 1989)

or sexual reinforcement (Everitt, 1990; Everitt et al., 1989) in rats. Therefore, it is possible that psychostimulant- and sex- induced changes in accumbens and basolateral amygdala result in potentiated reward salience of Meth.

Sensitizing regimens of drugs have been shown to facilitate sexual behavior. Sensitizing pretreatments of D-amphetamine (10 daily injections of 1.5 mg/kg) facilitate sexual behavior (Fiorino and Phillips, 1999a,b) as well as approach behavior to sexual stimuli (Nocjar and Panksepp, 2002). Studies in female rats pretreated with Meth (3 daily injections of 5 mg/kg) resulted in increased receptive behaviors (Holder et al., 2010). In contrast, the current study did not show effects of a sensitizing regiment of Meth treatment on sexual behavior. Possible explanations for this discrepancy include the lower drug dosing used in the current study, different assessments of sexual motivation, and sex differences (Becker and Hu, 2008).

Studies on rodent models of Meth addiction have recently been focused on drug bingeing paradigms to investigate Meth-induced behavioral impairments (Belcher et al., 2007; Izquierdo et al., 2009; O'Dell et al., 2011), neuroplastic changes (Brennan et al., 2010), and neurotoxicity (Graham et al., 2008; Kuczenski et al., 2007; Moszczynska et al., 1998). The main objective of these studies is to achieve plasma drug levels in the rat closely to those found in human Meth addicts. In contrast, the current study demonstrated that once daily passive administration of a low Meth dose was sufficient to cause long lasting compulsive sex behavior. A Meth Bingeing paradigm was not utilized for practical reasons: high doses of Meth impair sexual behavior (Frohmader et al., 2010a)

and human users often use sexual performance enhancing drugs to maintain sexual function (Semple et al., 2009). The focus of the current sets of studies was to investigate sexual reward and compulsive mating in animals with unimpaired mating behavior. The results demonstrate that compulsive sex behavior and altered drug and sex reward can already be caused by very low drug exposure once concurrent with sexual experience and are not dependent on inducing bingeing levels of Meth in the brain.

Together, the current set of studies form an important step towards a better understanding of the effects of Meth on compulsive sex behavior and associations between drug and sex reward. Moreover, these data parallel those reported in human addicts; thus, the male rat model can be further utilized to examine molecular and structural mechanisms of Meth effects on sex behavior and potentially contribute to future drug addiction therapies.

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CHAPTER 5:

Neural substrates controlling inhibition of sexual behavior in male rats

5.1 INTRODUCTION

High incidence of hypersexual behavior overlaps with psychiatric disorders including drug addiction, anxiety, and mood disorders (Bancroft, 2008) as well as in response to medications such as those prescribed for Parkinson's disease (Claassen et al., 2011; Park and Stacy, 2011; Reiff and Jost, 2011). It is well recognized that psychostimulants, opiates, and alcohol alter sexual behavior in men and women (Frohmader et al., 2010b; Peugh and Belenko, 2001; Pfaus, 2009; Rawson et al., 2002). Moreover, the prevalence of sexual health related diseases within addict populations has raised awareness of the effects these drugs have on sexual behavior, as chronic drug use is associated with hypersexual behavior and unsafe sexual practices, resulting in increased rates of sexually transmitted infections including Human Immunodeficiency Virus (HIV). In particular, the effect of drugs of abuse on hypersexuality is well documented for the psychostimulant methamphetamine (Meth). Meth users report hypersexual behavior and engage in unprotected sex more often than other drug users. This sexual risk behavior is mainly due to loss of inhibitory control (Green and Halkitis, 2006; Halkitis et al., 2001; Mckirnan et al., 2001; Rawson et al., 2002).

Recently, our laboratory developed a paradigm to study hypersexuality in male rats (Davis et al., 2010) using a sex aversion conditioning paradigm in which males mating is paired with subsequent visceral illness (Agmo, 2002; Peters, 1983). Following repeated pairings of mating and the aversive stimulus, animals ceased to engage in copulatory behaviors (Davis et al., 2010; Frohmader et al., 2010a). However, Meth pretreatment (acute or repeated) weeks prior to conditioning, disrupted the acquisition of

inhibited sexual responses (Frohmader et al., 2010a). Thus, Meth-pretreated male rats displayed sex-seeking behavior even though mating was associated with an aversive stimulus, termed maladaptive sex-seeking behavior.

Currently, the neural substrates of hypersexuality are not well understood. Understanding the neural circuits mediating the inhibition of mating is a critical step for understanding the underlying mechanisms causing hypersexuality in general as well as Meth-induced hypersexuality. Therefore, the main goal of the current study was to investigate the neural circuits activated by cues associated with conditioned sex aversion using the phosphorylation of MAP Kinase or extracellular-regulated protein kinase (pERK) as a marker for neural activation. The expression of pERK is highly dynamic and occurs 5-20 minutes after neural activation (Valjent et al., 2000; Valjent et al., 2004; Valjent et al., 2005). Moreover, pERK is an adequate marker for neural activation by conditioned cues as it's expression is critical for learning and memory during different task such as conditioned taste aversion (Berman et al., 1998), fear conditioning (Atkins et al., 1998; Schafe et al., 2000), spatial learning (Selcher et al., 1999), recognition memory (Kelly et al., 2003), food-reward conditioning (Ribeiro et al., 2005), and sexual behavior (Frohmader and Coolen, unpublished data; abstract SBN 2009). More importantly, the expression of pERK is involved in the mechanisms of drug action and synaptic plasticity (Girault et al., 2007; Valjent et al., 2006; Valjent et al., 2004), which may be critical for the understanding of drug-induced alterations in the inhibition of sexual behavior following aversive conditioning.

5.2 MATERIALS AND METHODS

5.2.1 Subjects

Adult male Sprague-Dawley rats (210-225 g) were obtained from Charles River Laboratories (Montreal, QC, Canada) and dual housed in standard Plexiglas cages (home cages). Food and water were available *ad libitum.* The animal room was maintained at a 12/12 h reversed light/dark cycle (lights off at 10.00 h). Testing was performed during the first half of the dark cycle under dim red illumination. Stimulus females for sexual behavior were bilaterally ovariectomized under deep anaesthesia (87 mg/kg ketamine and 13 mg/kg xylazine) and received a subcutaneous implant containing 5% estradiol benzoate (EB) and 95% cholesterol. Sexual receptivity was induced by a subcutaneous (s.c.) injection of 500 μg progesterone in 0.1 ml sesame oil 4 h prior to testing. All experimental procedures were approved by the Animal Care Committee at the University of Western Ontario and conform to the guidelines outlined by the Canadian Council on Animal Care.

5.2.2 Experimental design

5.2.2A Sexual experience

Male rats (n=20) gained sexual experience in separate test cages (mating arenas; 60 x 45 x 50 cm^3) containing clean bedding during five twice-weekly mating sessions. During each mating session, males were allowed to mate with a receptive female until the display of 1 ejaculation or for 1 hr, whichever occurred first. During each mating session, all standard parameters for sexual performance were recorded, including: mount latency

(ML; time from introduction of the female until the first mount), intromission latency (IL; time from introduction of the female until the first mount with vaginal penetration), ejaculation latency (EL; time from the first intromission to ejaculation), post ejaculation interval (PEI; time from ejaculation to first subsequent intromission), number of mounts (M), and number of intromissions (IM) (Agmo, 1997).

5.2.2B Conditioned copulation aversion

One week following the last mating session, animals were subjected to a conditioned copulation aversion paradigm using lithium chloride (LiCl). During seven conditioning trials, all males were placed in the mating arena for a 10 min habituation period after which a receptive female was introduced. Stimulus females were scented by swabbing almond oil on the neck and base of the tail prior to mating. Olfactory cues have been shown to facilitate male approach behavior and to strengthen conditioning (Agmo, 2002; Lawrence and Kiefer, 1987). Males were allowed to mate for 30 min or until 1 ejaculation. If intromissions did not occur within the first 15 min, the trial was terminated. One minute following ejaculation or trial termination all males received a 127.2 mg/kg intraperitoneal (i.p.) injection of LiCl administered at a volume of 10 ml/kg. LiCl was administered regardless of whether mating occurred.

5.2.2C Final test

Following the seventh conditioning trial, when animals displayed significant inhibition of sexual behavior, they were exposed to sex aversion conditioned cues to examine pERK expression or removed from their home cage prior to brain collection to serve as controls

(n=6). Males were either exposed to the mating arena alone (n=6) or a scented female (n=8), and perfused 10 min later to visualize conditioned cue-induced pERK.

5.2.3 Tissue preparation

Animals were anesthetized with pentobarbital (270 mg/kg; i.p., Bimeda-MTC, Animal Health Inc., Cambridge, ON, Canada) and perfused transcardially with 5 ml of saline followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and post-fixed for 1 h at room temperature in the same fixative, then immersed in 20% sucrose and 0.01% Sodium Azide in 0.1 M PB and stored at 4°C. Coronal sections (35 μm) were cut on a freezing microtome (H400R, Micron, Germany), collected in four parallel series in cryoprotectant solution (30% sucrose and 30% ethylene glycol in 0.1 M PB) and stored at 20°C until further processing.

5.2.3A Immunohistochemistry

All incubations were performed at room temperature with gentle agitation. Free floating sections were washed extensively with 0.1 M Phosphate-buffered saline (PBS) between incubations. Sections were incubated in 1% H_2O_2 for 10 min, then blocked in incubation solution (PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100) for 1 h. Following staining, the sections were washed thoroughly in 0.1 M PB, mounted onto Superfrost plus glass slides (Fisher, Pittsburgh, PA, USA) with 0.3% gelatin in ddH_2O. Following dehydration, slides were coverslipped with dibutyl phthalate xylene (DPX; Sigma-Aldrich, St Louis, MO, USA).

pERK: Tissue was incubated overnight with a rabbit polyclonal antibody against p42 and p44 MAP Kinases ERK1 and ERK2 (pERK; 1:2.000 lot 26; Cell Signaling Technology, Danvers, MA, USA Cat # 9101), followed by a 1 h incubation with biotinylated donkey anti-rabbit IgG (1:500; Jackson Immunoresearch Laboratories, West Grove, PA, USA) and avidin-horseradish peroxidase complex (ABC Elite; 1:1000; Vector Laboratories, Burlingame, CA, USA). Next, tissue was incubated for 10 min in a chromogen solution containing 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St Louis, MO, USA) in 0.1 M PB containing 0.015% hydrogen peroxide, resulting in a reddish-brown reaction product. The primary antibody has been extensively characterized in the literature (Murphy and Blenis, 2006; Roux and Blenis, 2004). Moreover, omission of the primary antibody prevented all immunoreactivity and Western blot analysis of rat brain tissue revealed two bands at the appropriate molecular weights.

5.2.4 Data analysis

5.2.4A Sexual behavior

Standard parameters for sexual performance were recorded as described above and analyzed using analysis of variance (ANOVA). Data analysis of sexual behavior prior to or during conditioned sex aversion revealed no significant differences between groups in any of the parameters of sexual performance. Percentages of males that displayed mounts, intromissions, or ejaculation during each conditioning trial were calculated and differences between conditioning trials were compared using McNemar's chi-square analysis at a significance level of 0.05.

5.2.4B pERK cell counts

Single labeled cells for pERK were counted in the middle levels of nucleus accumbens (NAc) core and shell subregions, the caudate putamen (CPu), basolateral amygdala (BLA), lateral amygdala (LA), central amygdala (CeA), the orbitofrontal cortex (OFC), and the anterior cingulate area (ACA), prelimbic (PL), and infralimbic (IL) subregions of the medial prefrontal cortex (mPFC) using standard areas of analysis using a camera lucida drawing tube attached to a Leica DMRD microscope (Leica Microsystems, Wetzlar, Germany). Standard areas of analysis were: 400 x 600 □m in the NAc core and shell subregions and LA, 800 x 800 □m in the CPu, 600 x 600 □m in the OFC and CeA, 600 x 800 □m in the BLA and each mPFC subregion. Two sections were counted bilaterally for each brain region and were averaged per animal. Group averages were compared using one way ANOVA (factor: *stimulus*) and Fisher's LSD for *post hoc* comparisons at a significance level of 0.05.

5.2.5 Images

Digital images were captured using a CCD camera (Macrofire, Optronics, Goleta, CA, USA) attached to a Leica microscope (DM5000B, Leica Microsystems, Wetzlar, Germany) with fixed camera settings. Images were imported into Adobe Photoshop 9.0 software (Adobe Systems, San Jose, CA, USA). Images were not altered in any way except for adjustment of brightness.

5.3 RESULTS

5.3.1 Conditioned copulation aversion

Conditioned copulation aversion induced by LiCl significantly inhibited sexual behavior. Specifically, decreased percentages of LiCl-paired males that mated were first evident on the fourth conditioning day ($p=0.008$, M and I; $p=0.002$, E) and persisted through conditioning days 5 ($p=0.01$, M and I, $p=0.005$, E), 6 ($p=<0.001$, M, I, and E), 7 ($p=0.001$, M and I; $p=<0.001$, E) (Figure 5.1A and B). Moreover, during the final test, none of the males exposed to the scented female displayed mating behavior ($p=<0.001$, M, I, and E) (Figure 5.1A and B).

5.3.2 Neural activation of the limbic system following conditioned sex aversion

Analysis of numbers of pERK cells revealed that exposure to conditioned cues associated with sex aversion significantly increased pERK expression in the regions of the frontal cortex, striatum, and amygdala ($F(2,14)=3.45-5.64$; $p=0.03-<0.016$). Specifically, exposure to the scented female increased pERK expression in all subregions of mPFC, the OFC, CPu, and CeA compared to home cage controls (Figure 5.2 and 5.3). The mPFC was also activated by exposure to the conditioning environment; however, only trends in increased numbers of pERK-expressing cells compared to controls were detected ($p=0.08$, ACA; $p=0.07$, PL; $p=0.09$ IL). In contrast, significant pERK expression was not observed in the NAc core and shell subregions, and in the lateral and basolateral amygdala (Figure 5.2B and C).

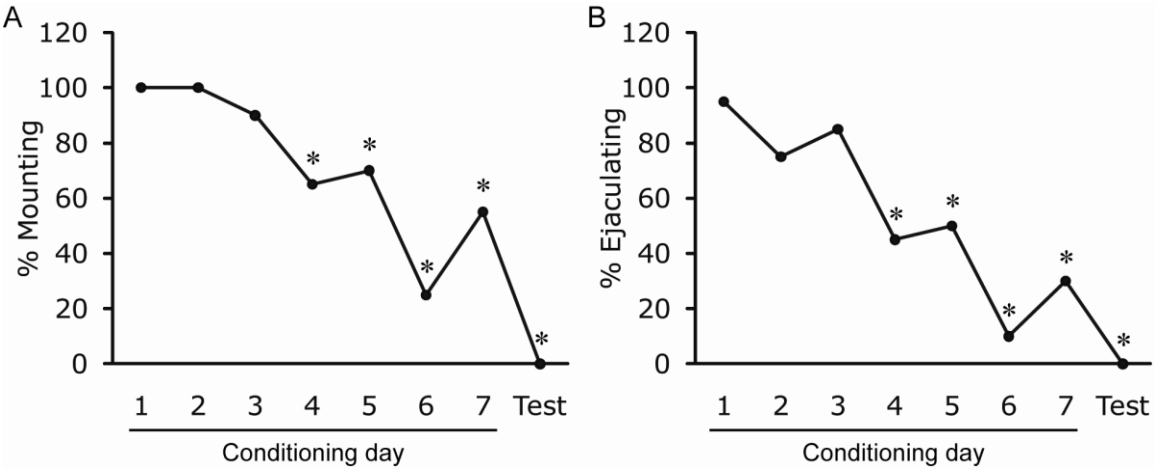


Figure 5.1 Sexual behavior during LiCl-induced conditioned sex aversion. Percentages of males mounting (A) and ejaculating (B) during seven conditioning trials and final test. * indicate significant differences from conditioning trial 1 ($p<0.05$).

Figure 5.2 Conditioned sex aversion-induced pERK expression. Mean SEM number of Perk cells in the frontal cortex (A), striatum (B), and amydgala (C) 10 min following exposure to cues associated with sex aversion. * indicate significant differences from home cage controls ($p<0.05$).

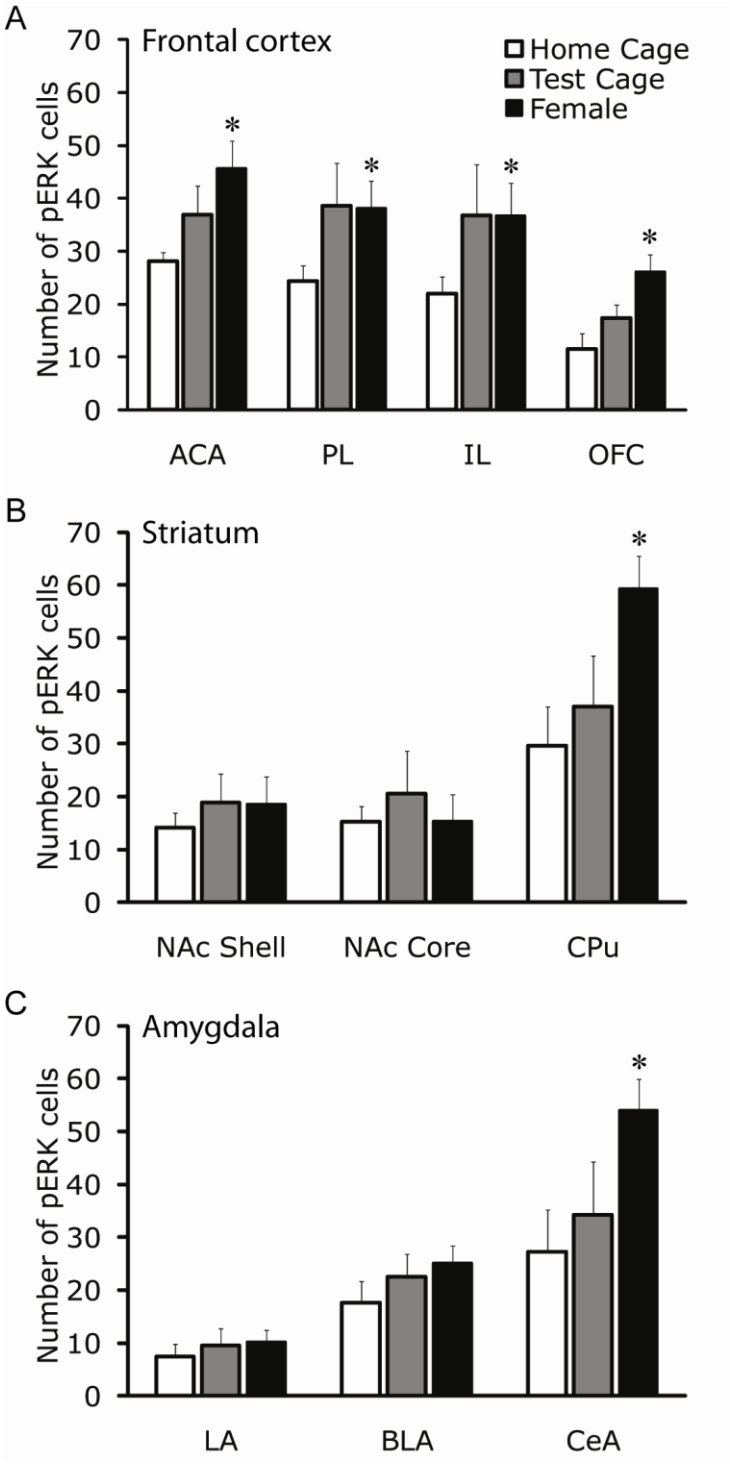
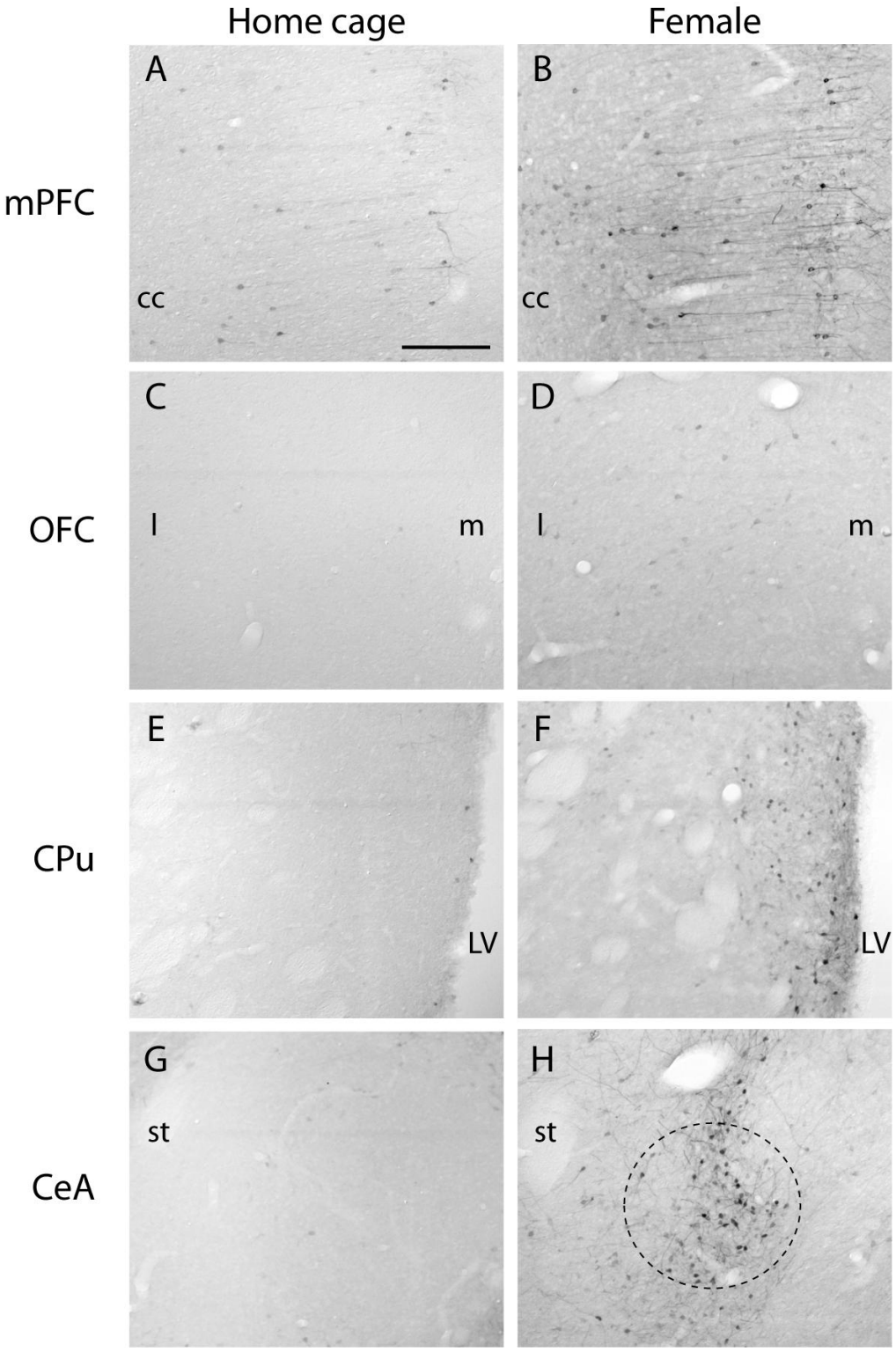

A
Frontal cortex
Home Cage
Test Cage
Female
Number of pERK cells
70
60
50
40
30
20
10
0
*
*
*
*
ACA
PL
IL
OFC
B
Striatum
Number of pERK cells
70
60
50
40
30
20
10
0
*
NAc Shell
NAc Core
CPu
C
Amygdala
Number of pERK cells
70
60
50
40
30
20
10
0
*
LA
BLA
CeA

Figure 5.2 pERK expression in the brain following exposure to cues associated with sex aversion. Representative images of pERK expression in the mPFC (A and B), OFC (C and D), CPu (E and F), and CeA (G and H) of home cage controls (A, C, E, and G) and males exposed to the conditioning environment with a scented female (B, D, F, and H). Scale bar indicates 200 μm. Circle in panel F represents the standard area of analysis for the CeA. Abbreviations: cc, corpus callosum; l, lateral; m, medial; st, stria terminalis; VL, lateral ventricle.


Home cage
Female
mPFC
OFC
CPu
CeA
A
B
C
D
E
F
G
H
cc
cc
l
m
l
m
LV
LV
st
st

5.4 DISCUSSION

The current study attempted to elucidate the neural substrates for controlling the inhibition of sexual behavior. Here, it was demonstrated that exposure to cues associated with conditioned inhibition of sex behavior resulted in significant ERK activation in all subregions of the mPFC, the OFC, CPu, and CeA in animals that learned to inhibit mating following LiCl conditioning.

Activity in brain regions such as the prefrontal cortex, striatum, and amygdala have been shown to be critical for action–outcome learning and performance of goal-directed behaviors during Pavlovian conditioning (Shiflett and Balleine, 2011). Studies have shown that the OFC is important for cue-outcome prediction and expectancy, an important component of goal-directed behavior in animals and humans (Schoenbaum et al., 2009). Similarly, the mPFC is essential for decision making, in particular when it involves response, conflicts, and effort (Heidbreder and Groenewegen, 2003). The CPu is thought to support motor habits by promoting the acquisition (Carelli et al., 1997) and storage (Atallah et al., 2007) of stimulus-response associations (Balleine et al., 2009). The CeA is required for the expression of a conditioned fear response, and CeA specific lesions prevent the display conditioned behavior (Nader et al., 2001).

In the current study we hypothesize that pERK expression is reflecting neural activation following “proper” cue-response behavior; hence, males inhibit mating when exposed to cues associated with aversion. However, in the case of compulsive behaviors, the neural circuits are altered in such a way that they lead to “improper” or maladaptive

behaviors where an animal displays mating despite learned adverse consequences. Loss of control over drug use is a central feature of addiction that has been modeled in animals. It is characterized by unsuccessful efforts to stop responding for drug despite adverse consequences (Vanderschuren and Everitt, 2004).

In line with the addiction literature, ERK has been shown to be activated in limbic structures including the striatum, amygdala, prefrontal cortex, and ventral tegmental area after acute or repeated treatment with psychostimulant drugs (Zhai et al., 2008). There is ample evidence that the mPFC is involved in drug craving and relapse to drug-seeking and drug-taking behavior in both humans and rats (Capriles et al., 2003; Childress et al., 1999; Grant et al., 1996; Kalivas and Volkow, 2005; McLaughlin and See, 2003; Shaham et al., 2003). Cocaine administration induces pERK expression in the rat prefrontal cortex (Valjent et al., 2004). Furthermore, in self-administration studies locally blocking pERK expression in the prefrontal cortex prevents cue- and cocaine prime-induced drug reinstatement (Whitfield et al., 2011). In addition, cocaine injections induce pERK expression in the CPu and inhibition of ERK blocks drug-seeking behavior (Valjent et al., 2000). Finally, ERK phosphorylation in the CeA has also been shown to be critical for cue-induced drug seeking in rats. Using self-administration paradigms, exposure to cocaine-paired cues increased ERK phosphorylation in the central amygdala following drug abstinence (Lu et al., 2004). Moreover, inhibition of central amygdala ERK decreased enhanced cocaine craving (Lu et al., 2005). Together, these findings indicate that ERK activation in specific brain regions is important for cue-induced drug-seeking behavior.

Previous research has showed that the expression of pERK is involved in mechanisms of synaptic plasticity, drug action, and learning and memory (Girault et al., 2007; Valjent et al., 2006; Valjent et al., 2004). Therefore, drug-induced alterations in brain areas critical for inhibition of mating may be responsible for compulsive sexual behaviors. Therefore, these brain regions may be associated with the effects of Meth on maladaptive sex seeking. Altered functioning of these neural circuits by repeated exposure to drugs of abuse is hypothesized to be responsible for reduced impulse control (Brewer and Potenza, 2008; Fineberg et al., 2009) and increased sex-directed behavior as observed in many addicts (Bancroft, 2008; Jentsch and Taylor, 1999).

The current study provides an important initial step towards understanding the neural substrates for controlling the inhibition of sexual behavior and may lead to further understanding of hypersexuality. As high incidence of compulsive sexual behavior overlaps with psychiatric conditions including drug addiction (Bancroft, 2008), future studies will aim to investigate Meth-induced alterations on neural circuits shown to be involved in the inhibition of mating during conditioned sex aversion. It is hypothesized that Meth will alter conditioned cue-induced pERK expression in brain areas that mediate inhibition of sexual behavior.

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CHAPTER 6: GENERAL DISCUSSION

6.1 SUMMARY OF RESULTS AND CONCLUSIONS

6.1.1 Meth activates subpopulations of neurons that are also activated by sexual behavior

The findings in Chapter 2 form a critical first step towards a better understanding of how drugs of abuse act on neural pathways that normally mediate natural reward. Moreover, these data show that in contrast to current belief, drugs not only act on the same brain regions that regulate natural reward, but in fact, drugs activate the same cells involved in natural reward behavior. Specifically, methamphetamine (Meth) activated neurons that had previously been activated by sexual behavior in the nucleus accumbens (NAc) core and shell, basolateral amygdala (BLA), anterior cingulate area (ACA) subregion of the medial prefrontal cortex (mPFC) (Frohmader et al., 2010b), and orbitofrontal cortex (OFC; (Frohmader and Coolen, 2010).

Comparisons of the similarities and differences in neural activation of the limbic system induced by mating and Meth may lead to a better understanding of substance abuse and associated alterations in natural reward.

6.1.2 High doses of Meth decrease sexual motivation and performance

Studies included in Chapters 3 and 4 demonstrated that acute and repeated regiments of Meth impair sexual function in male rats. Meth administration 30 min prior to mating inhibited sexual performance in a dose dependent matter evidenced by decreased percentages of males that mated (Frohmader et al., 2010a) and increased latencies to initiate sexual behavior (Frohmader et al., 2010a; Frohmader et al., 2011). Moreover, effects of Meth on sexual function dissipated following drug abstinence.

These results are consistent with male addicts reporting erectile dysfunction and delayed ejaculation while using Meth (Frosch et al., 1996; Peugh and Belenko, 2001).

6.1.3 A model for compulsive sex-seeking behavior was established

Although compulsive sexual behavior is one of the most commonly reported effects of Meth on sexual behavior, there is a lack of research focused on investigating these behaviors in an animal model. Therefore, a critical step of the work included in my thesis was the establishment of a model for compulsive sexual behavior in male rats. In Chapters 3, 4, and 5, a conditioned sex aversion paradigm was utilized in which sexual reward was paired with visceral illness induced by lithium chloride (LiCl). Specifically, it was shown that acute and repeated administration of a low Meth dose (1 mg/kg) resulted in long-term effects on maladaptive sexual behavior. Males pretreated with Meth were unable to inhibit mating despite adverse consequences of sexual behavior weeks following drug pretreatment (Frohmader et al., 2010a; Frohmader et al., 2011). Furthermore, it was shown that the effects of Meth on maladaptive sex-seeking were not due to learning impairments or diminished sensitivity to LiCl (Frohmader et al., 2011). Following the establishment of a model for compulsive sexual behavior, studies included in Chapter 5 elucidated the neural substrates involved in mediating the inhibition of sexual behavior following conditioned sex aversion. Specifically, cues associated with sex aversion resulted in neural activation in the mPFC, OFC, caudate putamen (CPu) and central amygdala (CeA) in male rats that displayed inhibited sexual behavior.

Together, results in male rats parallel those reported in human addicts, suggesting that this animal model can be used to further investigate the cellular mechanisms by which Meth alters sex behavior and potentially contribute to future treatment of Meth addiction.

6.1.4 Concurrent exposure to Meth and sexual behavior is required for maladaptive sexual behavior and altered drug and sex reward

One of the main finding of this work was the discovery that the effect of Meth on compulsive sex behavior was dependent on the association between Meth and mating. Experiments in Chapter 4 showed that concurrent Meth and mating pretreatments resulted in maladaptive sex-seeking behaviors. In contrast, non-concurrent Meth experience and sexual experience had no effects during conditioned copulation aversion. Moreover, concurrent Meth and sex experience was required for conditioned place preference (CPP) for Meth and for concurrent Meth and sex compared to Meth or sex alone. However, CPP for mating alone was decreased (Frohmader et al., 2011).

Together, these data indicate that an association between Meth and mating is critical for the development or expression of compulsive sex behavior and changes in sex and drug reward. In addition, these data parallel those reported in human addicts (Green and Halkitis, 2006; Schilder et al., 2005; Semple et al., 2002), thus validating the male rat model to further examine molecular and structural mechanisms of Meth effects on sex behavior and potentially contribute to future drug addiction therapies.

6.2 FUTURE DIRECTIONS

Although the set of studies included in my thesis provide critical steps for understanding the effects of Meth on sexual behavior and the underlying neural substrates, several questions remain. As mentioned above, studies included in Chapter 2 elucidated brain regions in which populations of neurons were co-activated by both mating and Meth. However, these findings create a map for potential sites where Meth is acting to alter sexual behavior. Future studies will utilize local manipulations to elucidate the functional relevance of this co-activation, investigate the neurochemical phenotypes and connections of activated neurons, and determine whether neuroplastic changes are occurring in neurons co-activated by mating and Meth.

First, as the association between mating experience was shown to be required for altered drug and/or sex reward as well as the expression of maladaptive sexual behavior, it is important to investigate how this memory is formed and how it can be disrupted once it is formed. Therefore, targeting the processes that mediate acquisition and subsequent stabilization of memory (consolidation and reconsolidation), as well as unlearning these memories is critical for understanding the robust drug-sex nexus identified in the studies of my thesis. In line with this, memory consolidation refers to the stabilization that a new memory has to undergo in order to persist, while reconsolidation refers to process by which consolidated memories return to a transient and unstable state following retrieval, from which they must again stabilize in order to persist. Reconsolidation can be achieved by exposure to cues associated with an initial memory and its subsequent retrieval, thus returning these fear memories to an unstable state (Reviewed by Nader and Einarsson, 2010). Processes of reconsolidation have been reported in drug reward

(Miller and Marshall, 2005; Valjent et al., 2006), habituation (Rose and Rankin, 2006), incentive learning (Wang et al., 2005), fear conditioning (Deębiec et al., 2006; Nader et al., 2000), instrumental learning (Hernandez and Kelley, 2004), and inhibitory avoidance (Milekic and Alberini, 2002). Furthermore, reconsolidation has been shown to be effectively manipulated with receptor antagonists and more commonly by protein synthesis inhibitors such as anisomycin (Nader et al., 2000). Direct infusions of anisomycin into BLA, which is critical for mediating fear memory consolidation (LeDoux, 2000; Schafe and LeDoux, 2000) have been shown to block memory reconsolidation (Nader et al., 2000). More recently, the molecule Protein Kinase M zeta (PKMzeta), a constitutively active isoform of protein kinase C, has received particular interest as it has been suggested to mediate memory maintenance long after memory consolidation (Pastalkova et al., 2006; Sacktor, 2011). Local inhibitions of PKMzeta with zeta inhibitory peptide (ZIP) in the NAc and BLA have been shown to reverse long-term potentiation induced by consolidated memory and leads to long-term impairment of memory. Specifically, ZIP infusions into the NAc abolish long-term memory for drug-reward (Li et al., 2011), while intra-BLA infusions induced robust memory loss following fear conditioning (Migues et al., 2010; Serrano et al., 2008). Future studies will aim to investigate mechanisms by which Meth and mating associations can be disrupted. Therefore, future aims will also examine the functional relevance of brain regions in mediating this long term associative memory. We hypothesize that the NAc is critical for mediating concurrent sex and Meth-induced alterations of drug and/or sex reward. In addition, we believe the mPFC, OFC, and BLA may be important for mediating Meth-sex association leading to compulsive sex-seeking behaviors.

Next, we hypothesize that the co-activated neurons are not only being activated by mating and Meth, but also undergoing neuroplastic alterations. Recent studies from our laboratory investigated morphological alterations in neurons of the mPFC using diolistic labeling following sex and Meth pretreatments. Preliminary data showed that concurrent mating and Meth pretreatments resulted in increased dendritic spine density in mPFC pyramidal neurons compared to males that received concurrent mating and saline pretreatments when neurons were visualized one week following the last drug-sex pairing. Morphological alterations including dendritic branching and spine density reflect changes in synaptic connectivity, altered neural function, and subsequently reflect on persistent behavioral responses associated with repeated drug-seeking (Reviewed by Robinson and Kolb, 2004). Previous research has shown increased dendritic branching and spine density in both NAc medium spiny neurons and mPFC pyramidal neurons following repeated D-amphetamine and cocaine administration (Crombag et al., 2005; Robinson et al., 2001; Robinson and Kolb, 1999) and are strongly linked with behavioral sensitization and incentive sensitization (Robinson and Kolb, 1997, 1999).

In contrast to NAc and mPFC neurons, OFC neurons show decreased spine density following repeated amphetamine (Crombag et al., 2005), which are in line with structural abnormalities (Fein et al., 2002), altered metabolic activity (Volkow et al., 1992), and monoamine depletions (Volkow et al., 1993), as well as OFC-dependent cognitive deficits (Volkow et al., 1992) reported in human psychostimulant users. Moreover, OFC-dependent behavioral impairments have also been reported in monkeys and rats following repeated psychostimulant administration. Cocaine-administered monkeys display deficits in behavioral inhibition during object recognition task where a new response is required (Jentsch et al., 2002).

Similarly, It has been shown that cocaine-treated rats display impaired reversal learning and reinforcer devaluation (See review by Schoenbaum and Shaham, 2008). In line with these data, it is hypothesized that Meth is altering OFC function and in turn resulting in cognitive inflexibility. There is evidence that Meth results in long-term reductions in dopamine and serotonin in the brain (Chapman et al., 2001; Kuczenski et al., 1995; Walsh and Wagner, 1992). Interestingly, serotonin and particularly serotonin in the OFC is an important mediator of reversal learning (Clarke et al., 2004; Clarke et al., 2005). These impairments may reflect the addict's inability to disrupt maladaptive behaviors despite aversive consequences (Jentsch and Taylor, 1999; Schoenbaum et al., 2004).

Together, the studies included in this thesis and proposed future studies are and will be critical for further understanding of the behavioral and molecular effects of Meth on the neural circuits that regulate sexual behavior including sexually compulsive behaviors.

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APPENDIX A

March 22, 2010

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2014

Dear Dr. Coolen:

Your Animal Use Protocol form entitled:
Neural Regulation of Rewarding Behavior and Substance Abuse
Funding Agency CIHR - RN 014705

has been approved by the University Council on Animal Care. This approval is valid from **March 22, 2010 to March 31, 2011.** The protocol number for this project is **2010-211 which replaces 2006-036 which has expired**.

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ANIMALS APPROVED FOR 4 Years

Species	4 Year Total Numbers Estimated as Required	List All Strain(s)	Age / Weight
Rat	15,960	Sprague Dawley	3-6 months/210-650 grams

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

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April 8, 2010

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ANIMALS APPROVED

Species	4 Year Total Numbers Estimated as Required	List All Strain(s)	Age / Weight
Rat	640	Sprague Dawley	3-6 months/210-650 grams

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CURRICULUM VITA

Name: Karla Stephanie Frohmader

Educational Background:

PhD in Neuroscience
University of Western Ontario, London, ON, Canada
Started MSc in September 2006, transferred to PhD in May 2008
University of Michigan, Ann Arbor, MI, USA
Visiting scholar in the Department of Molecular and Integrative Physiology
December 2010 – October 2011

Bachelor of Medical Sciences: Honors Specialization in Medical Sciences
The University of Western Ontario, London, ON, Canada
September 2002 – May 2006

Teaching Experience:

Department of Anatomy and Cell Biology
The University of Western Ontario, London, ON, Canada
September 2006 – May 2009
Teaching Assistant, Systemic Human Anatomy 3319

Scholarships and awards:

2006-2011: Western Graduate Research Scholarship
2006-2007: Graduate Research Thesis Awards Fund
2005-2006: Dean's Honors List

Publications:

Concurrent exposure to methamphetamine and sexual behavior enhances subsequent drug reward and causes compulsive sexual behavior in male rats (2011) Karla S. Frohmader, Michael N. Lehman, Steven R. Laviolette, and Lique M. Coolen. Research Article, Journal of Neuroscience, in press.

Effects of methamphetamine on sexual performance and compulsive sex behavior in male rats (2010) Karla S. Frohmader, Katherine L. Bateman, Michael N. Lehman, and Lique M. Coolen. Research Article, Psychopharmacology: 212(1):93-104

DeltaFosB in the nucleus accumbens is critical for reinforcing effects of sexual reward (2010) Kyle K. Pitchers, Karla S. Frohmader, Vincent Vialou, Ezekiell Mouzon, Eric J. Nestler, Michael N. Lehman, and Lique M. Coolen. Research Article, Genes Brain and Behavior: 9(7):831-40.

Mixing Pleasures: Review of the effects of drugs on sex behavior in humans and animal models (2010) Karla S. Frohmader, Kyle K. Pitchers, Margaret E. Balfour, and Lique M. Coolen. Invited Review, Special Issue: Hormones and Behavior: 58(1):149-62

Methamphetamine acts on subpopulations of neurons regulating sexual behavior in male rats (2010) Karla S. Frohmader, Joost Wiskerke, Roy A. Wise, Michael N. Lehman, and Lique M. Coolen. Research Article, Neuroscience: 166(3):771-84

Seminar Presentations:

Effects of methamphetamine on sexual behavior and underlying substrates. Presentation was demonstrated for the Anatomy and Cell Biology Departmental Seminar Series Friday, June 11th, 2010.

Taking the risk: effects of methamphetamine on sexual behavior in male rats. Presentation was demonstrated at the 15th Annual Murray Barr, Anatomy and Cell Biology Departmental Research Day Thursday, October 30th, 2008.

Abstracts:

Karla S. Frohmader and Lique M. Coolen (2011) Neural substrates of inhibition of sexual behavior in the male rat: Effects of methamphetamine. Abstract accepted in June 28. Poster will be presented at the Society for Neuroscience annual meeting in November in Washington DC [International Conference].

Karla S. Frohmader and Lique M. Coolen (2011) Neural substrates of inhibition of sexual behavior in the male rat. Abstract accepted May 17. Poster was presented at the Society for Behavioral Neuronendocrinology annual meeting held in June in Queretaro, Mexico [International Conference].

Karla S. Frohmader and Lique M. Coolen (2010) Effects of methamphetamine on maladaptive sexual behavior and underlying neural substrates. Abstract accepted in June 29. Poster will be presented at the Society for Neuroscience annual meeting in November in San Diego, CA [International Conference].

Karla S. Frohmader and Lique M. Coolen (2010) Effects of Methamphetamine on sexual behavior and underlying neural substrates. Abstract accepted June 3. Poster was presented at the Translational Research in Methamphetamine Addiction Conference held in July in Pray, MT [International Conference]

Lique M. Coolen, Susanne Schmid, Karla S. Frohmader, Caroline M. Coppens, and Kyle K. Pitchers (2009) Sexual behavior induces neural plasticity in the mesolimbic system. Abstract accepted March 6th. Presentation was demonstrated at the Society for Behavioral Neuronendocrinology annual meeting held in June in Lansing, MI [International Conference]

Karla S. Frohmader, Allisha M. Bailey, Cleusa V.R. Oliveira, Joost Wiskerke, and Lique M. Coolen (2009) Taking the risk: effects of methamphetamine on sexual behavior in male rats. Abstract accepted March 6th. Poster was presented at the Society for Behavioral Neuronendocrinology annual meeting held in June in Lansing, MI [International Conference].

Karla S. Frohmader, Katherine L. Bateman, and Lique M. Coolen (2008) Taking the risk: effects of methamphetamine on sexual behavior in male rats. Abstract accepted May 15th. Poster was presented at the Society for Neuroscience annual meeting in November in Washington DC [International Conference].

Karla S. Frohmader, Katherine L. Bateman, and Lique M. Coolen (2008) Taking the risk: effects of methamphetamine on sexual behavior in male rats. Abstract accepted March 14th. Poster will be presented at the Society for Behavioral Neuronendocrinology annual meeting held in July at the University of Groningen, Groningen, The Netherlands [International Conference].

Karla S. Frohmader, Katherine L. Bateman, and Lique M. Coolen (2008) Taking the risk: effects of methamphetamine on sexual behavior in male rats. Abstract accepted March 14th. Poster presented at the Southern Ontario Neuroscience Association annual meeting held May 9th at the University of Western Ontario, London, ON, Canada [Southern Ontario Conference].

Karla S. Frohmader, Katherine L. Bateman, and Lique M. Coolen (2008) Taking the risk: effects of methamphetamine on sexual behavior in male rats. Abstract accepted February 29, 2008. Poster presented at the Margaret Moffat Reaserch Day held March 20th at the University of Western Ontario, London, ON, Canada [Schulich School of Medicine and Dentistry event].

Karla S. Frohmader, Joost Wiskerke, and Lique M. Coolen (2007) Do drugs and sexual behavior activate the same cells in the limbic system? Abstract accepted July 31st. Poster presented at the Society for Neuroscience annual meeting in November in San Diego, CA [International Conference].

Karla S. Frohmader, Joost Wiskerke, and Lique M. Coolen (2007) Sexual behaviour and Methamphetamine activate overlapping populations of nucleus accumbens neurons. Poster presented at the annual Murray Barr Research and Lecture Day held October 11th at the University of Western Ontario, London, ON, Canada [Department of Anatomy and Cell Biology and Schulich School of Medicine and Dentistry event].

Karla S. Frohmader, Joost Wiskerke, and Lique M. Coolen (2007) Sexual behaviour and Methamphetamine activate overlapping populations of nucleus accumbens neurons. Abstract accepted April 10th. Poster presented at the Society for Behavioral Neuronendocrinology annual meeting held in June in Pacific Grove, CA [International Conference].

Karla S. Frohmader, Joost Wiskerke, Cleusa V.R. Oliveira, and Lique M. Coolen (2007) Inhibiting ERK phosphorylation in the mPOA facilitates male sexual behaviour. Poster presented at the Margaret Moffat Reaserch Day held March 22nd at the University of Western Ontario, London, ON, Canada [Schulich School of Medicine and Dentistry event].