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Interaction of stress and stimulants in female rats : role of dopamine in the stress-induced reactivity to methamphetamine

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ABSTRACT

INTERACTION OF STRESS AND STIMULANTS IN FEMALE RATS:
ROLE OF DOPAMINE IN THE STRESS-INDUCED
REACTIVITYTO METHAMPHETAMINE

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Previous research in humans and animals suggests that exposure to stress alters the susceptibility and behavioral responses to drugs of abuse, including methamphetamine. Female rats have been shown to be more sensitive to the effects of stimulants and stress than males, but few studies have investigated the interaction between stress and stimulants in female rats. Therefore, the current study investigated whether stress potentiated the behavioral and dopaminergic responses to a methamphetamine injection in female rats. Adult female rats were either exposed to 10 days of stressors that varied by day and time or were left undisturbed except for daily weighing (control rats). Fourteen days after the last stressor, all rats received an injection of 7.5 mg/kg methamphetamine and distance traveled and stereotypy was measured in an open field box (Experiment 1) or dopamine increases were measured in the dorsal striatum (Experiment 2). Female rats exposed to chronic unpredictable stress (CUS) had significantly higher locomotion in the open field immediately following an injection of methamphetamine, with no significant differences at any other time points. In Experiment 2, female rats exposed to CUS had significantly higher levels of dopamine in the dorsal striatum at all time points following an acute injection of methamphetamine compared to control rats. Estrous cycle was not found to be a significant predictor of distance traveled following a methamphetamine injection. This is the first study inves-

tigating the interaction between stress and methamphetamine in female rats. Interestingly, these findings parallel previous findings from our lab with male rats exposed to CUS showing both an increase in locomotion and dopamine in the dorsal striatum following an injection of methamphetamine compared to control rats. The current findings characterize the interaction of females to stress and stimulants, which may provide insight into potential drug addiction treatments for women.

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REACTIVITY TO METHAMPHETAMINE

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CHAPTER 1

INTRODUCTION

The use of illicit drugs is a serious problem in the United States with 9.2% of Americans reporting that they are current drug users and over 1.2 million people trying non-medical stimulants in the previous year (SAMSHA, 2013). National surveys report that both males and females use methamphetamine, with males reporting a slightly higher rate of methamphetamine use in "the past year" (0.7%) compared to females (0.5%; SAMHSA, 2005). Interestingly, a growing literature suggests that males and females respond differently to stimulants. Although a greater percentage of the male population reports using methamphetamine, females tend to be more sensitive to stimulants compared to males (Becker & Hu, 2008). Drug use in women escalates more quickly to addiction than in men as measured by increased frequency of drug use (Becker & Hu, 2008). Females are also more likely than males to enter treatment for cocaine addiction and have shorter drug-free abstinent time periods than their male counterparts (Becker & Hu, 2008; Kosten, Gawin, Kosten & Rounsaville, 1993). The goal of the current research is to better understand the effects of stimulants on females as such knowledge will allow for better treatment of drug addiction within the female population.

Stimulants

The illicit stimulants that individuals abuse include cocaine, methamphetamine and amphetamine. While all activate the central nervous system, these drugs have different mechanisms through which they act on the peripheral and central nervous systems. Cocaine

blocks the reuptake of the monoamines norepinephrine, dopamine and serotonin from the terminal, therefore increasing the levels of these neurotransmitters within the synapse (Heal & Pierce, 2006; Kimko, Cross, & Abernethy, 1999; Rothman et al., 2001). Methamphetamine and amphetamine also increase the levels of monoamines within the synapse but by reversing the direction of the catecholamine transporters and increasing the release of dopamine from the synaptic vesicles. Methamphetamine is thought to directly affect the vesicular monoamine transporter (VMAT) by decreasing the ability to package dopamine into vesicles and therefore increasing available dopamine within the terminal to be reverse transported into the synapse (Fleckenstein, Volz, Riddle, Gibb, & Hanson, 2007; Sulzer et al., 2005). The different mechanisms of action between stimulants are important as they may explain the differences in behavioral outcomes and patterns of drug use.

The behavioral and neurochemical responses in the brain due to acute compared to chronic exposure to drugs differ and are important to consider in understanding addiction. Much of the research on the mechanism of action has focused on acute exposure to stimulants, frequently on a drug-naïve nervous system. However, repeated exposure to stimulants can result in changes to the nervous system and behaviors that differ from the acute effects. Similarly, recent hypotheses suggest a shift in involved brain regions during acute drug use compared to chronic drug use (Piazza & Deroche-Gamonet, 2013). The following sections will discuss both the acute and chronic behavioral and neurochemical effects of stimulants, highlighting the studies that have used females.

Sex Differences in the Behavioral Effects of Stimulants

Exposure to a stimulant produces fast effects in the brain and on behavior. The behavioral

effects in rodents often have been measured in open field tasks that assess locomotion. Several studies have found differences in male and female responses following an injection of a stimulant. Schindler, Bross, and Thorndike (2002) measured locomotion in male and female rats following injections of methamphetamine, varying the dose from 0.1 mg/kg to 3.0 mg/kg with a two-day saline washout period between doses. All rats showed an increase in forward locomotion in a dose-dependent manner to methamphetamine. Female rats traveled significantly greater distances compared to males after equivalent methamphetamine doses (i.e., 1.0 and 3.0 mg/kg). Likewise, Milesi-Halle and colleagues (2005) measured forward locomotion after administering methamphetamine subcutaneously (s.c.) or intravenously (i.v.) to male and female rats. Their study found that female rats had a greater increase in locomotion compared to male rats immediately following an injection of 1.0 or 3.0 mg/kg i.v. methamphetamine when given a primer injection of 1.0mg/kg s.c. methamphetamine three days prior. The behavioral effects of the 3.0 mg/kg methamphetamine dose lasted a longer amount of time in females than males, although these differences were not noticeable at a lower dose of 1.0mg/kg (Milesi-Halle, Hendrickson, Laurenzana, Gentry, & Owens, 2007). Overall, the few studies that have compared the effects of methamphetamine on male and female rats suggest that females may be more behaviorally sensitive to the effects of methamphetamine, as indicated by the greater increase in locomotion in the open field.

Similar sex differences have also been reported with other stimulants, including amphetamine and cocaine. An injection of amphetamine increased the distance traveled for female rats to a greater degree than males when assessed in an open field arena (Milesi-Halle et al., 2007). The behavioral effects of a 3.0mg/kg amphetamine dose, but not a 1.0mg/kg dose,

lasted a significantly greater amount of time in females than males (Milesi-Halle et al., 2007). This sex difference also has been found after an acute injection of cocaine; female rats had greater locomotor activity than male rats when measured after the injection (Chin et al., 2001; Walker et al., 2001). Collectively, these findings suggest that female rats are more sensitive to the locomotor effects of a variety of stimulants compared to males.

Stereotypy is another behavioral measure used to assess the effects of stimulants. It is defined in rodents as the presence of repetitive movements, excessive licking and grooming, repetitive exploration, and rotational behavior (Ellinwood & Balster, 1974). Stereotyped movements are seen in multiple animal species, as well as humans who repeatedly use stimulants (for review see Lewis, Gluck, Bodfish, Beauchamp, & Mailman, 1996). Similar to the findings with forward locomotion, an injection of 3.0mg/kg methamphetamine or amphetamine induced stereotypy behaviors in both sexes, but female rats had longer lasting stereotypies with more head weaving and sniffing than male rats (Milesi-Halle et al., 2007). Likewise, after a single injection of 15.0mg/kg cocaine, drug-naïve female rats showed overall higher levels of stereotypy compared to male rats as measured on a 10-point rating scale (Chin et al., 2001). Walker and colleagues (2001) also found that female rats have a greater increase in stereotypy and horizontal activity than male rats after an injection of 10.0mg/kg cocaine. Interestingly, the presence of stereotypy can affect locomotion by decreasing horizontal locomotion while increasing the amount of repetitive behavior (Milesi-Halle et al., 2007). However, reports that have characterized both behaviors have found that females respond to stimulants both with enhanced locomotion and stereotypy compared to male rats. Thus, overall female rats naïve to stimulants show greater behavioral activation in both locomotion and stereotypy after

administration of methamphetamine, amphetamine, or cocaine compared to male rats. Their increased sensitivity to these drugs may contribute to an increased vulnerability to repeated use and addiction.

Repeated exposure to stimulants can also alter behavior over time. In some cases, the behavioral effects of a drug become more pronounced or "sensitized" with repeated administration, whereas in other circumstances stimulants can cause a decrease or "tolerance." Interestingly, the reports discussed above by Milesi-Halle and colleagues (2007) used repeated injections but varied the dose and administration route to ensure that neither sensitization nor tolerance developed. While chronic stimulant exposure has been well characterized in male rats (Steketee & Kalivas, 2011), few studies have systematically investigated it in females and those that have will be considered below.

Chronic administration of methamphetamine has been shown to alter locomotion and stereotypy scores in female rats. Female rats were injected with 8.0mg/kg methamphetamine once a week for 16 weeks and tested for methamphetamine-stimulated locomotor activity on weeks 1, 8 and 16 after a 30-minute habituation period to the open field. There were no differences between the weeks for forward locomotion, but increased repetitive head movement was observed, suggesting sensitization as measured by stereotypy (Clemens, Cornish, Hunt, & McGregor, 2007). Schindler and colleagues (2002) assessed sex differences with repeated exposure to methamphetamine by injecting male and female rats with 0.3mg/kg methamphetamine for a total of four injections with two days between each injection. Females travelled a greater distance than males overall, but there was no difference in distance travelled across days for either males or females (Schindler et al., 2002). Thus, there were sex differences

in response to an injection of methamphetamine, but neither sex showed behavioral sensitization to methamphetamine. The lack of locomotion changes across days is likely related to the low dose of methamphetamine administered, as sensitization studies with male rodents usually use a higher dose. The injection procedures used in both studies are fairly unusual and a more common experimental approach developed in male rats involves daily stimulant injections. Increasing the frequency of administration or dose of methamphetamine may increase the likelihood of sensitization to stimulants, as expressed by an increase in locomotion or stereotypy.

Behavioral sensitization has been observed in female rats following repeated administration of amphetamine or cocaine. Female rats exposed to 2.6mg/kg d-amphetamine once every two days for a total of 10 injections showed an increase in locomotion immediately following the last day of injection compared to female control rats that did not receive any prior exposure to drug (Bisagno et al., 2004). The increase of amphetamine-induced locomotion observed at the end of the injection regimen is considered behavioral sensitization. Doremus-Fitzwater and Spear (2010) injected female adult rats with 1.5mg/kg amphetamine or 3.0mg/kg amphetamine for four days and tested immediately following the injection on each day. The rats in both dosing groups had greater stereotypy scores on day four compared to day one, another measure of behavioral sensitization. However, only rats in the 1.5mg/kg group showed a sensitized locomotor response on day four compared to day one. The females injected with 3.0mg/kg had no differences in forward locomotion, which could be explained by a greater increase in stereotypy. To investigate the effects of chronic cocaine, Chin et al. (2001) injected female rats with 15.0mg/kg cocaine for fourteen days. As expected, the chronic cocaine group had significantly greater locomotion compared to the saline group on days 1, 7 and 14 when

tested 30 minutes after the injection. The cocaine-treated group also showed increased horizontal activity on the 14th treatment day compared to the 1st day, indicating behavioral sensitization. However, there were no differences in overall locomotion, rearing counts, or in stereotypy on days 1, 7 or 14. Daily administration of a lower dose of cocaine (5.0 mg/kg) for seven days also resulted in behavioral sensitization in female rats. Locomotion was recorded 30 minutes following the cocaine injection on days 1, 3, 5, and 7; there was a significant increase in locomotion exhibited on days 5 and 7, indicating locomotor sensitization to cocaine (Mandt, Allen, & Zahniser, 2009). In conclusion, female rats have shown behavioral sensitization to varying doses of chronically administered amphetamine and cocaine as measured by an increase in locomotion and stereotypy.

The above studies investigated drug exposure through passive administration (i.e., experimenter injected stimulants); however more recently, repeated exposure to stimulants has been investigated through self-administration paradigms. Self-administration procedures allow the animal to administer a drug by pressing a lever at a specific rate. These self-administration studies have investigated a variety of parameters related to addiction, including the likelihood of self-administration, the amount of drug administered, if an animal chooses the drug over some other reward such as food, or the likelihood of relapsing after extinction to the drug has occurred. Self-administration studies often use a progressive ratio schedule, which measures the motivation associated with working for a drug. This paradigm progressively increases the number of responses needed to receive a drug and therefore assesses how "hard" the rat will work to receive the drug.

Similar to the behavioral effects following passive administration of stimulants, female

rats react differently than male rats in a self-administration paradigm. Roth and Carroll (2004) found a greater percent of female rats reached the self-administration criterion for methamphetamine at a faster rate than male rats. Females also self-administered more methamphetamine than male rats under a progressive ratio schedule. Similarly, female rats administered more methamphetamine than males over a six-hour time period following one week of one hour fixed-ratio access to methamphetamine self-administration, although there were no differences found between males and females in the one-hour time period following the one-week self-administration training period (Reichel, Chan, Ghee, & See, 2012). The self-administration studies show that females take higher doses of methamphetamine more quickly than males, which suggests that their susceptibility to drug addiction may be greater. Behavioral sensitization following chronic exposure to methamphetamine through self-administration has also been characterized in male rats, but not in females. Across exposure days, rats' behavior changed from increases in primarily locomotion to the presence of stereotypy (Hadamitzky, McCunney, Markou, & Kuczenski, 2012). These findings are consistent with previous findings indicating the presence of stereotyped sensitization following increased chronic doses of stimulants, whether self-administered or experimenter administered.

Whether stimulants are self-administered or experimenter administered, they have been shown to induce behavioral sensitization in rats, and the timing between repeated drug administrations and testing has been shown to be critical. The one study that tested female rats found that those treated twice daily with escalating amphetamine dosing five days a week over 42 days showed increased sensitization to an amphetamine challenge at later time points compared to female rats treated with saline (Paulson, Camp, & Robinson, 1991). The

amphetamine-treated female rats showed similar stereotypy and locomotion to saline-treated rats when challenged with 2.6mg/kg amphetamine three days after the last treatment but increased stereotypy and no differences in locomotor crossovers 7 days later. These amphetamine-treated rats also have increased stereotypy and decreased locomotor crossovers compared to saline-treated female rats when given the amphetamine challenge 14, 28, 90 and 180 days following the last treatment exposure, indicating increased sensitization at longer time periods rather than shorter time frames following prior exposure (Paulson, Camp, & Robinson, 1991). Similarly, male rats exposed to an escalating dosing regimen of amphetamine over 6 weeks followed by a challenge injection of 0.5mg/kg amphetamine either 3, 7, or 28 days later showed similar findings. Only following a 28-day withdrawal did male rats show sensitized behavioral responses and dopamine release in both the dorsal and ventral striatum following the challenge injection. Rats tested 3 and 7 days after the amphetamine regimen showed no sensitized effect (Paulson & Robinson, 1995). Based on this research, sensitization is most robust with increased withdrawal periods between chronic administration and a challenge injection of a stimulant.

Taken together, the behavioral findings indicate that female rats have increased behavioral responses to stimulants compared to male rats. Multiple studies demonstrate that female rats show greater increases in locomotion and stereotypy following administration of amphetamine, methamphetamine or cocaine and these increased effects last longer. Female rats also self-administer more than males when given free access to stimulants. Most importantly, prior exposure to stimulants leads to greater behavioral sensitization in female rats than males. These findings together indicate that females are more sensitive compared to their male counterparts to the acute effects of stimulants and the long-term behavioral changes associated

with repeated use.

Role of Dopamine in Stimulant-Induced Behaviors

Various neural mechanisms may mediate the behavioral activation observed with stimulant exposure. One possible mechanism is dopamine. As discussed above, stimulants increase extracellular dopamine in many brain regions that receive dopaminergic input, such as the dorsal striatum, which in turn activates behavior (Kelly, Seviour, & Iverson, 1975; Paulson & Robinson, 1995). In male rats, methamphetamine has been shown to increase dopamine in the dorsal striatum in a dose-response fashion. Male rats were injected with either 1.0mg/kg or 5.0mg/kg methamphetamine and dopamine levels were measured using in vivo microdialysis in the caudate putamen. Extracellular dopamine levels peaked 30 minutes following the injection of methamphetamine and an 11-fold and 3-fold increase of extracellular striatal dopamine was reported after 5.0 or 1.0 mg/kg methamphetamine, respectively (Pereira et al., 2006). This dose-dependent increase in dopamine in the striatum parallels the observed longer lasting increases in distance traveled and stereotypy after an injection of 3.0mg/kg methamphetamine compared to 1.0mg/kg methamphetamine in both male and female rats (Milesi-Halle et al., 2007). Thus, methamphetamine injection increases dopamine in the dorsal striatum as expected given its mechanism of action at dopamine terminals.

There is some evidence that the dopamine response to methamphetamine may be more sensitive in females than males. McFadden, Carter, and Matuszewich (2012) found that adult female rats had higher levels of dopamine release following an injection of 2.0mg/kg methamphetamine compared to male rats when combining all rats in the study. However, when just comparing control female and male rats, there were no significant sex differences,

suggesting that the effect size of that sex difference may be small. Studies with cocaine support the increased sensitivity of female compared to male rats. Walker, Ray, and Kuhn (2006) injected male and female rats with 40.0mg/kg cocaine i.p. and used in vivo voltammetry to determine cocaine-stimulated dopamine release within the dorsal striatum. Female rats had higher extracellular dopamine concentrations following the cocaine injection compared to male rats, indicating increased sensitivity neurochemically in female rats. One potential explanation for differences in dopamine release following administration of stimulants is the dopamine membrane (DAT) and vesicular monoamine transporters (VMAT). The dorsal striatum has high concentrations of both transporters, which may render it particularly sensitive to the effects of methamphetamine (Volz, Fleckenstein, & Hanson, 2007). Previous research has suggested that sex differences following an injection of methamphetamine may be due to VMAT2 and DAT functioning, with female rats having greater DAT activity than males and a more active VMAT2 (Dluzen & McDermott, 2008).

Dopamine receptors are critical to methamphetamine-stimulated behaviors. There are two major families of dopamine receptors, D1 and D2. Both types of receptors have been implicated in the behavioral effects of stimulants. In male rats, both D1 and D2 antagonists decreased stereotypy of an amphetamine challenge injection, suggesting the importance of dopamine receptor activation for stimulant-induced sensitization (Kucenzski & Segal, 1998; Hamamura et al., 1991; Ujike, Onoue, Akiyama, Hamamura, & Otsuki, 1989). Moreover, mice lacking the D1 receptor show overall decreases in amphetamine- and cocaine-induced locomotor responses compared to wild-type mice (Xu, Guo, Vorhees, & Zhang, 2000; Zhang, Walsh, & Xu, 2000). However, studies with agonists are more difficult to interpret. Injections of either a D1 or

D2 receptor agonist alone produced hyperactivity with no significant sex differences (Thomsen, Ralph, & Caine, 2011). Conversely, Capper- Loup, Canales, Kadaba, and Graybiel (2002) found that neither D1 nor D2 receptor agonists alone could produce increased behavioral activity in rats, although the combination of the two agonists significantly increased stereotyped behaviors. The two studies differed on the particular agonists injected, the dosing, and the drug history of the rats. The Capper-Loup study used rats that had prior exposure to cocaine, whereas the animals were drug naïve in the Thomsen study. These findings suggest that both D1 and D2 receptors are necessary for stimulant-induced behavioral sensitization, although the exact mechanism and relationship are not completely understood.

Recent studies have focused on changes in the dopamine systems following chronic exposure to methamphetamine or other stimulants through self-administration procedures. Male rats given access to methamphetamine for 15 hours per day for 8 days were then assessed for dopamine levels 1, 7, or 14 days after completing the self-administration paradigm. Intracellular dopamine levels in the dissected striatum tissue were depleted after drug use ceased at all three time points. The dopamine transporter, although not measured at 1 and 7 days after self-administration paradigm, was concurrently decreased 14 days later (Krasnova et al., 2010). Similarly, free access to methamphetamine for six hours a day for 10 to 14 days in male rats resulted in decreases in dopamine transporter protein in the dorsal striatum when measured after a 14-day withdrawal period. These alterations in transporter protein levels were found without the presence of alterations of tissue basal dopamine levels (Schwendt et al., 2009). Decreases in dopamine transporter function were also found in male rats after five days of four-hour free access sessions to methamphetamine when the rat was sacrificed one hour after the final session

(McFadden, Stout, et al., 2012). These findings indicate that chronic exposure to methamphetamine through self-administration paradigms can have long-lasting effects on the dopamine transporter.

Overall, previous studies suggest that dopamine in the striatum may mediate stimulant-induced behaviors and that females differ from males in their striatal dopamine systems and responsiveness to stimulant drugs (Bobzean, DeNobrega, & Perrotti, 2014). Currently, the data for females is fairly limited, especially in response to methamphetamine. It is critical to better understand the mechanisms underlying females, increased sensitivity to stimulant drugs to allow for better interventions or treatment of addiction.

Role of Gonadal Hormones in Stimulant-Induced Behaviors

One major difference between males and females is the gonadal hormones estrogen, progesterone and testosterone. Gonadal hormones may mediate the sex differences observed with stimulant exposure. Becker and Cha (1989) found that female rats in the estrus phase, when estrogen levels are high, showed more amphetamine-stimulated stereotyped behaviors compared to females in diestrus. Likewise, the intensity of cocaine-stimulated stereotypy and locomotion was greatest during the estrus phase (Quiones-Jenab, Ho, Schlussman, Franck, & Kreek, 1999). The importance of estrogen in the response of female rats to stimulants was further supported in studies that remove endogenous estrogen through ovariectomy with hormone replacement. Ovariectomized female rats had a greater response to either cocaine or amphetamine if given estrogen compared to females that did not receive estrogen (Becker, Molenda, & Hummer,

2001). Sircar and Kim (1999) found that female rats ovariectomized and given both estradiol benzoate and progesterone had a potentiated behavioral response to 15.0 mg/kg cocaine compared to rats not given any treatment or given estradiol benzoate or progesterone alone. Ovariectomized female rats not treated with hormones had behavioral sensitization scores similar to males' scores, as measured by a blind observer and classified on a 12-point rating scale (Sircar & Kim, 1999). In rats, ovariectomized females showed a decrease in choosing a cocaine-paired chamber compared to intact females or ovariectomized females receiving estrogen benzoate (Russo et al., 2008). Russo and colleagues found that administration of 500μg of progesterone in female rats four hours before the conditioning phase decreased conditioned place preference to 5.0mg/kg cocaine. These data suggest that the greater circulating levels of estrogen and progesterone in female rats may mediate the behavioral sex differences observed in response to a stimulant injection. Gonadal hormones may modulate some of the rewarding effects of stimulants in women as well. In a review by Hudson and Stamp (2011) it was found that ovarian cycles in women could have an impact on withdrawal from drugs and on relapse when exposing the user to the drug. Previous studies have indicated that cocaine-dependent women who were administered progesterone reported a decrease in the rewarding properties after using the stimulant (Evans, 2007; Evans & Foltin 2006). It is possible that progesterone treatment for stimulant female users may decrease subjective reinforcement experienced with the stimulant, which may not be effective for male users. Based on these studies, it appears that the levels of circulating gonadal hormones can impact behavioral responses to stimulants and argues for the importance of controlling for stages of the estrous cycle when conducting experiments, either through statistical analyses in naturally cycling females or through manipulation of hormones.

Role of Gonadal Hormones in Stimulant-Induced Dopamine Levels

Gonadal hormones may alter the brain's response to stimulants that contribute to behavioral differences observed between males and females. As discussed above, stimulants activate dopaminergic pathways in the brain and gonadal hormones may modulate these pathways. Castrated male rats and ovariectomized female rats received 5µg estradiol benzoate or vehicle followed 30 minutes later with 10.0mg/kg cocaine (Cummings, Jagannathan, Jackson, & Becker, 2014). Female rats that received estradiol benzoate had a greater increase in extracellular striatal dopamine in vivo following the cocaine injection compared to the female rats that received the vehicle injection 30 minutes prior. There were no overall differences between males and females when combining both groups receiving estradiol benzoate and those receiving vehicle. Male rats that received the estradiol benzoate did not differ from those that received the vehicle, indicating that only females were sensitive to the effects of estrogen following stimulant exposure. Interestingly, no differences were found in the nucleus accumbens in either male or female rats treated with vehicle or estradiol benzoate, suggesting that the dorsal striatum would be an area of interest for sexual dimorphisms in the dopaminergic activation following cocaine.

Other studies in the dorsal striatum have also shown dopaminergic response to stimulants to be dependent upon gonadal hormones. Female rats that received a sham ovariectomy had greater dopamine release in the dorsal striatum following an injection of 10 mg/kg cocaine compared to ovarietcomized females (Walker et al., 2012). Castner and colleagues compared castrated male and ovariectomized female rats primed with either 2.0µg/100g estradiol benzoate

or 1.5μg/100g 17B-estradiol and then challenged with d-amphetamine 30 min later. Priming with either form of estrogen increased the extracellular dopamine following an injection of d-amphetamine in the ovariectomized females, but neither had an effect on extracellular dopamine in the castrated males following an injection of d-amphetamine (Castner, Xiao, & Becker, 1993). Dluzen and McDermott (2000) also found that ovariectomized female mice implanted with a 21-day estradiol pellet had a greater percent increase in dopamine concentrations within the striatum following four injections of 20.0mg/kg methamphetamine over an eight-hour time frame compared to female mice that received the vehicle. Overall, these findings suggest that the dopaminergic response to stimulants may be dependent upon estrogen, and clear sex differences exist in this relationship.

Role of Gonadal Hormones in Basal Dopamine Levels

Both basal levels of dopamine and stimulant-induced release of dopamine are dependent upon the function and number of dopamine-related targets (i.e., DAT, dopamine receptors). Previous research in humans and rodents has examined DAT in the striatum of males and females. Consistent with possible gender differences in dopamine neurotransmission, one study has found that women have more dopamine transporters within the striatum compared to men, which would support the suggestion that females are more sensitive to drugs such as stimulants that act on DAT (Mozley, Gur, Mozley, & Gur, 2001). However, other studies in humans have not found similar sex differences in dopaminergic markers. Using single-photon emission computed tomography (PET), no differences were found between men and women in dopamine transporters within the striatum (Best et al., 2005). The discrepencies between the prior two

studies have been explained as potentially due to low statistical power for Best et al. or different radioligands used during the imaging techniques (Best et al., 2005). When meauring the D2 receptors in the striatum, there were no differences following an i.v. injection of saline between men and women through the use of binding potential during PET scan (Munro et al., 2006). At this point, there seems to be no consistent conclusions made regarding sex differences in dopamine transporters and the mixed results are likely due to differences in methodologies used.

To determine whether gonadal hormones may be important for dopamine function, several studies have compared the stage of the menstrual cycle in humans or estrous cycle in rodents. In women, no differences in dopamine transporters in the striatum of females were observed in the follicular phase compared to those in the luteal phase of the menstrual cycle. The dopamine transporter availability also was not correlated with the estradiol and progesterone levels associated with these phases of the menstrual cycle (Best et al., 2005). However, when injected with saline, dopamine binding was found to be lower in the putamen but not the ventral striatum in women in the luteal phase of the menstrual cycle compared to those in the follicular phase (Munro et al., 2006). In rats, DAT binding is greater during diestrus of the estrous cycle in intact females when hormones are low compared to proestrus when estrogen levels are high (Datla, Murray, Pillai, Gillies, & Dexter, 2003). Ovariectomized rats had a decrease in DAT binding in the middle striatum (.48-.2 from bregma). This effect was reversed when rats were treated with two weeks of oestradiol replacement, such that DAT binding was similar to that of intact females (Le Saux & Di Paolo, 2006). These findings suggest that gonadal hormones in female rats likely contribute to DAT binding.

DAT contributes to basal levels of extracellular dopamine by regulating the removal of

dopamine from the synapse, with increased DAT binding contributing to a decrease in extracellular dopamine (Becker, Perry, & Westenbroek, 2012). Thus, changes in DAT due to gonadal hormones can also explain changes in basal dopamine levels. In female mice, the highest basal dopamine levels were measured during proestrus; lowest levels were measured during the diestrus phase of the estrous cycle as measured through ex vivo levels in the striatum. When measuring basal dopamine levels through microdialysis, castrated male rats had a significantly higher level of dopamine than ovariectomized female rats (Castner, Xiao, & Becker, 1993; Cummings et al., 2014). Using fast-scan cyclic voltammetry in anesthesized unaltered rats, female rats had more extracellular dopamine in the caudate nucleus following electrical stimulation compared to male rats (Walker, Rooney, Wightman, & Kuhn, 2000). Although these findings differ, one study compares basal altered rats while the other study compares stimulated unaltered rats and likely contributes to the mixed findings. Taken together, animal and human studies suggest that gonadal hormones can influence basal and stimulated levels of dopamine in the striatum, which in turn could mediate behavior differences observed between males and females.

Stress

The hypothalamic-pituitary-adrenal (HPA) axis regulates secretion of glucocorticoids, which in turn can affect the brain and various behaviors. In the presence of stress, the hypothalamus secretes corticotrophin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH, in turn, stimulates the secretion of glucocorticoids from the adrenal gland. The HPA axis works through numerous feedback

loops to help the organism re-establish homeostasis, even in times of stress (Kudielka & Kirschbaum, 2005).

Sex Differences in Basal Glucocorticoids

Another process that may contribute to sex-specific activity levels and responses to stimulants is the HPA axis. Chisari, Carino, Perone, Gaillard, and Spinedi (1994) measured basal HPA axis function and plasma hormone levels of ACTH and corticosterone (CORT) of intact male and female rats. They found that intact females had higher levels of both ACTH and CORT compared to intact males when tested at the start of the light cycle (08:00-09:00). In support of this research, our laboratory has found that plasma CORT levels are higher under basal conditions in female than male rats when blood was collected 4-5 hours into the light portion of their diurnal cycle (McFadden et al., 2011). Similar findings were also reported in intact male and female rats where the male rats had lower basal CORT levels compared to intact female rats (Kokras et al., 2012). Taken together, multiple studies have found that female rats have higher basal glucocorticoid levels than male rats.

Time of day is an important variable as circadian rhythms have been shown to alter levels of glucocorticoids. Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb (1963) found basal levels of CORT and ACTH in plasma and adrenal glands differed by sex depending on the time of day. Male and female rats that were housed in a 14:00 light/dark cycle with lights on from 04:00 to 18:00 showed no differences in plasma or adrenal CORT levels at 03:00 (lights off); however, females had a higher peak of CORT in the early period of the dark cycle than did males (19:00, lights off). ACTH levels were also found to differ between males and females with females having low levels in the morning and higher levels in the early evening (19:00), whereas

males had higher levels in the morning (07:00) and lower levels in the afternoon (15:00-23:00). Taken together, these findings indicate that there are basal sex differences in the HPA axis between male and female rats but that these differences may be modulated by time of day and circadian rhythms.

Sex Differences in Acute Stress-Stimulated Glucocorticoid Responses

Females also have been shown to have different glucocorticoid responses to stress than males, as has been shown in a variety of acute stress paradigms. Female rats tend to have higher glucocorticoid levels than male rats after activation of the HPA axis (Kudielka & Kirschbaum, 2005). Acute swim stress, restraint stress and foot shock increased plasma corticosterone levels in females to a greater extent compared to males (Duchesne, Dufresne, & Sullivan, 2009; Galea et al., 1997; McFadden et al., 2011; Verma, Hellemans, Choi, Yu, & Weinberg, 2010; Weinstock, Razin, Schorer-Apelbaum, Men, & McCarty, 1998; Zareian, Karimi, & Dorneyani, 2011). These acute stress-induced changes in CORT levels are long lasting, with female rats having higher CORT levels than males as long as a week after acute restraint stress (Verma et al., 2010).

The sex differences under acute stress conditions may be dependent on the time of day similar to basal levels. Male rats exposed to acute restraint stress and then put in open field had significantly higher CORT levels in both the light and dark phases compared to the resting state. Likewise, male rats showed greater CORT levels after a five-minute forced swim test compared to basal levels. Conversely, female rats only had increased CORT levels following either acute stress paradigm (i.e., restraint and open field or forced swim) in the light phase but only had increases in the dark phase following the forced swim test (Verma et al., 2010). Studies looking at CORT levels after an acute stressor tend to suggest that female rats have a higher increase in

CORT levels than male rats, but time of day during which data collection takes place may produce varying results.

Sex Differences in Locomotion and Corticosterone Following Chronic Stress

Sex differences have been reported following exposure to chronic stress in behavioral measures and stress hormone levels. Chronic mild stress (CMS) is a procedure that consists of exposing rodents to a series of mild stressors over a period of weeks (Willner, 1997, 2005). Many behaviors are altered following exposure to CMS. For general locomotion, male and female rats exposed to CMS lasting for six weeks had decreased movement compared to control rats in the open field (Dalla et al., 2005). Females exposed to CMS had greater decreases in time spent rearing and moving compared to males (Dalla et al., 2005). Following 21 days of repeated restraint stress (6 hours/day), female rats did not show an increase in locomotion immediately following the last restraint stress compared to female control rats, suggesting a behavioral tolerance or habituation to the restraint stress. However, there was no comparison made to male rats (Bisagno et al., 2004). The same repeated restraint stress was found to decrease grid crossings in an open field during the dark in both male and female rats on the day following the last day of stress. When tested in the light, females had an increase in locomotor grid crossings compared to a decrease in grid crossing in male rats (Huynh, Krigbaum, Hanna, & Conrad, 2011). Another study, exclusively focused on male rats, found that male rats exposed to 14 days of restraint stress (1 hour/day) showed an increase in locomotion to a novel open field on day 15 compared to rats exposed to unpredictable stress or non-stressed rats (Araujo, DeLucia, Scavone, & Planeta, 2003). Similarly, chronic unpredictable stress (CUS) lasting ten days in male rats was shown to increase locomotion in an open field on the day following the last day of stress (Cox et

al., 2011). These discrepancies of the behavioral effects are difficult to interpret and would suggest that many factors, such as the type of stress, length of stress and time of day, may be critical.

CMS and other chronic stress procedures have also been shown to alter CORT levels to a greater degree in female rats than in male rats. Female rats exposed to six weeks of CMS had a significant increase in basal CORT levels while males did not (Dalla et al., 2005). In this study, there were no sex differences in basal CORT levels, contrary to previous research in rats. Exposure to chronic restraint stress (6 hour/day) for 21 days also resulted in sexually dimorphic CORT responses in stress-induced CORT release. Bowman, Zrull, and Luine, (2001) reported that female rats exposed to restraint stress (21 days for 6h/day) had higher levels of CORT compared to baseline levels, prior to the start of stress. This finding was confirmed by Galea et al. (1997), who also showed females to have overall higher plasma CORT levels compared to male rats when tested 30 minutes into restraint on multiple days. Chronic 21-day footshock stress also significantly increased CORT levels when measured two hours after the last stress exposure in male and female rats, but females had a significantly higher increase (Kuipers, Trentani, van der Zee, den Boer, 2013). In our laboratory, we found sex differences in swim stress-induced CORT levels following 10 days of unpredictable stress. However, prior exposure to unpredictable stress attenuated the acute swim stress-induced CORT increases compared to female control rats, but not in male rats (McFadden et al., 2011). Taken together, these findings indicate that female rats may be more sensitive to chronic stress than male rats as measured by increases in circulating CORT levels, but the type or length of chronic stress exposure may be critical to the effects on the function of the HPA axis.

Overall, the previous research suggests that CORT response to stress may differ between males and females, with females showing greater changes in CORT to physical or social stressors compared to males. Stimulant drugs also increase glucocorticoids (Goeders & Guerin, 1996) and manipulating the stress-hormones in males can influence stimulant-dependent dopamine release and behavior (Piazza & Le Moal, 1997). However, very few studies have investigated whether stress sensitizes the dopamine response in female rats despite their sensitivity to stress and stimulants. Dalla et al. (2008) exposed male and female rats to 6 weeks of CMS then took brains 24 hours later following the last stressor. Ex vivo dopamine content in tissue was then assessed within the striatum. Female rats were found to have lower dopamine content than male rats, irrespective of condition, and CMS did not significantly alter dopamine levels in either male or female rats. Scheggi et al. (2002) exposed male rats to 21 days of chronic stress that consisted of both restraint and unavoidable stress every other day throughout the protocol then tested dopamine content in the caudate putamen three days following the last stressor. There were no basal dopamine level differences when comparing the unavoidable stress group and the control group. Likewise, male rats exposed to CUS for ten days or no stress (control) showed no significant differences in extracellular basal dopamine as measured by microdialysis one day following the last stressor (Matuszewich and Yamamoto 2004; Tata, Raudensky, & Yamamoto, 2007). Similarly, baseline dopamine levels were found not to differ between control male rats and rats tested the day following unpredictable stress or one to two weeks after the stress protocol (Matuszewich, Carter, Anderson, Friedman, & McFadden, 2014). Together, these findings indicate that chronic stress does not alter ex vivo or in vivo unstimulated basal dopamine, at least within the striatum.

In summary, female rats overall have higher basal levels of stress hormones than male rats. Similarly, exposure to either acute or chronic stress increases stress hormones to a greater extent in females than in males and these increases are longer lasting after stress exposure. However, there is no evidence that suggests that chronic stress alters basal levels of dopamine in the striatum for either males or females. It is not known in female rats if stress alters stimulant-induced dopamine increases in the brain, but given their increased sensitivity to stress, females may have greater cross-sensitization, which may contribute to an increased risk of addiction.

Stress and Stimulant Interaction

Exposure to stress can increase the likelihood of drug abuse in humans. Koob (2008; 2009) has proposed that stress, stress-related neurological mechanisms, and stimulant drugs interact to create a unique drug by stress response. Previous studies have suggested that stress can sensitize the nervous system to stimulant drugs, leading to an augmented response in individuals who are drug naïve and this is called cross-sensitization (Piazza & Le Moal, 1997, 1998). Koob's theory of cross-sensitization suggests that prior exposure to stress may increase dopamine release in reward centers of the brain by increasing glucocorticoids, which leads to an increase in the reinforcing properties of stimulants. Although both stress and stimulants can separately have an effect on the brain and behavior, many individuals experience both together and therefore understanding the interaction is critical for improving treatment of drug addiction.

Behavioral Findings

Cross-sensitization is an increase in responsiveness to a stimulus, such as a stimulant drug, due to prior exposure to another type of stimulus, such as stress. Cross-sensitization

between stress and stimulants has been assessed through measuring behaviors in male rodents (for review see Piazza & Le Moal, 1996). For example, male rats exposed twice daily to a variety of stressors (including foot shock, restraint, individual cold housing, and swim stress) for six days and then given a two-week withdrawal had an increase in horizontal activity in response to a challenge injection of 15 mg/kg cocaine (Prasad, Ulibarri, & Sorg, 1998). Araujo and colleagues (2003) reported similar findings following 14 days of either repeated restraint stress or unpredictable stress (including restraint stress, cold room isolation, and changes of the light/ dark cycle). When challenged the day after stress exposure ended, 10.0mg/kg cocaine significantly increased locomotion in males exposed to restraint stress compared to controls. There were no differences between the groups following a saline injection or between the unpredictable stress group and control group following the cocaine injection. The restraint stress group had the highest locomotion followed by the unpredictable stress group and last the control group, suggesting that the restraint group was most sensitive to cocaine. Thus, exposure to either predictable or unpredictable stress for 14 days does not influence general locomotor activity but increases the behavioral sensitization when injected with cocaine. Haile, GrandPre, and Kosten (2001) also found male rats exposed to unpredictable stress for 10 days had an increase in behavioral activity when injected with 7.5 mg/kg cocaine the day after the stress exposure ended, whereas those rats exposed to chronic predictable stress did not have a significant increase in locomotion compared to the control non-stressed group. The sensitivity following the particular stress exposure differs between the Araujo and Haile papers, but there were many methodological differences in the administration of stress (e.g., duration/intensity of stressors and length of stress protocol) as well as in the protocol for the open field (e.g., habituation

periods and drug dose). In general, though, all of these studies show evidence for cross-sensitization between prior stress exposure and stimulant-induced behavioral activation in males.

Depriving male rats of food has been used as a stressor to induce sensitization to stimulants as well. Male rats food restricted to 80% of their free-feeding weight over a period of one to two weeks were then tested with three doses of cocaine (0, 2.5, 5.0, 10.0 mg/kg) with a washout day in between test days. There were no significant differences in locomotion between the food-deprived group and the group that had unlimited food access following a saline injection or the low dose of cocaine (2.5 mg/kg). However, the food-deprived group had an increase in locomotion after the cocaine injection compared to the rats that were not food-deprived following an injection of either of the two higher doses of cocaine (5.0 mg/kg & 10.0 mg/kg; Bell, Stewart, Thompson, & Meisch, 1997). Similarly, it had been found that male food-deprived rats have an increase in locomotion after an injection of amphetamine directly into the nucleus accumbens, compared to male rats that had not been food deprived (Deroche et al., 1995). Taken together, these findings indicate that several types of stress exposures increase the sensitivity to cocaine or amphetamine and this may indicate a more general mechanism underlying stress-induced cross-sensitization.

Social stress also potentiates the behavioral response to stimulant drugs. Nikulina and colleagues (2004) exposed male rats to intruder resident social defeat stress every third day for a total of four days over a ten-day period. The social defeat stress consisted of the experimental animal being placed into a defensive resident animal's cage. In the resident's cage, the intruder or stressed rat could be bit and/or pursued by the resident multiple times. Rats were then challenged with either saline or d-amphetamine 7 or 60 days after the last day of social stress.

Although there were no differences between the social stress group and the control group when challenged with a saline injection, when challenged with 1.0mg/kg d-amphetamine, the rats that experienced social stress had an increase in locomotion compared to the control non-stressed rats at both time points. Similarly, Covington and Miczek (2001) exposed male rats to social stress four times across a period of ten days and found a greater increase in locomotion after 1.0mg/kg amphetamine challenge ten days after the last social defeat stress compared to the control group when assessed 10, 35, and 60 minutes after the injection. Collectively, these findings indicate that prior exposure to social stress can increase behavioral locomotor responses to a stimulant compared to non-stressed rats, indicating that the predisposing stress need not be physical to elicit sensitization to a later challenge of a stimulant.

In a study examining cross-sensitization in both female and male rats, Holly, Shimamoto, DeBold, and Miczek (2012) assessed locomotor activity following a challenge of cocaine. The rats were exposed to either resident intruder social defeat stress or daily handling and then challenged with an acute injection of 10.0 mg/kg cocaine ten days after the last defeat session. Male and female rats that were stressed showed a significant increase in locomotion five minutes after the injection of cocaine compared to the rats that received daily handling. Interestingly, the female rats continued to show a sustained elevation in locomotion 25 minutes after the injection, whereas the male rats' locomotion at this time did not differ from baseline levels. Similar to the potentiated response to an acute injection of stimulants, female rodents appear to be more responsive to the behavioral effects associated with cross-sensitization.

The few studies investigating the stress sensitization to stimulants in female rats have found similar effects to that of male rats. Female juvenile (PD21-22) and adult (PD 64-66) rats

were exposed to 60 min/day for four days of restraint stress or were left undisturbed. The rats were challenged with 1.5mg/kg amphetamine 2 and 21 days after the last stressor. Compared to females that were not exposed to restraint stress, juvenile and adult female rats exposed to restraint stress had an increase in locomotion to 1.5mg/kg amphetamine as measured by the number of crosses in a test chamber on either the 2- or 21-day challenge. There were no differences in stereotypy scores after the amphetamine injection on either day, although all rats tested on day 21 showed higher levels of stereotypy compared to the challenge on day two (Doremus-Fitzwater & Spear, 2010). In a more complex design, female rats were exposed to 6 h/day restraint stress for 21 days and 2.6 mg/kg amphetamine was injected every other day for a total of 10 injections. The combination of stress and stimulant exposure showed significant increases in locomotion compared to rats only exposed to stress, only exposed to drug, and the control group exposed to neither stress nor drug (Bisagno et al., 2004). Overall, these findings suggest that female rats also show cross-sensitization between stress exposure and stimulants; however, the number of studies investigating this cross-sensitization is quite limited at this point, with previous data only examining restraint and social defeat stress.

Role of Dopamine in Stress-Induced Cross-sensitization

Dopamine is critical to stimulant-induced behavioral activation. In male rats, prior exposure to stress or stress hormones sensitizes the dopamine response to stimulant drugs. Male rats food deprived to 90% of their free-feeding weight show a significant increase in nucleus accumbens dopamine after exposure to 10.0mg/kg i.p. cocaine compared to free-feeding rats (Rouge-Pont, Marinelli, Le Moal, Simon, & Piazza, 1995). Similarly, male rats exposed to ten days of CUS then injected with 7.5mg/kg methamphetamine during microdialysis show a

significant increase in dopamine release in the nucleus accumbens compared to control animals (Raudensky & Yamamoto, 2007). Using the same stress procedures, dopamine efflux in the dorsal striatum of male rats was also augmented compared to control rats after four injections of 10.0mg/kg or a single injection of 7.5 mg/kg methamphetamine (Matuszewich & Yamamoto, 2004; Matuszewich et al., 2014). The sensitized response of dopamine following a single injection of the lower dose (7.5mg/kg methamphetamine) is interesting because extracellular dopamine within the striatum after multiple injections of methamphetamine (every two hours for a total of four injections) did not differ compared to rats that were not stressed (Matuszewich & Yamamoto, 2004; Tata, Raudensky & Yamamoto, 2007). The Matuszewich et al. (2014) and Matuszewich and Yamamoto (2004) studies measured dopamine at different time intervals (15 minutes compared to every 60 minutes), which may be important for stress-potentiated effects (Matuszewich et al., 2014). Thus, while the data is not entirely consistent, most studies have found that prior exposure to stress increases the dopamine response to stimulant drugs in male rats.

Although no differences have been found in basal dopamine levels of stressed compared to non-stressed rats (Scheggi et al., 2002), prior stress likely lays a foundation for a synergistic effect with stimulant drugs. Prior exposure to stress has been shown to increase dopamine within the dorsal striatum following a stimulant injection, such as methamphetamine, which may lead to the observed increases in locomotor activation. In male rats, the evidence suggests that prior exposure to stress increases the behavioral and dopaminergic response to stimulant drugs. Similar cross-sensitization studies have not been conducted in female rats, despite their sensitivity to both stimulant drugs and stress alone. Therefore, the current study examined

whether females demonstrated behavioral and dopaminergic cross-sensitization between CUS and the stimulant drug methamphetamine. Overall, it was predicted that female rats exposed to chronic stress would show sensitized responses through increased locomotor and striatal dopamine release following an injection or methamphetamine.

Hypotheses

Experiment 1 Hypothesis: It was hypothesized, based on male data, that female rats exposed to CUS would show greater forward locomotion following an injection of methamphetamine compared to control rats.

Experiment 2 Hypothesis: It was predicted that dopamine levels in the striatum would follow a similar pattern to that of the locomotion data. Therefore, it was predicted that female rats exposed to CUS two weeks prior to microdialysis would have a greater increase in dopamine levels within the dorsal striatum compared to female control rats following an injection of methamphetamine.

Exploratory Hypothesis: It was predicted that female rats in estrus phase of the estrous cycle would have an increased locomotion in the open field and increased extracellular dopamine release following an injection of methamphetamine compared to rats classified as being in the diestrus or proestrus phase.

CHAPTER 2

METHODS

Subjects

A total of 76 female Sprague-Dawley rats (~75 days old) from the colony at Northern Illinois University were used in this study. Experiment 1 included 48 female rats (control n=23, CUS n=25) and experiment 2 included 28 female rats (control n=14, CUS n=14). Rats were housed in polycarbonate cages in a room maintained at a temperature of 22±2ºC and a 12-hour light/dark cycle (lights on at 06:00h). Rats had access to food and water ad libitum except for CUS rats when specified in the protocol. All procedures were approved by the Northern Illinois University Institutional Animal Care and Use Committee and followed National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication 8th edition, 2011).

Stress

In experiments 1 and 2, rats were randomly divided into one of two groups: CUS or control. The rats in the CUS group received two stressors a day at varying times lasting ten consecutive days (see Table 1) as adapted from Gouirand and Matuszewich (2005). For experiment 1, rats in the control group were handled daily for weighing and vaginal smears similar to the stress group but no additional stressors were applied. In experiment 2, the control group received daily handling and received surgery one week before microdialysis.

Table 1
Schedule of CUS Protocol

Day 1	13:00 wet bedding stress, 4 hr. (add 400ml of water to cage)
	17:00 lights on, overnight
Day 2	11:00 cold room, 60 min.
	12:00 shaker table, 50 min.
Day 3	11:00 lights off, 3 hr.
	15:00 restraint stress, 60 min.
Day 4	16:00 shaker table, 50 min.
	17:00 food/water deprivation, overnight
Day 5	15:00 cold room, 15 min.
	17:00 isolation housing, overnight
Day 6	11:00 wet bedding stress, 4hr. (add 400ml of water to cage)
	16:00 lights off, 2 hrs.
Day 7	13:00 shaker table, 30 min.
	18:00 lights on, 60 min.
Day 8	10:00 shaker table, 20 min.
	15:00 restraint stress, 60 min.
Day 9	10:00 wet bedding stress, 4 hr. (add 400ml of water to cage)
	17:00 food water deprivation, overnight
Day 10	17:00 isolation housing, overnight
	17:00 lights on, overnight

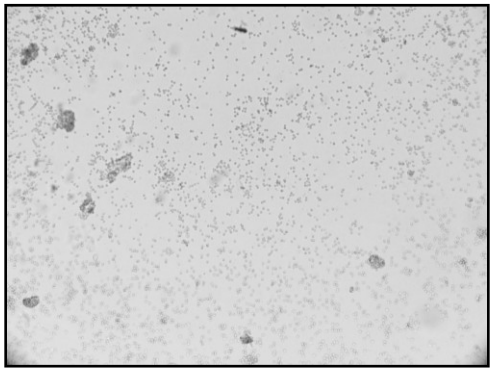
Drug Treatment

For behavioral testing in experiment 1, saline (0.9% NaCl) was injected on day one of the open field paradigm. Methamphetamine HCl (7.5mg/kg) was dissolved in 0.9% saline (1ml/kg) and injected on day two of the open field paradigm. For experiment 2, all rats received methamphetamine HCl (7.5mg/kg) dissolved in 0.9% saline (1ml/kg) during the microdialysis protocol.

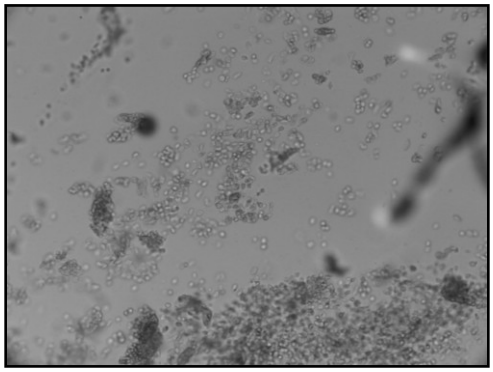
Vaginal Smears

Vaginal smears were done daily between 09:00h-10:00h to assess the estrous cycle of each female rat in all groups. Clean 0.9% saline was taken up into an eyedropper for a total of 100 µl. The tip of the eyedropper was inserted into the vagina, the bulb of the eyedropper squeezed and then released so as to gently push saline into the vagina and then draw it back into the eye dropper. The fluid was placed on a slide and viewed under a microscope at 10x power. Diestrus was classified when the sample had a dominance of leukocytes and larger cells that were not nucleated (see Figure 1a). Proestrus was classified when the sample had a dominance of nucleated cells (see Figure 1b), and estrus was classified when the sample had a dominance of cornified cells (see Figure 1c; Becker et al., 2005). All female rats were smeared beginning five days before stressors started. Since stress has been shown to have an effect on the estrous cycle (Mourlon, Naudon, Giros, Crumeyrolle-Arias, & Dauge, 2011), female rats continued to have vaginal smears each day to determine whether the stress procedures altered their cycle. Once the female began the stress procedures, she remained in the study regardless of her cycle length. The number of cycles were counted during the 10-day period (CUS procedures or matched time frame for control rats) and compared.

A



B



C

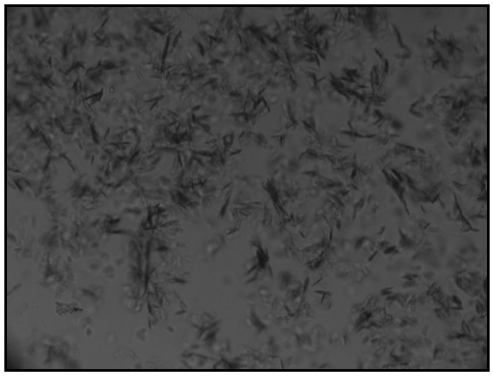


Figure 1. Representative vaginal smear samples for (A) diestrus, a predominance of leukocytes (B) proestrus, a predominance of nucleated cells and grape-like clumping and (C) estrus, a predominance of cornified cells.

Experiment 1: Open Field

The open field test was conducted two weeks after the last stressor. On day one of testing, a rat was individually placed into the back right corner of the 48 x 48cm wood box with a camera overhead attached to a Panasonic DMR-ES25 DVD recorder used to record all behavior. The rat was allowed to explore the box for 30 minutes and then injected with 0.9% saline i.p. and returned to the back right corner of the box. She was then recorded for 90 minutes until testing was done for the day. On day two of testing, similar procedures were followed, with a 30-minute habituation period and then an injection of 7.5mg/kg methamphetamine i.p. dissolved in 0.9% saline, and the female rat was returned to the box for 90 minutes. After day two of testing, rats were isolated overnight to monitor hyperthermia and then returned to their home cage the following morning.

Stereotypy

Stereotypy was analyzed using a quantitative scale based on a previous scale by Ellinwood and Balster (1974) to quantify behaviors that include repetitive head movements, rotational behavior, and slow patterned behaviors. Stereotypy was classified based on the predominant behavior in the open field recording beginning at 35 minutes into the experiment (five minutes after the time of methamphetamine injection). The behavior was based on a 30-second observation every 5 min from 35 min to 120 min into day two of the open field testing. The rating scale used to assess rat behavior is listed in Table 2 with behavioral scores ranging from 1 (sleep) to 8 (continuous stereotypy movements).

Table 2
Rating Scale Used to Assess Stereotypy on Day Two of Open Field

Rating	Behavior
1	Sleep
2	Awake but not moving
3	Normal exploration
4	More active than normal but with normal movements
5	Running from edge to edge
6	Slow patterned at a normal level
7	Continuous rotational movements
8	Repetitive head movement

Adrenal Gland Weights

For experiment 1, rats were sacrificed using rapid decapitation three to six weeks following the open field test. Brains were immediately removed and frozen on dry ice and stored in -80ºC for a future study. Adrenal glands were removed from rats in all groups and immediately weighed for comparisons.

Statistical Analyses

A 7 (time- 15 minute blocks) x 2 (condition- CUS or CON) x 2 (drug challenge- saline or methamphetamine) repeated-measures analysis of variance (ANOVA) was used to assess differences in locomotion during the open field test. For all statistical analyses, the first time point of 0-15 minutes was not used and 15-30 minutes was used as a baseline; therefore, a total of seven time points were used in statistical analyses. Stage of estrous cycle on the test day was used as a covariate but was not significant for any measure and therefore was not used for the analyses presented below. Separate 7 (time- 15 minute blocks) x 2 (condition- CUS or CON) ANOVAs were run for each day of the open field. A 17 (time- 5 minute blocks) x 2 (condition) repeated-measures ANOVA was used to assess differences in stereotypy ratings during the open field test following 7.5mg/kg methamphetamine. Independent-samples *t* test for group comparisons at specific time points was used for post-hoc analyses. Adrenal weights were analyzed using an independent-samples *t* test with adrenal weight ratio as the dependent variable and condition as the independent variable. Adrenal weight ratio was calculated by dividing the adrenal weight by the body weight. The number of estrous cycles during the 10 days of CUS or a matched time period for controls was compared with an independent *t* test. Greenhouse-Geisser correction to the degrees of freedom was used to correct for all Mauchley's test of sphericity

assumption violations, although corrections were rounded to the nearest whole number.

Experiment 2: Microdialysis

Surgery

One week before microdialysis, a guide cannula was implanted above the dorsal striatum using procedures based off of Matuszewich and Yamamoto (2004). For rats in the CUS exposure group, rats underwent surgery one week after the last day of stress exposure. Rats were anesthetized with a combination of xylazine (6 mg/kg) and ketamine (70 mg/kg) at a dose of 0.75mg/kg xylazine/ketamine mixture. The female rat's head was shaved, the area cleaned with Betadine solution (povidone-iodine, 10%; Purdue Frederick) and then placed into a Kopf stereotaxic frame. One drop of mineral oil was placed onto each eye to prevent drying and was re-administered as needed. A 21-gauge stainless steel guide cannula (11 mm in length, Small Parts, Inc., Miami Lakes FL USA) was implanted above the striatum (+.05 mm anterior, $\pm$.30 mm medial, -.10mm dorsal to bregma) and secured by three metal screws with super glue and cranioplastic cement. A 27-gauge stainless steel stylet was placed into the cannula until the day before microdialysis to prevent debris from blocking the cannula. Triple antibiotic treatment (equate) was put on the incision site and the rat was monitored during recovery from anesthesia and weighed daily until microdialysis to assess for weight, eating, drinking, activity, and possible distress.

Microdialysis

The afternoon before the microdialysis test day, the stylet was removed from the cannula, and the cannula area was cleaned for possible obstruction. The female rat was briefly

anesthetized using an oxygen/isoflurane mixture. The box for anesthetization was primed for five minutes and then the rat exposed to the oxygen/isoflurane mixture for four minutes. Immediately following anesthetization, the microdialysis probe was slowly inserted into the cannula. The rat was returned to its polycarbonate cage and attached to a tether and swivel (Instech Laboratories, Inc., Plymouth Meeting, PA, USA). Food and water were freely available overnight and the light cycle was maintained. Dulbecco's phosphate-buffered saline medium (NaCl 138 mM, 2.1 mM KCl, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 8.1 mM NaH_2PO_4, 1.2 mM $CaCl_2$, and 5 mM d-glucose, pH 7.4) was perfused at a rate of 0.2 µl/min through the microdialysis probe using a KD Scientific syringe infusion pump (Fisher Scientific, Inc., Pittsburg PA) until the next morning. The morning of microdialysis testing, the rate of perfusion of the Dulbecco's phosphate-buffered saline medium was increased to 2.0 µl/min. After a two-hour equilibration period, samples were collected every 15 minutes with a total of five baseline samples and eight methamphetamine samples, which began five minutes after a 7.5mg/kg i.p. methamphetamine injection. At the end of the sample collection, the rat was given an injection of Euthasol to deeply anesthetize for euthanasia and the brain collected (see below, Probe Placement).

Microdialysis Probes

The cannula was made using 21-gauge stainless steel tubing (Small Parts, Inc.) cut down to exactly 11mm. Microdialysis probes were constructed within our lab, based off of methods described in Matuszewich and Yamamoto (2004). The probe construction allowed for the inlet of Dulbecco's through PE20 tubing, which led into 26-gauge stainless steel tubing and through a dialysis membrane, allowing for the diffusion of dopamine into the probe. Dulbecco's from the membrane was then pumped through silica tubing into outlet tubing and into a 250 µl vial for

collection.

High-Performance Liquid Chromatography

Microdialysis samples were analyzed for dopamine with high-performance liquid chromatography with electrochemical detection (HPLC-EC). A Rheodyne injector (Cotati, CA, USA) with a 20 µl loop delivered the dialysis sample onto a reverse-phase Synergi 4 µm C18 column 150 x 2 mm (Phenomenex, Torrance, CA). A Shimadzu 10ADVP pump continuously pumped mobile phase (32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM ethylenediaminetetraacetic acid, 0.32mM octyl sodium sulfate and 6% acetonitrile) at a flow rate of 0.20 ml/min. Compounds were detected on a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA) with a 3 mm glassy carbon working electrode, maintained at a potential of +0.5 V relative to an Ag/AgCl reference electrode. Data were collected using ChromPerfect Spirit Software (Justice Innovations, Inc., Denville, NJ, USA).

Probe Placement

Following the microdialysis experiment, rats were overdosed with an i.p. injection of 0.12 ml Euthasol. Green McCormick food coloring was perfused through the microdialysis probe, the rat was decapitated and the brain quickly removed and frozen. Forty-micron coronal sections were sliced from A/P coordinates of +3.0mm to -2.0mm. The slides were examined under a microscope to assess probe placement. Only data from rats with probes located in the dorsal striatum were used for statistical analysis.

Statistical Analyses

Microdialysis data was analyzed with a repeated-measures ANOVA using the GLM

procedure on SPSS 21.0 software (New York, NY, USA). For each dependent measure, the two groups (non-stressed control, CUS) were compared over time. Microdialysis baseline data were analyzed with an independent-samples *t* test on the average of the five baseline samples. The average baseline sample and the eight samples after the methamphetamine injection were converted to percent of the average baseline and compared with repeated-measures ANOVA. Greenhouse-Geisser correction was used to correct for violation of the sphericity assumption, but degrees of freedom were rounded to the nearest whole number. Significant group differences ($p<0.05$) were further analyzed for all tests using a *t* test at each specific time point. All data are expressed as the mean±SEM.

CHAPTER 3

RESULTS

Open Field

Body Weights

CUS rats overall weighed more than the rats in the control group over the ten days of stress as indicated by a main effect of condition ($F(1, 46)= 5.452$, $p=.024$). In addition, there was a day by condition interaction ($F(3, 139)= 39.261$, $p<.001$), which was likely attributed to the decrease in weight of CUS animals following food and water deprivation overnight in the stress protocol (see Figure 2). As expected, both groups showed an increase overall in body weight across the ten days of stress as indicated by a main effect of day ($F(3, 139)= 27.298$, $p<.001$) on body weight.

When comparing the weights across the two days of open field testing, there was no difference in overall weights between days one and two of open field ($F(1, 46)= .361$, $p=.551$) nor was there a condition by day interaction ($F(1, 46)= .017$, $p=.897$). The CUS rats did have greater average weights compared to the control group for the open field testing ($F(1, 46)= 11.559$, $p=.001$) similar to the difference during the stress protocol.

Adrenal Gland Weights

There was no significant effect of condition on adrenal gland weight ($t(38) = .668$, $p=.508$) between groups, indicating that CUS did not have an effect on adrenal gland weight

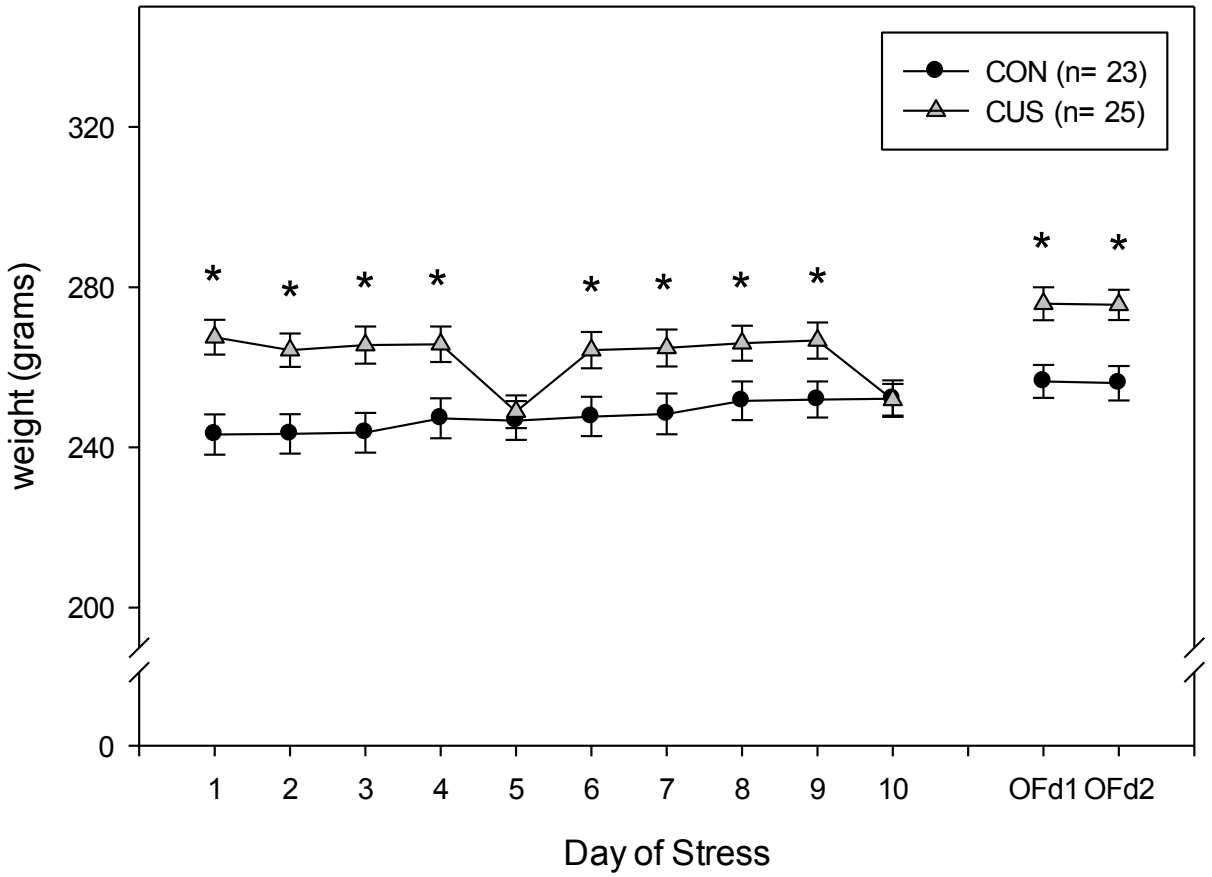


Figure 2. Average body weight of female rats in the CUS and CON condition during ten days of the stress protocol (Days 1 – 10) and then on both days of open field (OFd1 and OFd2). Body weight was recorded prior to behavioral testing in the open field. The CUS group had overall higher body weights during the stress protocol and open field compared to the control group. *p<.05 for an independent-samples *t* test.

when measured three to six weeks after the end of the stress protocol (see Figure 3).

Estrous Cycle

There was a fairly even distribution of rats in each stage of the estrous cycle. Each day of open field and each condition had at least five rats in each stage of the estrous cycle (see Table 3). When looking at the ten days of stress, there was no difference in average number of cycles during the ten days between female rats receiving CUS ($X=1.31 \pm 0.82$ cycles) and the control group ($X=1.33 \pm 0.76$ cycles; $t(60) = .104$, $p=.918$). Estrous cycle was not a significant covariate on either day one or day two of the open field for distance traveled and therefore was not used for any further analyses.

Table 3. Distribution of Rats in Each Stage of the Estrous Cycle on Day One and Day Two of the Open Field.

	Diestrus	Proestrus	Estrus	No data
Open Field D1				
CUS	43% (9)	33% (7)	24% (5)	(4)
CON	55% (12)	18% (4)	27% (6)	(1)
Open Field D2				
CUS	24% (5)	33% (7)	43% (9)	(0)
CON	48% (11)	22% (5)	30% (7)	(4)

Distance

As expected, a 7 (time) x 2 (condition) x 2 (day) ANOVA indicated a main effect of day ($F(1, 45) = 65.695$, $p<.001$) and a time by day interaction ($F(3, 142) = 46.866$, $p<.001$) for distance traveled in the open field (see Figure 4). There was also a significant time by day by

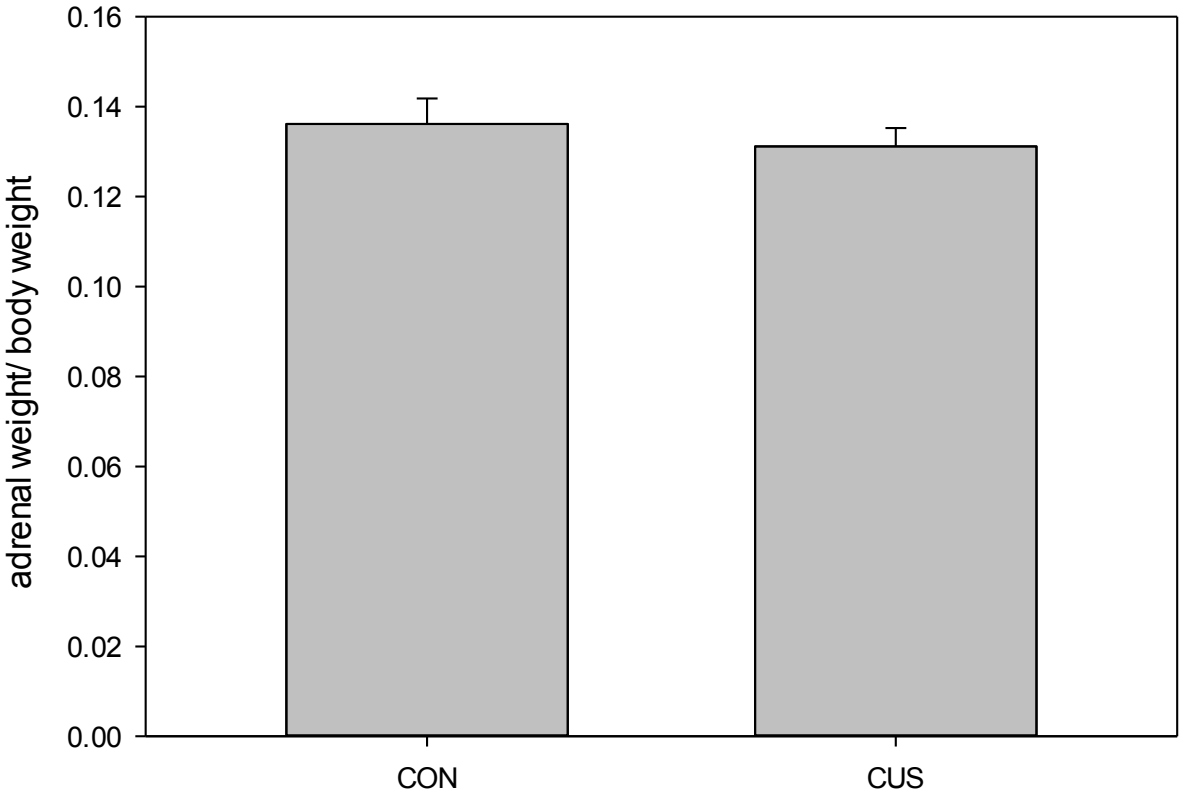


Figure 3. Adrenal gland weight to body weight ratio for both the control and CUS group when taken three to six weeks following open field. There were no differences in adrenal gland weight ratio of the CUS group compared to the CON group.

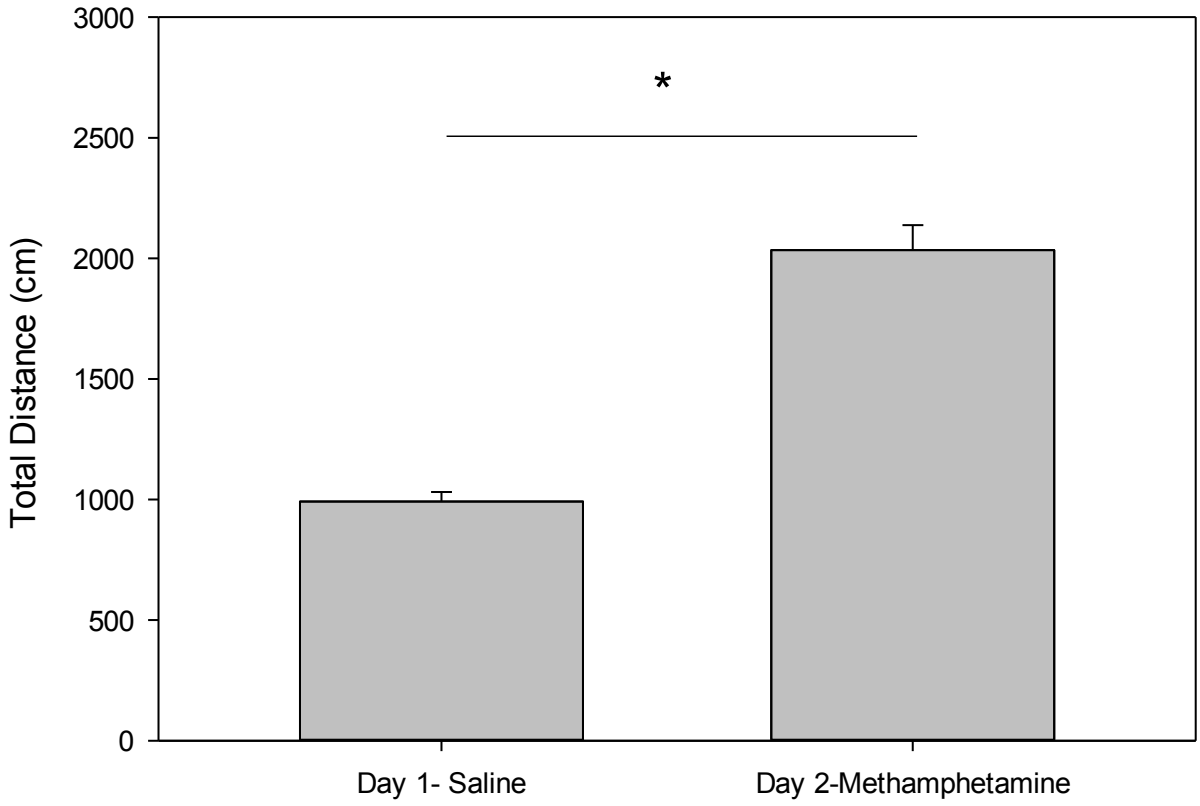


Figure 4. Total average distances traveled in the open field on day one when given an injection of saline or day two when given an injection of methamphetamine when combining both the CUS and the control groups. Overall, day two of open field had greater total distances compared to day one of the open field. *p<.05 when comparing day one to day two.

condition interaction ($F(3, 142) = 2.765$, $p=.041$). The interaction was further investigated by examining each day of the open field separately.

When looking more specifically within each day, distance traveled on day one of the in distance travelled as time progressed ($F(5, 218) = 46.517$, $p<.001$). There was no time by condition interaction ($F(5, 218) = .792$, $p=.550$) nor a main effect of condition ($F(1, 46) = .767$, $p=.386$), indicating that all rats traveled similar distances on day one as a whole and after an injection of saline, irrespective of previous exposure to stress or not (see Figure 5).

On day two of the open field, rats were habituated for a half hour then injected with 7.5mg/kg methamphetamine and recorded for an additional 90 min. As hypothesized, there was a significant effect of time ($F(3, 131) = 63.556$, $p<.001$) with a significant time by condition interaction ($F(3, 131) = 2.989$, $p=.035$); rats exposed to CUS two weeks prior differed in their responses following an injection of methamphetamine than females rats that were not stressed (see Figure 6). Post-hoc *t* test shows that CUS rats had a higher distance immediately after the methamphetamine injection compared to nonstressed controls ($t(46) = -2.041$, $p=.047$) with no other significant differences at specific time points. There was no overall main effect of condition on day two of the open field ($F(1, 45) = .349$, $p=.557$), indicating that female rats receiving prior chronic stress and non-stressed rats had similar distances when the entire 120 minutes of open field testing on day two was collapsed.

Stereotypy

From the open field recording, stereotypy was measured at 5 min intervals following the methamphetamine injection on day two. There was a significant main effect of time ($F(16, 736) = 84.010$, $p<.001$), suggesting that both groups entered into stereotypy. There was no main effect

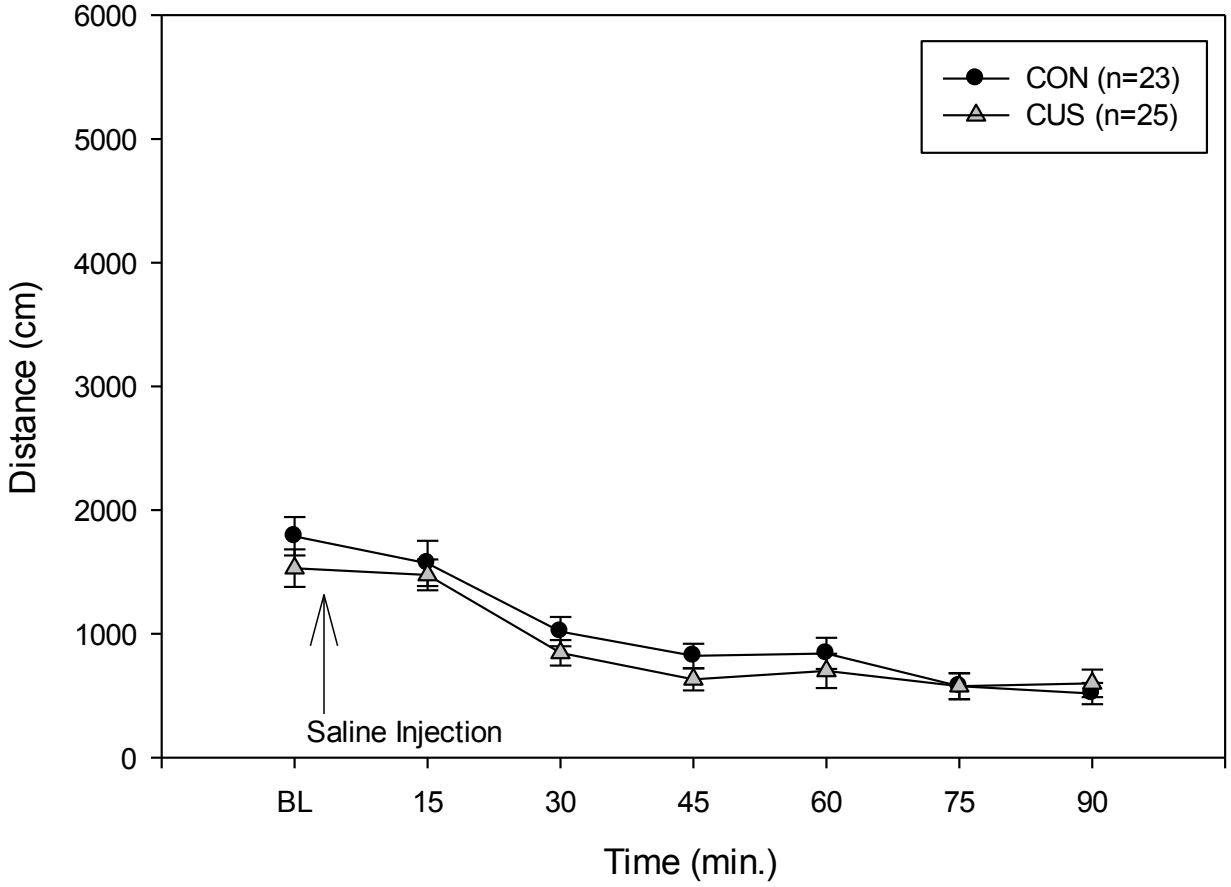


Figure 5. Average distance traveled on day one of the open field both before (BL) and after an injection of saline. All groups had a decrease in distance as time in the open field progressed but there was no difference comparing distance between the CON and CUS group.

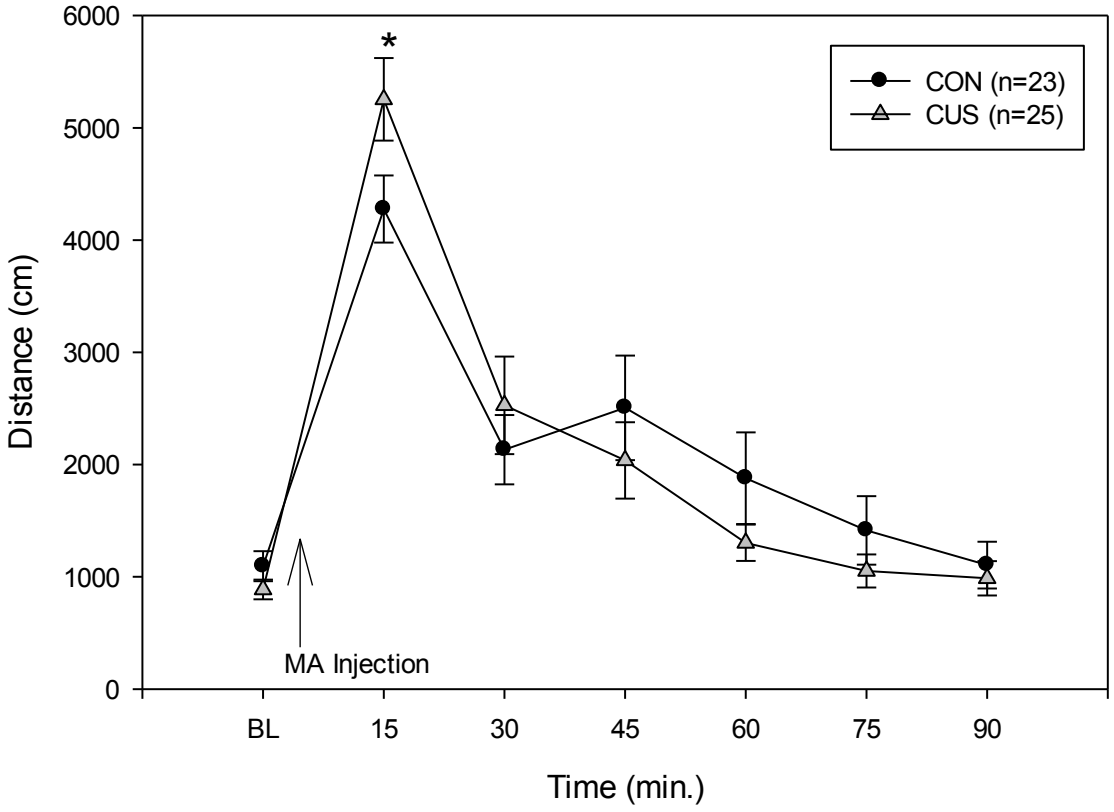


Figure 6. Average distance traveled on day two of the open field before (BL) and after an injection of 7.5mg/kg methamphetamine. Rats in the CUS group had a significant increase in distance following the methamphetamine injection compared to the CON group. *p<.05 comparing CUS to CON.

of condition ($F(1, 46) = .211$, $p=.648$) or a time by condition interaction ($F(16, 736) = 1.157$, $p=.297$); all animals entered into stereotypy after methamphetamine at a similar rate, irrespective of being exposed to CUS two weeks prior or not (see Figure 7).

Microdialysis

Body weight

The body weights of both groups were compared across the 10 days of stress and again the day before the dialysis experiment. Over the 10-day stress procedures, there was a significant main effect of day ($F(3, 33) = 5.115$, $p=.008$) and a day by condition interaction ($F(3, 33) = 8.528$, $p<.001$), both of which are likely influenced by the decreased weights in the CUS group following food and water deprivation overnight (see Figure 8). Body weights on the day before dialysis did not significantly differ between the CUS group and the control group, $t(16)= 1.44$, $p=.887$.

Histology

Microdialysis data was used from rats when the probe was placed accurately in the dorsal striatum; the probe had to be located within A/P -1.6 - +3.0, M/L +2.4 - 4.0, and D/V -6.5 - -9.0 (for representative picture of probe placement within the dorsal striatum, see Figure 9). Of the 28 total animals that received surgery, 18 were used in the data analyses for dopamine. Three animals were dropped from analyses as the probe did not fall within these specified coordinates. Five animals were dropped due to data collection issues. One animal was dropped from analyses due to issues with data output on the HPLC-EC and one animal was dropped due to being an

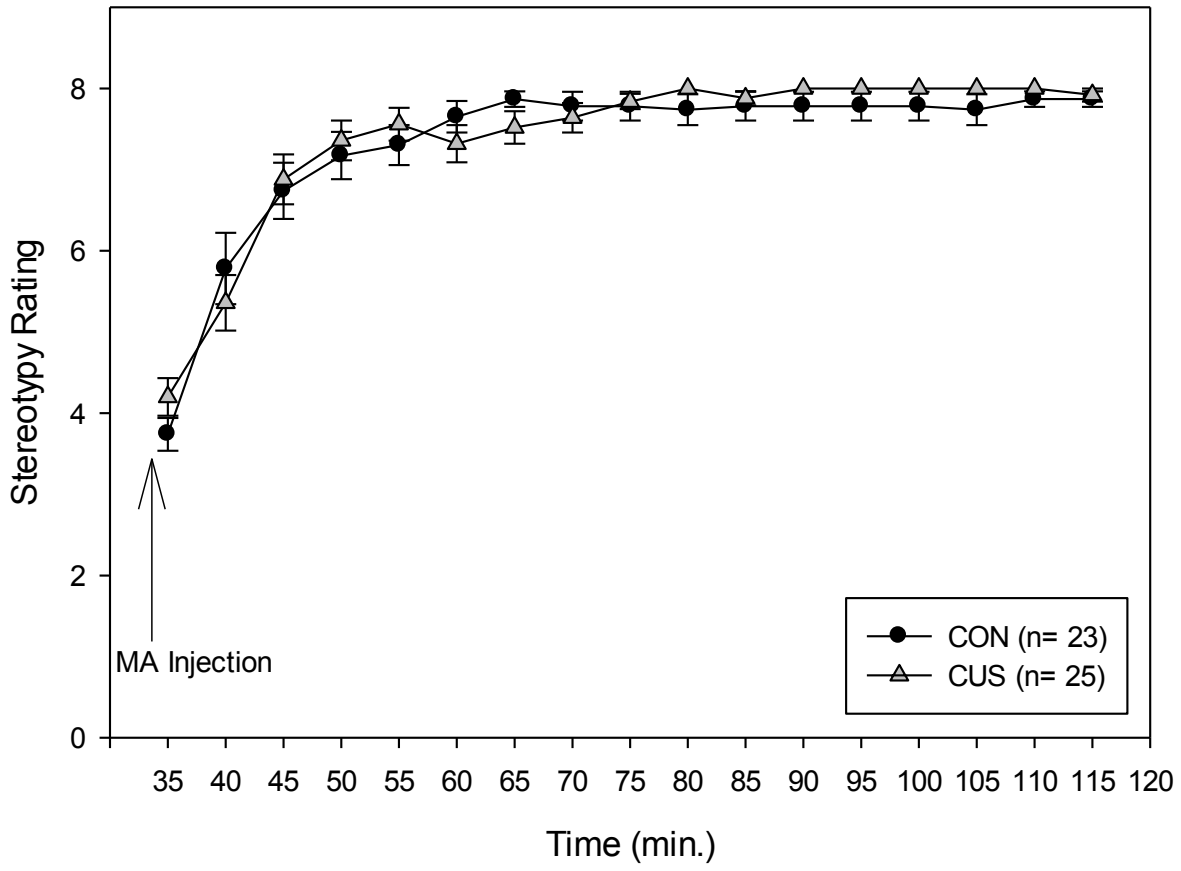


Figure 7. Average stereotypy ratings during open field day two following an injection of 7.5mg/kg methamphetamine. All groups entered into stereotypy following the injection, but there was no effect of condition.

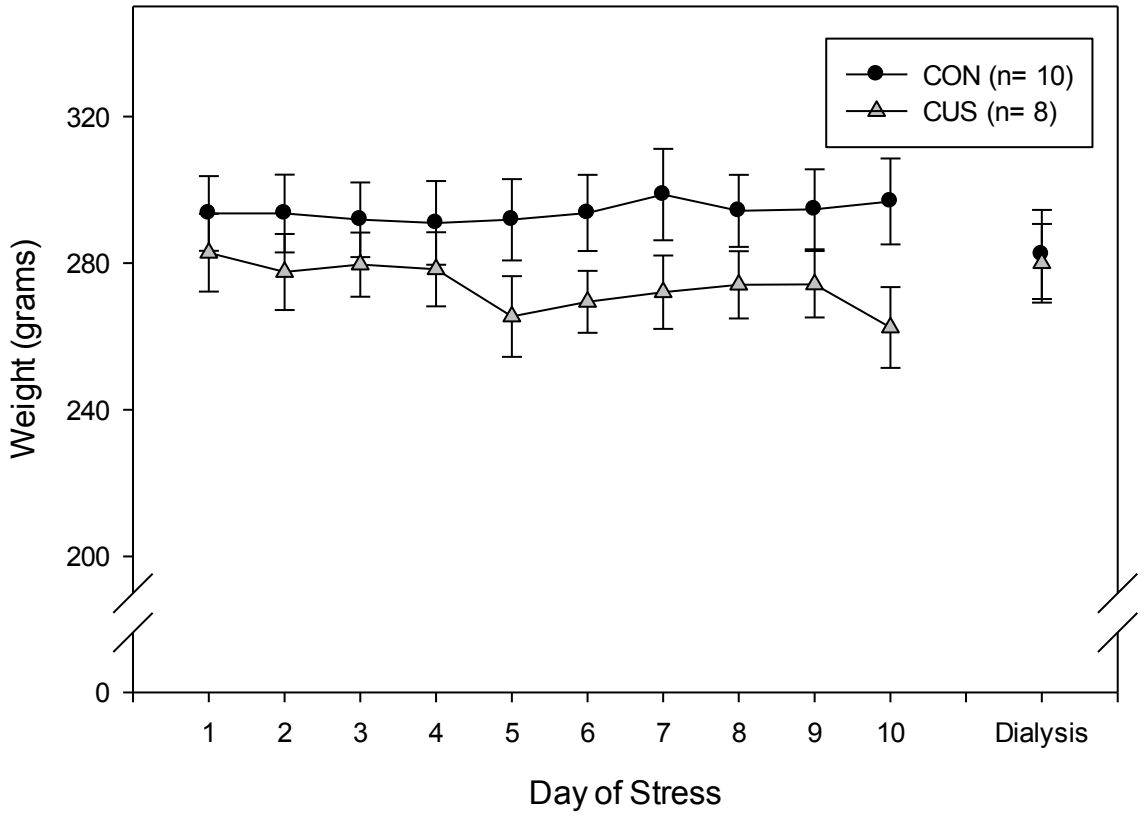


Figure 8. Average body weight of female rats in the CUS and CON condition during ten days of the stress protocol (Days 1 – 10) and then on the day before dialysis. There were no significant differences of average body weights between the CUS group and the CON group.

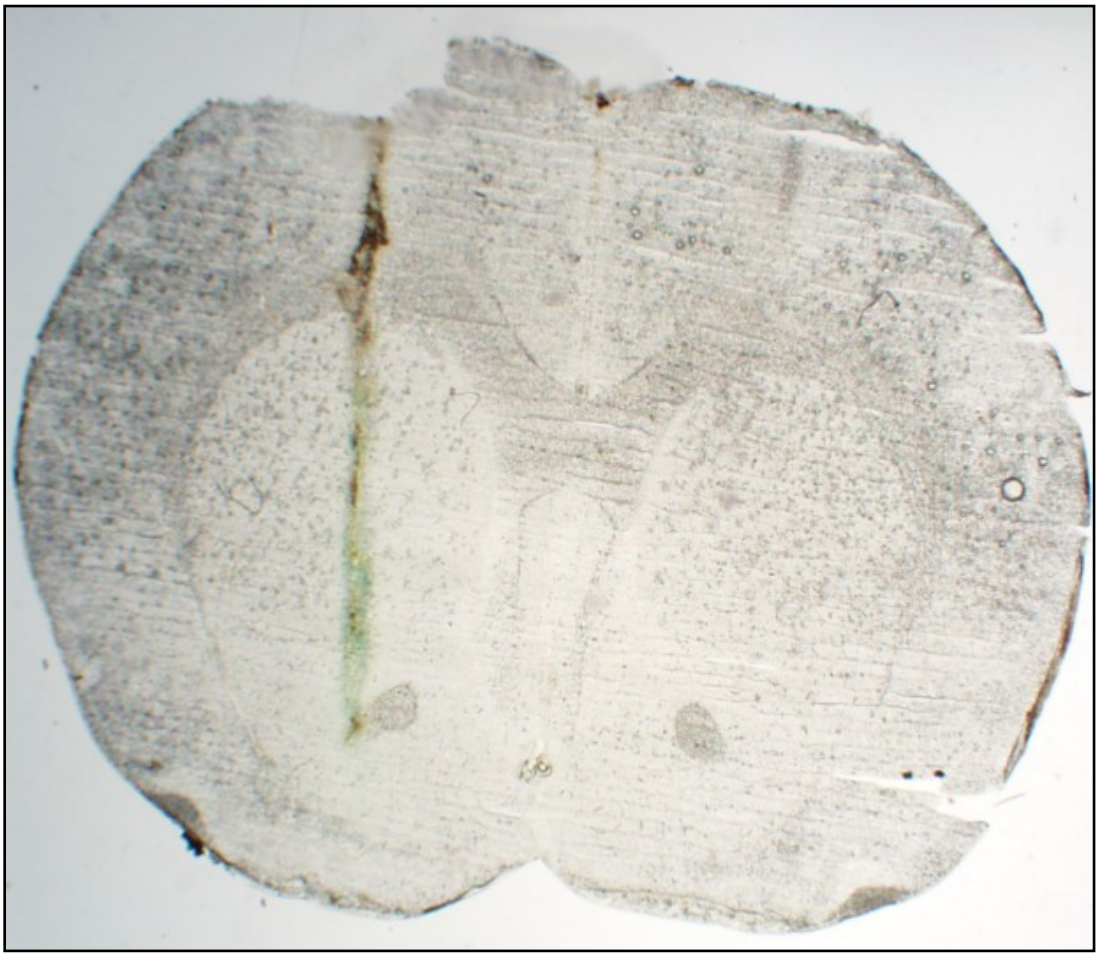


Figure 9. Example of probe placement verification in the dorsal striatum. Green dye was diffused through the probe membrane following microdialysis. The green area indicates where dopamine was being collected.

outlier on three of the eight methamphetamine samples, with two of the data points being more than two standard deviations from the mean and one data point being over one standard deviation from the mean.

Dopamine Concentrations in the Dorsal Striatum

Dopamine levels were measured from five baseline samples and then eight samples after an injection of 7.5 mg/kg methamphetamine. The average baseline dopamine levels significantly differed between groups ($t(16) = 2.575$, $p=.020$), with rats in the control group having overall higher levels ($X = 3.2848 \pm 0.990$pg) compared to CUS rats ($X= 2.2320 \pm 0.663$pg). Therefore, the five baseline samples were averaged and change scores were calculated for all samples and converted to a percentage with 100% equaling the average baseline.

When looking at percent change from baseline, there was a significant main effect of time ($F(1, 23) = 77.112$, $p<.001$). There was also a significant time by condition interaction with rats previously exposed to CUS having a significant increased percent change in dopamine levels following the methamphetamine injection compared to control rats ($F(1, 23) = 7.299$, $p=.007$), which was also shown as a main effect of condition ($F(1,16) = 7.217$, $p=.016$; see Figure 10).

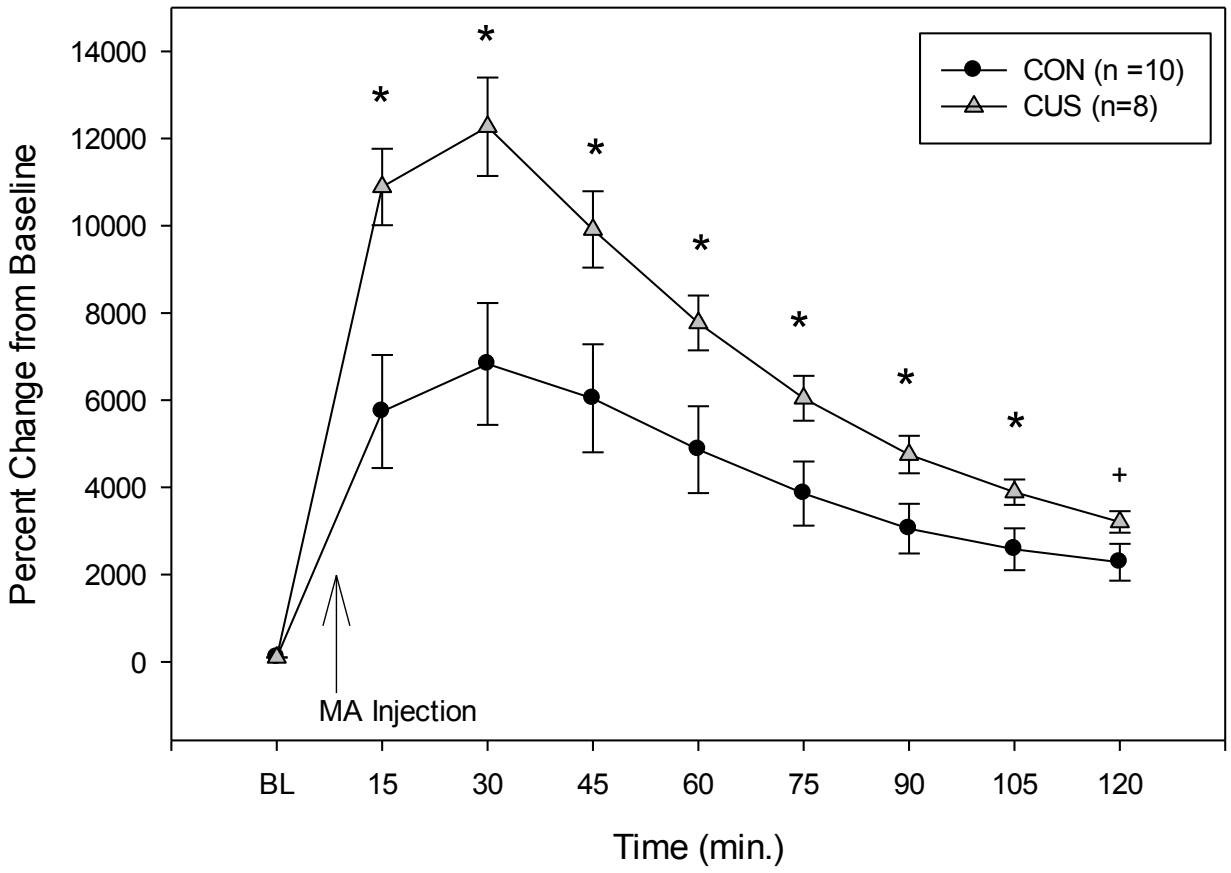


Figure 10. Percent change of dopamine in the dorsal striatum compared to baseline (pre-methamphetamine) levels. Female rats exposed to CUS two weeks prior showed a greater percent change of dopamine from baseline in the dorsal striatum following an injection of methamphetamine compared to CON rats. * $p<.05$, + $p<.10$ compared to CON rats at each time point.

CHAPTER 4

DISCUSSION

The current study investigated whether prior exposure to stress augmented the behavioral and neurochemical responses to a single injection of methamphetamine in female rats. As hypothesized, prior exposure to CUS increased the sensitivity of the female rat to an injection of methamphetamine two weeks after the last stressor. Rats exposed to CUS showed increased distance traveled in the open field compared to control rats, with no differences in stereotypy. Similarly, female rats exposed to CUS also showed a significant increase in dopamine within the dorsal striatum following an injection of methamphetamine and overall lower baseline dopamine levels. Estrous stage was not a significant covariate for locomotor response following exposure to either saline or methamphetamine. Adrenal gland weight did not significantly differ between those rats previously exposed to stress compared to control rats, although this may not be a sensitive enough measure for the assessment of HPA axis activation. Taken together, these findings indicate that previous exposure to CUS increases both the locomotor and dopaminergic response to an injection of methamphetamine in female rats.

This study is the first to examine behavioral cross-sensitization between chronic stress and an injection of methamphetamine in female rats. Although previous research has investigated the cross-sensitization in male rats between stress and stimulants (Araujo et al., 2003; Covington & Miczek, 2001; Haile, GrandPre, & Kosten 2001; Holly, Shimamoto, DeBold, & Miczek, 2012; Matuszewich et al., 2014; Nikulina et al., 2004; Prasad, Ulibarri, & Sorg, 1998), no research has looked at the cross-sensitization of stress and later exposure to methamphetamine in female rats. Interestingly, both sexes show a cross-sensitization between

CUS and a later injection of methamphetamine (Anderson, McWaters, & Matuszewich, 2014). Similar to the data in females (Figure 5), there were no differences in distance traveled in the open field following a saline injection between the CUS and CON group in male rats but a significant increase in distance traveled 15 minutes after an injection of methamphetamine. In exploratory analyses comparing the two sexes on distance traveled in the open field, there was a significant time by condition interaction ($F(3, 242)= 3.853$, $p=.008$), with CUS-exposed rats of both sexes showing greater increases following the methamphetamine injection. Male, but not female rats, then showed a decrease at later time points compared to the male control group. Female rats showed a more potentiated increase in distance traveled following the methamphetamine compared to the male rats regardless of condition ($F(3, 242)= 4.638$, $p=.003$), and females overall traveled greater distances than males ($F(1, 73)= 4.972$, $p=.029$; Anderson, McWaters, & Matuszewich, 2014; Figure 11). Thus, while stress exposure potentiated both male and female rats' locomotor response to methamphetamine, females still showed greater behavioral sensitivity to the drug, as has been found previously (Milesi-Halle et al., 2007).

The current study is also the first to examine the neurochemical cross-sensitization between stress and methamphetamine in female rats. Dopamine efflux in the dorsal striatum of female rats exposed two weeks earlier to CUS was significantly greater than control female rats. A similar potentiation of dopamine in the dorsal striatum was observed in male rats exposed to CUS (Matuszewich et al., 2014). In the exploratory analyses comparing the sexes, both male and female rats exposed to CUS before microdialysis showed a significant increase from

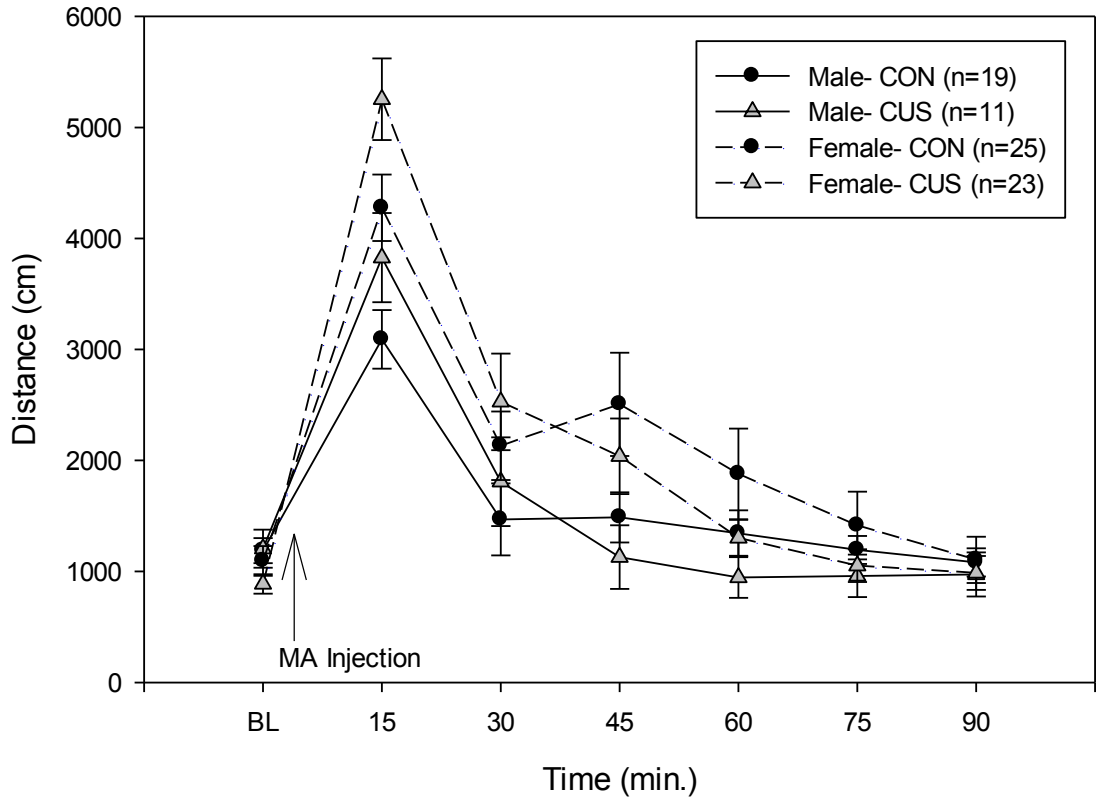


Figure 11. Average distance traveled on day two of the open field before (BL) and after an injection of 7.5mg/kg methamphetamine in both male and female rats. Females had a greater increase in locomotion immediately following the methamphetamine injection compared to male rats and also had overall greater locomotion compared to male rats. When combining both male and female rats, those exposed to CUS overall had a greater increase in distance following methamphetamine compared to CON rats.

baseline dopamine in the dorsal striatum compared to control rats as shown with a main effect of condition ($F(1, 32)= 13.275$, $p=.001$) and a time by condition interaction ($F(2, 49)= 10.533$, $p=.001$; Figure 12). Interestingly, while the potentiation of dopamine release in the dorsal striatum was comparable between males and females, the percent change of dopamine in the dorsal striatum was much greater for female compared to male rats ($F(2, 49)= 7.248$, $p=.004$). Together, these findings indicate that both male and female rats show a potentiated response to an injection of methamphetamine following exposure to CUS, but females have an increased sensitivity to methamphetamine compared to male rats as shown both behaviorally and neurochemically.

There are several factors that may contribute to the increased magnitude of the response of the exposed female rats compared to male rats. One critical factor for both drug and stress sensitivity may be gonadal hormones. For the microdialysis study, estrous smears were taken the day before microdialysis was conducted; therefore, it was not possible to determine the exact stage of the estrous cycle on the day of the dialysis. Unfortunately, no control rats were in estrus the day before microdialysis. This was unexpected but limits any potential interpretation of relating dopamine levels or response to methamphetamine to cycle stage or hormonal levels. Although this current study was not able to make conclusions about estrous stage influence on dopamine levels in vivo, previous research has indicated a role of gonadal hormones in dopamine release. Ovariectomy in female rats is shown to decrease basal dopamine release in the striatum with estrogen replacement increasing in vivo dopamine release (Becker 1990; Castner, Xiao, Becker, 1993; Cummings et al., 2014). In the future, it would be beneficial for estrous smears to be taken the morning of microdialysis or directly manipulating gonadal

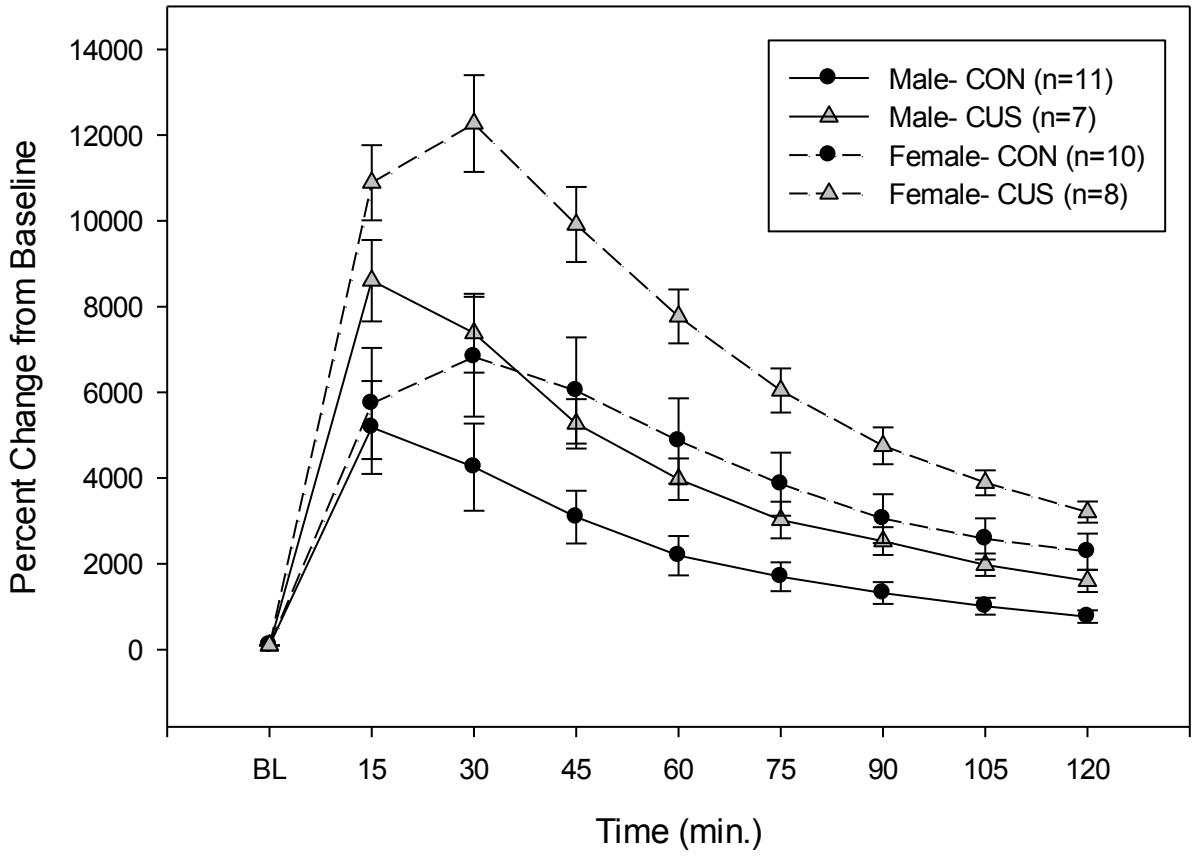


Figure 12. Percent change from baseline in extracellular dopamine within the dorsal striatum. Both male and female rats exposed to CUS show a greater percent change of dopamine in the dorsal striatum compared to CON rats following methamphetamine exposure. Females overall show a greater increase in extracellular dopamine compared to male rats following a methamphetamine injection.

hormones by ovarietcomizing females and providing hormone replacement for control of known hormone levels.

Another factor that could influence drug responsiveness is metabolic processes. Body weight was assessed for all rats throughout the studies. All rats were weighed daily during the ten days of stress, with the control rats weighed on parallel days, and then on the test day. In the microdialysis study, there were no differences between body weights of CUS and CON rats throughout either the ten days or the day before microdialysis. In the open field study, the CUS rats weighed significantly more throughout the ten days of stress (except following the two nights of food and water deprivation) and also weighed significantly more than CON rats on the days of open field (Figure 2). This weight difference is unlikely to alter the locomotor activating effects of methamphetamine for the amount of drug injected is determined by body weight, such that all rats receive the identical dose (7.5 mg/kg) regardless of weight. If body weight was important in the magnitude of the locomotor effects following an injection of methamphetamine, it would be expected that the CUS group, which weighed more, would have been more tolerant to methamphetamine due to drug metabolism, rather than more sensitive as was found in experiment 1. Drug metabolism was not measured in the current study, and there is no data to suggest how stress would influence metabolic function in response to stimulants. No studies were found investigating the influence that prior stress exposure has on methamphetamine metabolism, indicating that it is not yet known if stress influences stimulant metabolism. Similarly, no methamphetamine metabolism studies in rats mentioned the influence of mass or weight on pharmacokinetics, even those comparing males and females which are assumed to be of varying weights. One study examining the pharmacokinetics in humans did bring up the

issues of lean body mass versus adipose tissue in humans, although it was concluded that drug dosage does not need to be altered as a function of gender (Giudicelli & Tilliment, 1977). There have been studies comparing methamphetamine metabolism in male and female rats. Female rats have been shown to have slower metabolism of methamphetamine and clear drugs at a slower rate than male rats (Milesi-Halle et al., 2005; Mugford & Kedderis, 1998). This clearance rate has been suggested to be due to lower cytochrome P-450 activity but offers no indication of how weight differences may relate to P-450 activity (Milesi-Halle et al., 2005). Therefore, in the current study, it is possible that methamphetamine metabolism could differ as a result of previous stress or body weight differences, but there is no previous research to support this suggestion.

Although prior exposure to unpredictable stress increased both the locomotor and dopaminergic response within the dorsal striatum, the findings do not perfectly parallel each other. The CUS group had an increased behavioral response directly following methamphetamine (15 min) but then a decline in locomotion at later time points. These findings are slightly different from the sustained increase in dopamine in the dorsal striatum following an injection of methamphetamine in the CUS group compared to the CON group. Other areas of the brain besides the dorsal striatum, such as the nucleus accumbens, may be responsible for the stimulant-induced locomotor effects. A prior study found that 6-OHDA lesion of the nucleus accumbens reduced the locomotor effects of both 1.5mg/kg amphetamine and 20mg/kg cocaine. Similarly, a 6-OHDA lesion in the nucleus accumbens reduced the locomotor stimulating effects of 1.5mg/kg amphetamine but not a similar lesion in the caudate results (Kelly, Seviour, Iverson, 1975). These findings indicate that dopamine transmission in the nucleus accumbens, rather than the dorsal striatum, may be critical for stimulant-induced locomotion, at least for amphetamine.

Thus, although dopaminergic and behavioral cross-sensitization were both observed, it is not clear that the dopaminergic response in the dorsal striatum is responsible for the behavioral sensitization.

In male rats, much of the cross-sensitization literature has focused on the dopaminergic response in the nucleus accumbens. In a recent study, male rats were restrained for two hours once and then three weeks later challenged with an injection of 15.0mg/kg i.p. cocaine. As expected, the rats exposed to stress previously had a significant increase in locomotion following the cocaine injection compared to control rats. Similarly, rats previously exposed to stress show a significant increase in dopamine within the nucleus accumbens following the cocaine injection (Garcia-Keller et al., 2013). Prior exposure to social defeat stress also potentiated dopamine release within the nucleus accumbens following a single injection of 10.0 mg/kg cocaine i.p. in both male and female rats (Holly, Shimamoto, DeBold, & Miczek, 2012). Male rats exposed to CUS for ten days show a significant increase of dopamine release in the nucleus accumbens shell following an injection of 7.5mg/kg methamphetamine i.p. (Raudensky & Yamamoto, 2007). Overall, the nucleus accumbens may be a critical region to consider for the neural regulation of cross-sensitization. To date, there are no studies that have looked at chronic stress and dopamine release in the nucleus accumbens following methamphetamine in female rats. Therefore, in the future, determining dopamine release in the nucleus accumbens following CUS is important to fully understand cross-sensitization in female rats. It is likely that dopamine release in both the nucleus accumbens and the dorsal striatum contribute to stimulant activating locomotor effects, although possibly playing different roles (Kelly, Seviour, & Iverson, 1975).

Potential Mechanisms Mediating Cross-sensitization

Glucocorticoids

One obvious mechanism through which stress may potentiate the stimulant-induced increase in locomotion and dopamine in the dorsal striatum is CORT. In male rats, CORT has been investigated by blocking the production and reducing the levels of CORT. Previous studies of male rats have used metyrapone, a CORT synthesis inhibitor to attenuate circulating levels of CORT. Metyrapone disrupts the HPA axis by inhibiting the synthesis of CORT (Marrow, Statham, Overton, Brain, & Clark, 1999; Rotllant & Armario, 2005). Metyrapone has been shown to decrease CORT levels while increasing basal ACTH levels, indicative of the negative feedback loop through which the HPA axis works (Canini et al., 2009). In a study of male rats, metyrapone (150mg/kg i.p) or saline was injected following one-hour habituation and then distance traveled over the next two hours was measured. Metyrapone decreased locomotion the first hour after injection compared to the control group but the differences were diminished the second hour after injection. Metyrapone also significantly reduced CORT levels when measured two hours after the metyrapone injection as expected (Canini et al., 2009). However, male rats that received chronic metyrapone treatment (50mg/kg i.p.) for seven days showed no differences in locomotion or stereotypy when tested one day later compared to rats that were given a vehicle (Reid, Ho, Tolliver, Wolkowitz, & Berger, 1998). These studies suggest that metyrapone is effective in reducing CORT and CORT-stimulated locomotion quickly, but its effects do not last beyond 24 hours. Unfortunately, no similar studies have been conducted with female rodents and the effects on temporary inhibition of glucocorticoids are not known.

Interestingly, prior studies in non-stressed rats have shown that manipulation of glucocorticoid synthesis also influences drug-induced behavioral activation. Marrow et al. (1999) injected male rats with 100mg/kg s.c. metyrapone two and a half hours before testing for d-amphetamine-stimulated (3.4mg/kg) locomotion. The metyrapone group showed a significant attenuation of locomotor activity for three hours following the amphetamine compared to the group pretreated with the vehicle, with no differences in stereotypy. In the last three hours of testing, the metyrapone rats exhibited significant increases in stereotypy and locomotion compared to the vehicle-treated group (Marrow et al., 1999). This study indicates that metyrapone is able to reduce the effects of d-amphetamine but that this reduction is time sensitive. Marinelli and colleagues (1997) also assessed the effect of metyrapone (50mg/kg s.c.) on locomotion in male rats and found that rats pretreated with metyrapone had a decreased locomotion to subsequent injections of either saline or cocaine. The reduction in locomotion was greater to cocaine than to saline when compared directly. Thus, metyrapone can reduce locomotion, both drug free and stimulant induced.

Metyrapone has also been given with stress to disrupt the HPA axis and assess its role in cross-sensitization. Johnson and Yamamoto (2009) administered 50mg/kg i.p. 15 minutes prior to each stressor over 10 days before male rats experienced the stress. The chronic stress was shown to increase basal CORT levels, and metyrapone was able to block these increases when administered before the stressors. This finding verifies that metyrapone administered before stress decreases the stress-induced CORT increases. Previous studies have shown that metyrapone can reverse the behavioral effects that stress has on later exposure to stimulants. Reid, Ho, Tolliver, Wolkowitz, and Berger (1998) administered restraint stress two times a day

for five days over a total of eight days. One day after the last restraint, the male rats were administered 50mg/kg i.p. metyrapone once a day for seven days in a row. All male rats that were stressed and treated with metyrapone showed reduced locomotion in an open field paradigm after an injection of d-amphetamine (1.5mg/kg) compared to stressed rats not given metyrapone. Oddly, stereotypy was not significantly altered by the metyrapone treatment. Thus, the stress potentiation of amphetamine-induced activity appears to depend upon glucocorticoid activity in male rats.

Metyrapone also prevented stress-induced behavioral sensitization when food deprivation was used as a stressor. Male rats were divided into four treatment groups: food restricted to 90% of body weight with vehicle, vehicle alone, food restricted to 90% of body weight with metyrapone or metyrapone alone. Metyrapone or vehicle was injected and one hour later the rats began a two-hour habituation period in the locomotion apparatus and then either injected with saline or cocaine (10.0mg/kg, i.p.). The food-restricted rats had an increase in locomotion compared to controls following the cocaine injection and this increase was decreased when the food-restricted animals received metyrapone (Marinelli, Le Moal, & Piazza, 1996). Rouge-Pont et al. (1995) conducted a similar experiment, comparing the cocaine-stimulated locomotor activity in male rats that were either food restricted or free fed, with or without metyrapone treatment of 100mg/kg s.c. twice a day over eight days. Consistent with previous findings, the food-restricted animals had an increase in cocaine-stimulated locomotion (10.0mg/kg, i.p.) compared to animals that were fed ad libitum. Treatment with metyrapone significantly reduced locomotion in the food-restricted rats, regardless if the metyrapone was administered before the food-deprivation treatment or after food restriction. Taken together, these findings indicate that

metyrapone may be effective in reducing stress-induced increases in locomotion. All of the published cross-sensitization studies have been conducted only in male rats; and it is not known whether increases in glucocorticoids in females through stress exposure would affect their basal or stimulant-induced behavior. Together, these findings suggest that CORT may be an important mechanism underlying the cross-sensitization between CUS and increased behavioral response to an injection of methamphetamine.

CORT increases following CUS could act to increase striatal dopamine. For male rats, exposure to acute stress stimulates dopamine release in several forebrain regions (Piazza & Le Moal, 1997), including the striatum. To assess the impact of acute stress on dopamine, male rats that were divided into either sham group or adrenalectomy with the implantation of a CORT pellet, which was used to keep CORT levels similar to basal conditions and decrease stress-induced CORT. Both groups were given a 10-minute tail pinch acute stressor with dopamine release in the nucleus accumbens measured through microdialysis. Male rats in the adrenalectomized group with controlled basal levels of CORT had a significant decrease in dopamine release following the tail pinch stress compared to the sham rats, indicating the importance of glucocorticoids, specifically CORT, in stress-induced dopamine response (Rouge-Pont, Deroche, Le Moal, & Piazza, 1998). Male rats exposed to intermittent tail shock acute stress (1 second shock every ten seconds for one minute which was repeated every five minutes for thirty minutes) had a significant increase in extracellular dopamine in the dorsal striatum 15 minutes following the end of the tail shock stress as measured through microdialysis (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989). Male and female ex vivo striatum dopamine content was assessed twenty minutes following two days of forced swim test (Dalla et

al., 2008). Female rats overall had higher levels of dopamine content in the striatum than male rats but the forced swim test did not significantly increase dopamine content in either the male or female rats (Dalla et al., 2008). These findings indicate that type and pattern of stress exposure are crucial but also the method of measuring dopamine in the dorsal striatum.

Stress hormones have been proposed to be a mechanism mediating stress-induced dopamine release (Marinelli & Piazza, 2002). To assess whether glucocorticoids are necessary for acute stress-stimulated release of dopamine, male rats were adrenalectomized with the implantation of a CORT pellet to keep CORT levels similar to basal conditions but to decrease stress-induced CORT. A 10-minute tail pinch acute stress was applied and dopamine release in the nucleus accumbens measured through microdialysis. Male rats in the adrenalectomized group with low CORT levels showed lower dopamine release in response to the tail pinch stress compared to the sham adrenalectomized rats, indicating the importance of CORT in stress-induced dopamine response (Rouge-Pont, Deroche, Le Moal, & Piazza, 1998). These findings indicate that acute stress and the subsequent glucocorticoids released may moderate in vivo extracellular dopaminergic activity within the striatum.

In the current study, prior exposure to unpredictable stress may alter CORT in response to methamphetamine injection and subsequently the behavioral and dopaminergic response in the dorsal striatum. To better understand the role of CORT on the acute stimulatory action of methamphetamine in females, attenuating the stimulant-induced CORT response would be quite useful for future studies. In addition, assessing CORT and ACTH before and following the methamphetamine injection would help to understand the potential impact that CORT has at the time of drug presentation. In addition, the increases in CORT daily during CUS may be critical

to cross-sensitization. The immediate CORT release from each stressor may create a downstream cascade which leads to the sensitized response of methamphetamine two weeks later. It is also possible that the two-week delay between stress exposure and methamphetamine is critical for the sensitized response; therefore, stress hormone levels during this time would also be interesting to compare. Alterations of CORT through adrenalectomy, metyrapone (a CORT synthesis inhibitor), or exposure to CORT would help to answer the influence that CORT has on the cross-sensitization between stress and stimulants. Although glucocorticoids were not measured in the current study, it is likely that they play a role in the stress-induced sensitization of methamphetamine. In the future, alterations and measurements of glucocorticoids will help to further understand the impact that glucocorticoids have in stress-induced cross-sensitization in female rats.

Delta FosB

Delta FosB (ΔFosB) may be a potential molecular change that facilitates the cross-sensitization between stress and stimulants. ΔFosB is a stable isoform product of immediate early gene FosB and likely acts through the expression of other genes and targets downstream genes (Nestler, Barrot, & Self, 2001). It is a stable transcription factor, which has been shown to accumulate within the nucleus accumbens and dorsal striatum of male rats following chronic drug use (Hope et al., 1994; McDaid, Graham, & Napier, 2006). It has been suggested that ΔFosB contributes to the long-term gene expression changes that are present after drug use. An increase in ΔFosB also has been linked to drug-seeking behaviors and behavioral sensitization to drug exposure (Neslter, Barrot, & Self, 2001). Male rats exposed to two-hour self-administration sessions of methamphetamine for three weeks total showed an increase in ΔFosB within the

caudate putamen when measured immediately following the last of methamphetamine exposure (Cornish, Hunt, Robins, & McGregor, 2012). In another study, male rats were allowed to self-administer cocaine for four hours a day, six days a week, for a total of 18 days and brains collected either immediately or at various time points after the last session. Rats that self-administered chronic cocaine showed a significant increase in ΔFosB within the caudate putamen immediately and 24 hours after the last self-administration session, but by three weeks, rats showed no significant differences to the control group (Larson et al., 2010). Similarly, male rats subjected to passive administration of 15.0mg/kg i.p. cocaine twice daily for fourteen days then brains collected 18-24 hours after the last exposure showed a significant increase in ΔFosB within the caudate putamen compared to control rats (Perrotti et al., 2008). Although the research on stimulant induction of ΔFosB is quite limited, the current research suggests that chronic exposure to cocaine or methamphetamine increases ΔFosB within the caudate putamen.

ΔFosB has also been shown to increase after both acute and chronic stress. Male rats exposed to a single one-hour acute restraint stress showed an increase in ΔFosB within the striatum compared to the control non-stressed group up to two and four hours later (Alibhai, Green, Potashkin, & Nestler, 2007; Perrotti et al., 2004), but not 24 hours later (Perrotti et al., 2004). Multiple types of chronic stress have been shown to increase ΔFosB. For example, male rats exposed to a predatory cat collar had increased ΔFosB in the caudate putamen 24 hours after the last cat collar exposure, and the ΔFosB increase remained in rats perfused seven days after the last exposure (Staples, McGregor, & Hunt, 2009). Similarly, male rats that had chronic restraint stress for two to nine days had increased levels of ΔFosB within the striatum compared to control rats when tested one hour following the last restraint stress (Alibhai, Green, Potashkin,

& Nestler, 2007). One-hour restraint stress for either five or ten days resulted in a significant increase of ΔFosB within the caudate putamen when measured 24 hours later in male rats (Perrotti et al., 2004). These findings indicate that both acute and chronic stress are capable of inducing increases in ΔFosB within the striatum. In our current study, it is possible that the CUS paradigm increased ΔFosB within the striatum in a similar manner to what would be seen with previous stimulant use. Therefore, ΔFosB increases following stress could be a possible down-stream mechanism for the cross-sensitization between CUS and methamphetamine exposure in female rats.

Cue and Drug Reactivity

Individual differences in cue and drug reactivity may be important in understanding the effects of stress on drug responsivity. Various methods of categorizing individuals' responses to stimulant drugs have been used to better understand addiction processes. One recent approach has been to differentiate drug seeking or wanting (i.e., appetitive behavior required to procure the drug) compared to drug liking (i.e., reward properties of the drug itself for the individual). In the laboratory, this has been assessed in rats' inherent response prior to drug exposure as either goal trackers or sign trackers. Sign trackers tend to approach the cue which indicates that the reward is imminent rather than the reward itself (food), whereas the goal trackers will wait by the receptacle where the food is delivered (Flagel, Akil, & Robinson, 2009). Drug cues are an important part of drug use, as oftentimes a cue in the environment becomes rewarding itself (Berridge, 2001). Similarly, cues that were previously associated with drug taking can elicit reinstatement of drug-seeking behaviors and relapse to drug use after periods of abstinence (for review see Childress et al., 1993). Goal and sign tracking allow for assessment of individual

differences in rodents and then rats are divided into those that attribute greater salience to a conditioned stimulus such as a lever (i.e., sign trackers) and those that attribute greater salience to the unconditioned stimulus such a food cup where a sucrose pellet is delivered (i.e., goal trackers). Based on these differences, it is assumed that the rodents labeled as sign trackers are more prone to drug use and relapse because the cue (lever) itself becomes rewarding and will elicit or sustain the behavior without the primary reward itself (Robinson, Yager, Cogan, & Saunders, 2014). Similarly, goal trackers would have a decreased likelihood to cue-stimulated relapse because the cue is not as rewarding as the goal itself.

General behavioral and motivational differences have been found between sign trackers and goal trackers. Sign trackers tend to be greater novelty seekers and show greater locomotor activation compared to goal trackers (Flagel et al., 2010). Sign trackers also acquire cocaine self-administration quicker, are generally more impulsive, are more prone to attribute incentive salience to a cocaine cue, show higher reinstatement to food and cocaine and have a higher break point to cocaine compared to goal trackers (Beckmann, Marusich, Gipson & Bardo, 2011; Lovic, Saunders, Yager, & Robinson, 2011; Meyer, Ma, & Robinson, 2012; Saunders & Robinson, 2011; Yager & Robinson, 2010). All these behaviors are characteristic of animals that are more prone to self-administer drugs and can be generalized to humans, suggesting that this may be a good animal model for susceptibility to drug use and relapse (Tomie, Grimes, & Pohorecky, 2008).

Although sign tracking and goal tracking have been studied in male rats, little is known about these behaviors in female rats. Doremus-Fitzwater and Spear (2011) assessed adolescent and adult female rats to investigate possible age differences in sign tracking and goal tracking.

Surprisingly, they found that adult females tended to have increased sign tracking compared to adolescent females, suggesting a greater vulnerability to addiction in adult females. This finding contradicts our general understanding of adolescents as being more risk takers and vulnerable to addiction (for review see Spear, 2000). It is possible that adult females are more likely to attribute incentive salience to a drug cue more so than males, therefore increasing the overall likelihood towards addiction and relapse.

Little is known regarding the role of stress or stress-related hormones, such as CORT, in Pavlovian conditioned approach (PCA). Not surprisingly, plasma CORT levels increase following PCA assessment compared to basal levels prior to testing in all animals (Tomie, Silberman, Williams, & Pohorecky, 2002). The increase in plasma CORT levels was found to be higher in animals with higher conditioned responses, or sign trackers (Tomie, Silberman, Williams, & Pohorecky, 2002). These findings support previous research indicating that CORT influences self-administration, and sign trackers are more likely to self-administer (Beckmann, Marusich, Gipson, & Bardo, 2011; Piazza et al., 1991). Previous research has shown that environmental enrichment during PND21-51 increased the proportion of goal trackers, whereas social isolation during this time frame increased the number of sign trackers within a male rat sample (Beckmann & Bardo, 2012). Based on these findings, sign-tracking behavior may be affected by stress-induced CORT release and CORT differences may be driving the behavioral differences between goal and sign trackers. Taken together, these studies indicate that CORT, as well as stressful experiences, may alter CORT levels, which may increase sign-tracking behaviors. Male rats bred for high responsivity or locomotor response to a novel environment (bHR) have been shown to almost exclusively be sign trackers, whereas rats bred for low

response to novel environments (bLR) tend to have goal-tracking responses (Flagel et al., 2010). High responders have also been shown to have increased basal and stress-induced CORT levels compared to low responders (Dellu, Mayo, et al., 1996; Dellu, Piazza, Mayo, Le Moal, & Simon, 1996; Kabbaj, Devine, Savage, & Akil, 2000). Therefore, since sign trackers tend to have bHR to novel environments, it would be expected that they would have higher basal and stimulated CORT levels compared to goal trackers, who are often bLR. In the current study, it is possible that stress-induced changes in CORT during the unpredictable stress protocol increased the amount of sign-tracking behaviors and possibly shifted previous goal trackers into sign trackers, making them more sensitive to the later methamphetamine injection. In the future, whether the individual differences characterized by PCA autoshaping influences cross-sensitization between stress and methamphetamine in female rats will be assessed.

Clinical Implications

Individual differences are thought to play a critical role in both drug use and stress sensitivity. Health research has considered multiple predictive factors for such individual differences, and one that may be central is gender. Gender differences are evident in human research in terms of drug use and addiction, as well as sensitivity to stress and stress-related disorders. Females have been shown to be more susceptible to the effects of stimulants compared to males (Becker & Hu, 2008). For instance, women progress into drug dependence more quickly than men and report higher levels of craving (for review see Becker, Perry, & Westenbroek, 2012). Research in animals can help to pinpoint what factors may cause this increased susceptibility in women compared to men to help prevent the initiation and escalation

of illicit drug use and also suggest potential therapies that may help in the treatment of stimulant abuse disorders.

Females' greater sensitivity to stimulants, as well as the increased risk of relapse following drug abstinence, could be a result of gonadal hormones. Menstrual phase can have effects on withdrawal symptoms and also on general mood, which affect abstinence as well (Hudson & Stamp, 2011). In humans, women have reported a decrease in the subjective effects of cocaine following progesterone administration (Evans & Foltin, 2006). The influence of hormones on drug use has been studied in animals through altering levels of hormones and then studying the effect that these hormone levels have on behavior, drug use, craving, and relapse (Anker, Larson, Gliddon, & Carroll, 2007; Larson, Roth, Anker, & Carroll, 2005). Both estrogen and progesterone have been shown to influence stimulant-induced behaviors in female rats (Becker, Molenda, & Hummer, 2001; Sircar & Kim, 1999). Intact female rats given progesterone show a decrease in the escalation of cocaine self-administration and female rats in proestrus (highest levels of progesterone) show the lowest levels of cocaine seeking compared to other stages of the estrous cycle (Feltenstein & See, 2007; Larson, Anker, Gliddon, Fons, & Carroll, 2007). Findings from these studies have implications for therapies; if one hormone, such as progesterone, is found to be successful in reducing craving and drug use, it may be possible to administer this hormone as a way to offset the potential for relapse.

It is also likely that gender differences in response to different stressors may increase the susceptibility to different psychiatric disorders, which then could lead to drug addiction (Klein & Corwin, 2002). Women are also more likely than men to have a comorbid disorder such as anxiety or alcohol use, which may make therapy more difficult and relapse more likely (Brady &

Randall, 1999; Lex, 1991). The vulnerability of females to drug addiction during times of stress may be particularly tied to social environment and support, more so than males. A model of stress response has been proposed by Taylor et al. (2000) that differs from the historic flight or fight response model. They proposed that females follow the "tend and befriend" model when put into situations of stress, mostly driven by oxytocin and endogenous opioids (for review of literature see Slattery & Neumann, 2008). Tending involves behaviors that are nurturing to the self and increases safety while reducing distress. Befriending increases social networks that can increase support during distress. Within this model, it is suggested that social interactions are used to cushion against the possible effects of a stressor. Therefore, it is possible that women and men deal differently with stress; women may rely more on social interactions than men and therefore have a harder time staying abstinent when their social interactions are friends who are using drugs as well. Taken together, treatments that can alter and modify dopamine or stress responses may be beneficial for females in particular, with treatment of both disorders possibly being even more advantageous.

Stress has physiological and emotional effects that are likely to have altering effects on drug use and reaction. The general model of drug vulnerability and abuse suggests prior exposure to stress may increase the vulnerability of an individual to use, escalate use or relapse in use of illicit drugs (Piazza & Le Moal, 1996). As discussed previously, it is possible for stimulants to induce sensitization with extended use, but as the current study demonstrates, it is also possible for stress to sensitize the response to drug. In this thesis, female rats exposed to stress showed augmented locomotor responses and dopaminergic efflux in the dorsal striatum following a single injection of methamphetamine. In a study of women and men, exposure to

social stress, life event stress or perceived stress was positively correlated with dopamine release when exposed to a low dose (0.3mg/kg) of amphetamine as measured through PET (Oswald et al., 2007; Wand et al., 2007). These clinical findings indicate that stress, whether actual or perceived, modulates the effects of stimulants on the brain. Understanding stress as a risk factor for drug addiction may be critical to better treatment approaches in human addicts, especially for women. Addressing and reducing the stress or stress response may reduce the likelihood of drug use or relapse. The current findings have implications for understanding the complex interactions between stress and stimulants through the behavioral and dopaminergic response and can help establish the etiology and treatment of drug addiction and understanding of the potential for relapse in females.

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