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The Effect of early environmental manipulation on locomotor sensitivity and methamphetamine condition place preference reward

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THE EFFECT OF EARLY ENVIRONMENTAL MANIPULATION ON

LOCOMOTOR SENSITIVITY AND METHAMPHETAMINE

CONDITIONED PLACE PREFERENCE REWARD

by

Emily Hensleigh

Bachelor of Science in Psychology
Montana State University
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A thesis submitted in partial fulfillment
of the requirements for the

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ABSTRACT

**The Effect of Early Environmental Manipulation on
Locomotor Sensitivity and Methamphetamine
Conditioned Place Preference Reward**

by

Emily Hensleigh

Dr. Laurel Pritchard, Examination Committee Chair
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University of Nevada, Las Vegas

Early life stress has drastic effects on neurological development, affecting health and

well-being later in life. Instances of child abuse and neglect are associated with higher

rates of depression, risk taking behavior, and an increased risk of drug abuse later in life

(Chapman,D.P. 2004; Dube,S.R. 2003). This study used repeated neonatal separation of

rat pups as a model of early life stress. Rat pups were either handled and weighed as

controls or separated for 180 minutes per day during postnatal days 2-8.   In adulthood,

rats were tested for methamphetamine conditioned place preference reward and

methamphetamine induced locomotor activity. Tissue samples were collected and

mRNA was quantified from the following brain regions: prefrontal cortex

(norepinephrine transporter), nucleus accumbens (dopamine transporter), and ventral

midbrain (cocaine-amphetamine regulated transcript). Results indicated rats given

methamphetamine formed a conditioned place preference, but there was no effect of early

separation or sex. Separated males showed heightened methamphetamine-induced

locomotor activity, but there was no effect of early separation for females. Overall

females were more active than males in response to both saline and methamphetamine.

This suggests early neonatal separation may differently affect methamphetamine-induced

locomotor activity and methamphetamine reward. Additionally, these effects on

locomotor activity are likely sex-dependent.

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CHAPTER 1

INTRODUCTION

Early life stress, including child abuse or neglect, affects neurological development, negatively impacting health and well-being later in life. Studies suggest child abuse and neglect are associated with risk-taking behavior, increased risk of depression, and increased vulnerability to drug abuse (Chapman et al., 2004; Dube et al., 2003). Individuals who report early adverse experiences, such as abuse, are seven to ten times more likely to report substance use compared to individuals without a history of early adverse experiences. These individuals are also two to four times more likely to experiment with illicit drugs at an early age compared to those without a history of early adverse experiences. Finally, risk of developing an addiction drastically increases in individuals with significant life histories of stress (Dube et al., 2003). Based on clinical and animal studies, it is clear that early life stress causes neuroadapitve changes leading to persistent negative behavioral and neurological effects. The following will review the neurological correlates of stress, the relationship between stress and addiction, and the developmental effects of early life stress.

Several effects occur as a result of activation of stress pathways, such as the HPA axis. Minor stress, such as exercise, activates the stress response and results in slightly elevated stress hormone levels. This activation result in healthful benefits for the organism such as increased functioning of the HPA axis in response to a stressor. However, prolonged activation of the stress response produces neurological adaptations resulting in negative consequences such as prolonged elevated hormone levels in response to a stressor and decreased negative feedback of the HPA axis (McEwen, 2007;

Vazquez, 1998).The physiological stress response occurs after emotional or physiological strain and is an adaptive mechanism necessary for survival. Physically, the stress response prepares the organism to deal with an immediate threat by increasing heart rate and respiration, constricting blood vessels, enhancing attention, and increasing energy through glucose production. The paraventricular nucleus (PVN) of the hypothalamus and norepinephrine neurons in the locus coeruleus regulate the stress response (Levine, 2000; McEwen, 2007; Tsigos & Chrousos, 2002).

As suggested above, two main brain regions initiate and terminate the body's biological response to stress, the paraventricular nucleus (PVN) of the hypothalamus and norepinephrine neurons in the locus coeruleus. When an organism is under stress, norepinephrine is released from cells bodies in the locus coeruleus, activating the flight or fight response. Additionally, the paraventricular nucleus (PVN) of the hypothalamus releases corticotropin releasing factor (CRF) and arginine-vasopressin. CRF, the primary effector, in turn triggers the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH circulates systemically to the adrenal glands, eventually leading to the release of glucocorticoids, such as cortisol in humans and corticosterone in rats. These hormones bind to glucocorticoid receptors throughout the body and brain. Activation of glucocorticoid receptors in the hypothalamus and pituitary inhibits further release of CRF and ACTH, respectively, thus terminating the stress response. These events make up the hypothalamic-pituitary-adrenal (HPA) axis (Habib, Gold, & Chrousos, 2001; Tsigos & Chrousos, 2002).   Additionally, CRF binds to presynaptic receptors located on norepinephrine neurons in the locus coeruleus, leading to inhibition of norepinephrine release. Norepinephrine neurons also self regulate with α2-

autoreceptors and receive input from several other systems including GABA, serotonin, and opioid peptides. These components, the HPA-axis and norepinphrine functioning, regulate the body's homeostatic stress response (Koob, 1999; Tsigos & Chrousos, 2002).

Homeostatic functioning also relies on daily circulating levels of CRF. In humans, CRF and arginine-vasopressin maintain a daily rhythmic cycle, aiding in regulatory behaviors such as sleep and feeding. Elevated blood concentrations of these hormones occur during the onset of light (waking or morning) and gradually decrease throughout the day, with low levels occurring at the onset of darkness (sleep or evening) (McEwen, 2007). Rats follow a similar diurnal pattern with elevated CRF levels occurring during the onset of darkness (waking or evening) and gradually decreasing with low levels occurring at the onset of light (sleep or morning) (Lightman et al., 2002).

The HPA-axis aides in regulating daily homeostasis; however overactivation due to chronic stress leads to unfavorable consequences. Chronic stress increases the release of CRF and arginine-vasopressin leading to possible alterations in the natural circadian rhythm (Lightman et al., 2002; McEwen, 2007; Tsigos & Chrousos, 2002). Animal and human studies suggest overactivation of the HPA-axis leads to increased risk of cardiac disease, depression, type II diabetes, and drug abuse (McEwen, 2007). More specifically, chronic stress enhances susceptibility to addiction in both human and animal studies. The following paragraphs summarize supporting evidence for this claim.

Animals exhibit behaviors similar to relapse in humans. Recovering drug abusers report drug relapse commonly co-occurring with major life stressors. Similarly, stress alters drug self administration and drug reward in animal models (Koob, 2008; Sarnyai, Shaham, & Heinrichs, 2001; Sinha, 2008; Tsigos & Chrousos, 2002). Rats self-

administer more of a drug and acquire drug self administration more rapidly when

exposed to a stressor prior to training (Ahmed & Koob, 1997; Kitanaka, Kitanaka, &

Takemura, 2003; Pauly, Robinson, & Collins, 1993). Rats subjected to foot shock, a

known stressor, more readily self-administer cocaine (Ahmed & Koob, 1997). When

taught to lever-press for cocaine, animals extinguish lever-pressing when saline replaces

the drug. If given a priming injection of cocaine, the animal resumes lever-pressing

almost immediately, a phenomenon termed reinstatement. Experimental stressors

precipitate drug reinstatement without the need for a priming injection (Ahmed & Koob,

1997). The activation of the stress response via the HPA-axis affects many aspects of

drug self-administration and abuse. Neurological alterations in the HPA-axis after

repeated drug exposure further support this statement.

Neurological evidence indicates interactive effects between stress and drugs of abuse.

Acute administration of opiates, cocaine, alcohol, or amphetamine activates the HPA-axis

as well as the mesolimbic dopamine pathway (Majewska, 2002; Sarnyai et al., 2001).

Chronic administration of such drugs leads to over activation of the mesolimbic

dopamine pathway and the HPA-axis. This over activation may modify both systems,

contributing to withdrawal symptoms and drug relapse (Koob, 2008; Marinelli & Piazza,

2002).

Neurological alterations due to repeated exposure to drugs of abuse lead to

sensitization of certain behaviors in both humans and animal models. Sensitization refers

to the increased effect of a drug due to repeated use (Robinson & Berridge, 2001). In

humans, psychostimulants such as cocaine, amphetamine, or methamphetamine initially

increase activity levels. After chronic use of psychostimulants, several repetitive

behaviors arise which may include sorting small objects, or 'knick-knacking', and repeatedly scratching or picking at the skin (Darke, Kaye, McKetin, & Duflou, 2008; Winslow, Voorhees, & Pehl, 2007). In rodents, psychostimulants also initially increase locomotor activity. After multiple doses, the initial locomotor activating effects of the drug become sensitized and stereotyped behaviors become more prevalent (Vezina, 2004). These stereotyped behaviors include repeated rearing, constant head bobbing, inappropriate gnawing, or excessive grooming (Randrup & Munkvad, 1974).

Sensitization, exhibited by these repetitive behaviors, is a long-lasting phenomenon. After several months of drug cessation in rats, a challenge injection enhances stereotyped behaviors, indicating enduring effects of repeated drug administration (Robinson & Becker, 1986). In rodents and humans, drug effects become sensitized, such as the locomotor activating effects of amphetamines, whereas other effects, such as euphoria, become tolerant (Kitanaka et al., 2003). The neuroadaptations underlying sensitization likely reflect the chronic, relapsing nature of addiction, which is further explained by the Incentive-Sensitization Model of Addiction.

The Incentive-Sensitization Model of Addiction, proposed by Robinson and Berridge (2001), suggests sensitization plays an important role in addiction. The model proposes that repeated and persistent drug use leads to enduring neurological alterations, including those involved in reward. The model further suggests brain reward systems become hypersensitive to drugs of abuse and drug associated stimuli due to neuroadaptive changes. These sensitized systems mediate the "wanting" component of drug reward as opposed to the initial euphoric effects, or "liking" component, associated with the drug. The mesotelencephalic dopamine system mediates the incentive motivational aspect or

"wanting" of a drug, whereas the euphoric effect of a drug, or "liking", is mediated by different neuronal systems. Since separate systems underlie "liking" and "wanting" it is clear why repeated drug use sensitizes one behavior, but differently affects another behavior. This model explains why the craving, or "wanting", for a drug increases with repeated use, but the euphoric effects, or "liking", decreases with repeated use. (Robinson & Berridge, 2001).

In addition, stress hormones, glucocorticoids such as cortisol or corticosterone, can influence dopamine systems, regulating drug reward and craving. Glucocorticoids alter dopaminergic transmission leading to increased drug-induced behavioral sensitization. Artificial administration of glucocorticoids enhances amphetamine sensitization in rats, whereas suppression of glucocorticoid secretion, via adrenalectomy, inhibits amphetamine sensitization (Deroche et al., 1995; Pauly et al., 1993). Animals subjected to experimental stressors further exhibit increased locomotor activity and stereotyped behaviors induced by stimulants (Deroche et al., 1995; Majewska, 2002; Matuszewich & Yamamoto, 2004). Based on the incentive sensitization model, stress may potentiate the craving effects of drugs of abuse. Stressful early life experiences further illustrates the enduring nature of sensitization.

Chronic stress during development can lead to enduring changes in stress systems. In rodents, during the first two weeks after birth, the presence of the mother mediates the function of the HPA-axis. Absence of the mother, or dam, promotes activation of the HPA-axis and contact with the dam inhibits activation (De Kloet, Rosenfeld, Van Eekelen, Sutanto, & Levine, 1988). During the critical first two weeks, prolonged absence of the dam continually activates the HPA-axis, eventually leading to adaptations

in the regulation of this system (Vazquez, 1998). Several animal models of chronic early life stress attempt to elucidate the neural mechanisms that underlie such adaptations. Extended separation of neonatal rat pups from the dam results in behavioral and neurological deficits in adulthood. These animal models attempt to functionally represent the neurobiological and behavioral consequences of early life stress, such as child neglect.

Several rodent models have been used to reproduce the effects of early life stress. The maternal deprivation model separates the entire litter of pups from the dam once for 24 hours during the first week after birth. Adolescent and adult rodents which have undergone this procedure demonstrate deficits in both physical and cognitive development compared to control groups (Lehmann, Russig, Feldon, & Pryce, 2002; Rosenfeld, Wetmore, & Levine, 1992). The maternal separation model removes pups from the dam and houses them together as an entire group. Neonatal isolation removes pups from the dam and houses them individually until reunion with the dam. Separation and isolation occurs for one to six hours daily during the first one to two weeks of development. Control groups consist of either standard factory reared litters, which are never handled except for cage changes, or briefly handled litters, which are handled for approximately 15 minutes per day (Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Meaney, Brake, & Gratton, 2002). Social isolation also models early life stress but occurs after weaning (or approximately 21 days of age), rather than during early postnatal development. Socially isolated rats are housed in individual cages, while controls are housed in pairs or small groups (Lapiz et al., 2003). All the aforementioned

manipulations result in behavioral and neurological deficits later in life, though the effects vary, depending on which manipulation is applied.

Repeated maternal separation yields several behavioral deficits. In adulthood, separated rats exhibit decreased struggle time in the forced swim task, suggesting increased despair (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2007). Separated rats exhibit thigmotaxis and increased freezing behavior in an open field, suggesting fearful behavior in response to novelty (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000). Similarly, compared to handled controls, separated rats exhibit hypoactivity in an open field chamber, and later increased hyperactivity, indicating increased anxiety in a novel environment (Kalinichev et al., 2002). Socially isolated rats also show an increased activity level in a novel environment and augmented anxiety-like behavior similar to separated animals (Lapiz et al., 2003). Additionally, separated rats display decreased time in the open arms of an elevated plus maze, indicative of increased anxiety-like behavior (Aisa et al., 2007). These studies illustrate chronic early life stress, induced by separation or social isolation, results in depressive and anxiety-like behaviors later in life.

Control rats, standard factory reared, or handled rats also differ in behaviors in adulthood, which may be mediated by the dam. Standard factory reared pups (not handled for the first twenty one days of life) exhibit similar anxious behaviors compared to separated animals (Lehmann et al., 1998). Brief handling of neonatal pups (15 min/day) results in opposite behavioral patterns compared to separated and standard factory reared animals. As mentioned previously, separated animals show increased exploratory or hyperactive behavior in a novel environment compared to briefly handled controls (Kalinichev et al., 2002). The mother's behavioral response to separated and

handled pups may mediate this difference. After brief handling of pups, dams engage in

increased maternal behavior when compared to dams of separated or standard factory

reared pups. Reunion of pups with the dam after a brief handling separation (15 min/day)

elicits a high degree of maternal behavior, such as arched back nursing and licking

(Pryce, Bettschen, & Feldon, 2001). This increased maternal behavior likely organizes

brain development in a way which promotes a less anxious behavioral phenotype.

Conversely, dams of standard factory reared pups do not exhibit robust maternal

behavior. In adulthood these pups demonstrate more anxious behavioral patterns

compared to handled controls (Levine, 1960). Long, repeated separations deprive pups

access to maternal care and result in comparable anxious behavioral patterns. Early

maternal care affects neurological development, and separating pups from the dam alters

patterns of maternal care.

Neurological alterations in HPA-axis functioning and dopamine systems likely

underlie behavioral effects of maternal separation observed in adulthood. Handled

controls and separated rats differ in their physiological responses to stress (Ladd, Huot,

Thrivikraman, Nemeroff, & Plotsky, 2004; Plotsky & Meaney, 1993). Adult separated

rats show elevated levels of adrenocorticotropin hormone (ACTH) and corticosterone in

response to a brief stressor (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2008;

Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007). Importantly, handled

controls and separated animals do not differ in basal diurnal patterns of ACTH,

suggesting separated animals exhibit a potentiated stress response. After footshock, a

known stressor, separated animals show prolonged elevation of glucocorticoid blood

levels suggesting prolonged activation of the HPA-axis (Aisa et al., 2007; Aisa et al.,

2008; Ladd, Owens, & Nemeroff, 1996). Heightened and prolonged stress response also coincides with alterations in the HPA-axis negative feedback loop. Plotsky et al. (2005) subjected rats to two weeks of postnatal separation. In adulthood, these rats exhibited increased CRF mRNA levels in the paraventricular nucleus, central nucleus of the amygdala, locus coeruleus, and bed nucleus of the stria terminalis, suggesting compensatory mechanisms for elevated response to a stressor (Plotsky et al., 2005). Separated animals also exhibit reduced negative feedback of the HPA-axis regulated by glucocorticoid receptors, likely mediated by decreased glucocorticoid receptor density in the hippocampus (Ladd et al., 2004). Early life stress negatively impacts the stress response by reducing the effectiveness of the negative feedback shut-off loop.

Chronic stress early in development also alters the mesolimbic dopamine system, important in the rewarding properties of drugs of abuse (Arborelius & Eklund, 2007; Brake, Zhang, Diorio, Meaney, & Gratton, 2004). Separated animals show decreased dopamine transporter count in the nucleus accumbens and show elevated dopamine levels in the striatum after a stressor (Matthews, Dalley, Matthews, Tsai, & Robbins, 2001; Meaney et al., 2002). These alterations likely influence susceptibility to the effects of drugs of abuse.

Furthermore, repeated early life stress alters neurological systems likely underlying sensitization and rewarding properties of psychostimulants. Separated rats acquire cocaine self administration more rapidly and self administer cocaine at a lower dose compared to briefly handled controls (Kosten, Miserendino, & Kehoe, 2000; Moffett et al., 2006). Separated animals also exhibit increased sensitivity to the effects of an acute challenge injection of cocaine and an acute injection of amphetamine (Kikusui et al.,

2005; Matthews, Hall, Wilkinson, & Robbins, 1996). Compared to non handled controls, maternally separated rats exhibit increased sensitivity to the reward-enhancing effects of amphetamine in adulthood (Der-Avakian & Markou, 2010). However, no effects of amphetamine locomotor sensitization were found in separated or isolated rats (Brake et al., 2004; Weiss et al., 2001). Additionally, no effects of amphetamine locomotor sensitization across doses were found in neonatally separated rats (Hensleigh, Smedley, & Pritchard, 2010). These findings suggest early life stress may alter brain systems increasing vulnerability to drug reward in adulthood. Similarly, early life stress likely affects similar systems which may or may not lead to drug-induced locomotor sensitization.

Early environmental stress likely alters the rewarding properties of psychostimulants, such as cocaine and amphetamine, however the effect of early environmental stress on methamphetamine needs to be better characterized. Structurally and mechanistically, amphetamine and methamphetamine are similar; although differences exist between the two substances. In humans, different abuse patterns result in varying neurological effects. Amphetamine is typically obtained from prescription drugs and administered orally in single large doses. Illicit manufacturing of methamphetamine results in varying potencies, consumed typically by smoking dissolved crystals. Individuals who abuse methamphetamine administer the drug in a binge pattern with multiple high doses of the drug consumed over a few hours or days (Winslow et al., 2007).

The growing estimates of methamphetamine abuse increases demand for improved research. An estimated 731,000 individuals twelve or older (0.3 percent of the population) reported using methamphetamine at least once within the past year. Reports

of methamphetamine abuse remain elevated relative to 30 years ago, requiring the need for improved understanding of this drug (NIDA, 2008).

Methamphetamine is inexpensive to manufacture and relatively simple to obtain. Mass production of methamphetamine occurs by obtaining pseudophedrine from several over-the-counter drugs (Winslow et al., 2007). Increased regulations on the purchase of pseudophedrine-containing drugs have been implemented in an attempt to decrease the illegal production of methamphetamine. Regardless, methamphetamine production and illicit sales continue throughout the United States and other countries (Cunningham & Liu, 2003; Winslow et al., 2007). Typical administration of methamphetamine occurs through smoking crystals, which allows for rapid behavioral and neurological effects. Acute behavioral effects of methamphetamine result from increased release of monoamines and include euphoria, enhanced libido, elevated energy, and bruxism (grinding teeth). Many of these effects contribute to methamphetamine's addictive properties. Physiological effects result from systemic release of adrenaline (epinephrine) and include increased heart rate, increased blood pressure, and hyperthermia (Kish, 2008; Winslow et al., 2007).

In vitro and in vivo studies shed light on the pharmacological mechanisms of psychostimulants. Amphetamines enter the terminal buttons through the membrane transporters to exert their stimulant effects. Relative affinities for methamphetamine and amphetamine are highest at the norepinephrine transporter, followed by the dopamine transporter, and finally the serotonin transporter. Methamphetamine exerts a greater effect (potency) at the serotonin transporter compared to amphetamine (Han & Gu, 2006; Kuczenski, Segal, Cho, & Melega, 1995). Inside the terminal, methamphetamine

liberates monoamines from vesicles releasing them into the cytoplasm. Reversal of the

membrane transporter by methamphetamine pumps the newly dumped monoamines out

of the terminal button into the synapse. Additionally, methamphetamine and

amphetamine inhibit monoamine oxidase, slowing the breakdown of the available

monoamines (Fleckenstein, Volz, Riddle, Gibb, & Hanson, 2007). The perceived

rewarding effects relate to the dopamine transporter, although recent studies suggest an

important role for norepinephrine transporters in the rewarding properties of stimulants

(Fleckenstein et al., 2007; Schank et al., 2006; Sofuoglu & Sewell, 2009; Ventura, Cabib,

Alcaro, Orsini, & Puglisi-Allegra, 2003).

Several acute neurochemical effects occur after methamphetamine administration. In

rats, acute administration of methamphetamine results in elevated levels of monoamines

in the striatum (Fleckenstein, Metzger, Wilkins, Gibb, & Hanson, 1997; Haughey,

Brown, Wilkins, Hanson, & Fleckenstein, 2000). Methamphetamine also rapidly

regulates dopamine and norepinephrine transporters. Acute methamphetamine injection

rapidly down regulates expression of the dopamine transporter in vivo and in vitro. This

effect is reversible and occurs at high doses (Zahniser & Sorkin, 2004).

Methamphetamine acutely decreases clearance of dopamine in the synaptic cleft by

inhibiting reuptake through the dopamine transporter (Goodwin et al., 2009; Zahniser &

Sorkin, 2004). These short term effects can lead to several long term changes with

continual methamphetamine administration.

Long term methamphetamine abuse potentially leads to prolonged neuorotoxic

damage in dopamine systems. Neurotoxicity characteristically occurs after multiple

consecutive doses or a single high dose of methamphetamine. Clinical studies and

animal models of methamphetamine abuse exhibit neurotoxic damage. Individuals who abuse methamphetamine follow a characteristic dosage pattern, where methamphetamine administration occurs in 'runs'. This abuse pattern consists of short methamphetamine binges taken at multiple points over hours or days. Rats given a similar pattern of methamphetamine administration exhibit reduced dopamine levels and decreased dopamine transporter density in the nigrostriatal dopamine pathway (Hanson, Rau, & Fleckenstein, 2004; Kita, Wagner, & Nakashima, 2003; Segal, Kuczenski, O'Neil, Melega, & Cho, 2005). Imaging studies show decreased dopamine transporter density in the nucleus accumbens, prefrontal cortex, and caudate putamen of chronic methamphetamine abusers. Decreased dopamine transporter density furthermore suggests dopamine terminal damage associated with motor impairment and cognitive deficits in chronic methamphetamine abusers (Chang, Alicata, Ernst, & Volkow, 2007; Sekine et al., 2001; Wilson et al., 1996). These studies suggest chronic administration of methamphetamine can lead to prolonged alterations in monoamine systems.

Dopamine presumably underlies damage and behavioral effects of methamphetamine toxicity. Mainly, the dopamine transporter mediates the neurotoxic effects of methamphetamine. Substances which block the dopamine transporter, such as cocaine, show neuroprotective effects against methamphetamine toxicity. These neuroprotective effects occur even if administration of dopamine transporter blockers occurs several hours after neurotoxic doses of methamphetamine (Marek, Vosmer, & Seiden, 1990). Norepinephrine also allegedly mediates the neurotoxic damage of methamphetamine induced dopamine release. Blocking norepinephrine release pharmacologically, by lesions, or by genetic deletion results in increased dopamine release in the striatum,

enhanced methamphetamine induced stereotyped behaviors, and enhanced markers of neurotoxicity (Weinshenker et al., 2008). This suggests that without norepinephrine present, increased dopamine is released leading to detrimental neurotoxic effects. Chronic methamphetamine administration results in neurotoxicity, likely mediated by high levels of dopamine, leading to lasting injury in dopamine systems.

The majority of injury caused by methamphetamine occurs within dopamine systems; however deficits also occur in other monoamine systems. Methamphetamine abusers exhibit decreased serotonin transporters and vesicular monoamine 2 transporters in subregions of the striatum (Kita et al., 2003). Similarly, rats exhibit decreased serotonin transporter density and serotonin depletion after neurotoxic doses of methamphetamine (Friedman, Castaneda, & Hodge, 1998). Chronic methamphetamine administration in rats also results in damage to serotonergic terminals in the forebrain (Marshall, Belcher, Feinstein, & O'Dell, 2007). After an increasing dose regimen of methamphetamine, a challenge dose results in norepinephrine and serotonin depletion in the hippocampus, striatum, and cortex along with the marked depletions of dopamine observed in the striatum (Graham, Noailles, & Cadet, 2008). Methamphetamine neurotoxicity involves depletions throughout several neurotransmitter systems.

Animal models can allow us to elucidate neurological factors of addiction and contributing factors of drug abuse vulnerability otherwise difficult to tease out in the clinical population. Ethical constraints prohibit administration of illicit psychoactive drugs to human subjects. Beyond imaging techniques, studying the neurochemical effects of psychoactive drugs in living human subjects remains a challenge. Similarities across

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mammalian species allows for understanding predispositions to addiction and identifying

mechanisms which otherwise could not be elucidated in humans.

CHAPTER 2

RESEARCH QUESTIONS

This study asked two main research questions. First, does prolonged stress during development affect sensitivity to the locomotor effects and rewarding properties of methamphetamine in adulthood? To answer this question, this study employed the use of an animal model of early life stress. Separated and handled control rats were tested for locomotor sensitization and conditioned place preference. Conditioned place preference was used as a measure of the rewarding properties of methamphetamine. Control and separated rats were given injections of saline and methamphetamine that were repeatedly paired with distinct environments. A final place preference test determined the rewarding properties of methamphetamine (see materials and methods for a more in depth discussion of procedures). It was predicted that early life stress would increase the rewarding properties and locomotor activating effects of methamphetamine. Therefore, separated subjects were hypothesized to spend more time in the drug paired chamber compared to handled controls and exhibit heightened locomotor activity in response to methamphetamine.

The second research question consisted of: do neurobiological mechanisms involving brain monoamine systems underlie the behavioral effects of early separation on methamphetamine sensitivity? To get at this question, expression of messenger RNA for the following gene targets was quantified using RT-PCR: the dopamine transporter (DAT), the norepinephrine transporter, and the neuropeptide Cocaine-Amphetamine Regulated Transcript (CART).

For this study, dopamine transporter mRNA was quantified in the nucleus accumbens. Reduced levels of the dopamine transporter were found in the nucleus accumbens of separated rats (Meaney et al., 2002). The dopamine transporter (DAT) is important for stimulant addiction and is regulated immediately and long-term by stimulants (Haughey et al., 2000; Zahniser & Sorkin, 2004). Therefore, separated animals were hypothesized to have decreased DAT levels in the nucleus accumbens.

For this study, norepinephrine transporter mRNA was quantified in the prefrontal cortex. Alpha 2 adrenergic receptor density was greater in locus coeruleus of handled animals compared to separated animals in adulthood, suggesting early separation affects norepinephrine systems (Liu, Caldji, Sharma, Plotsky, & Meaney, 2000). Because α2 receptors function as autoreceptors, reduced expression in separated rats relative to handled controls suggests a deficit in noradrenergic regulation in separated rats. Norepinephrine transporter density was thought to have served as an adaptive mechanism to compensate for enhanced norepinephrine due to reduced autoreceptor activity. Increased levels of membrane NETs were found in rat prefrontal cortex after chronic cold stress (Miner et al., 2006). Therefore it was hypothesized that chronic early life stress would to result adaptive mechanisms leading to increased NET in the prefrontal cortex.

Lastly, Cocaine-Amphetamine Regulated Transcript (CART) was quantified in the ventral midbrain. CART is a peptide involved in regulatory behaviors, such as feeding and stress, and is further thought to be involved in the rewarding and reinforcing properties of psychostimulants. CART was named because mRNA of the peptide was upregulated after administration of cocaine and amphetamine (Douglass, McKinzie, & Couceyro, 1995; Jaworski & Jones, 2006; Vicentic & Jones, 2007). CART has been

localized in numerous areas of the brain including the hypothalamus, mesolimbic dopamine system, pituitary, and hindbrain (Vicentic & Jones, 2007). An increase in CART expression in the hippocampus was observed after chronic stress (Hunter et al., 2007). Injection of CART into the ventral tegmental area promoted locomotor behavior and potentiated conditioned place preference (Kimmel, Gong, Vechia, Hunter, & Kuhar, 2000). Suggesting possible similar underlying systems involved in cocaine and amphetamine induced locomotor activity. CART containing neurons in the VTA also synapse on dopamine containing neurons, which could play a role in mediating locomotor activity and CPP (Dallvechia-Adams, Kuhar, & Smith, 2002). The effects of chronic early life stress and methamphetamine dosing on CART expression were previously unknown. For the current study, CART mRNA was quantified in the ventral midbrain, which included the ventral tegmental area and substantia nigra.

CHAPTER 3

METHODOLOGY

Subjects

This was a 2 sex (male x female) x 2 condition (control x separated) x 2 drug (saline x methamphetamine) factorial design. Sixteen female and sixteen male Long-Evans rats were obtained from Charles River Inc. (Massachusetts) and used as breeders. Subjects had one week to habituate to the facility before being paired for breeding (see section on breeding procedures). Dams provided 73 offspring (34 males, 39 females) used for final analyses. Animals were kept on a 12:12 hour light/dark schedule with lights on at 0730 hrs. All animals were housed in polypropylene tub cages lined with corn cob bedding and had free access to food and water. Females were housed in pairs until breeding, after which each dam was housed singly. Males used for breeding were singly housed for the duration of the experiment, and cage changes occurred biweekly. Experimental manipulations took place between 0800 hrs and 1700hrs. All procedures were performed in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee at University of Nevada Las Vegas.

Breeding Procedures

One male and one female were placed together in a hanging wire cage for no more than five days. Fertilization was designated by the presence of a vaginal plug. After confirmation of a vaginal plug, females were removed and housed singly in polypropylene cages. If no plug was identified after five days, pairs were separated and females were singly housed and monitored for pregnancy.

Maternal Separation Procedure

The day of birth was designated post natal day (PND) 0 and litters were left undisturbed on this day. On PND 1, the dam was removed and pups were sexed and either culled or fostered to achieve roughly equal litter sizes and sex ratios. Separation and control procedures occurred on PND 2-8. Control litters were removed from the dam, transported to the testing room, and individually weighed. Individual pups were identified by number markings using a non-toxic pen. After all pups were weighed, the pups were rolled in soiled home cage bedding and returned to the dam. Control procedure lasted approximately 10-15 minutes. Separated (S) animals were removed from the dam, transported to the testing room, and individually weighed. Each pup was placed in an individual plastic cup covered with an air-vented lid for three hours. Temperature was monitored and kept at that of the nest, approximately 30 degrees Celsius, by use of a heating pad. Two to four pups from each litter were left with the dam during separation to decrease stress on the mother. After three hours, separated pups were transported back to the animal housing room, rolled in soiled cage bedding, and returned to the dam, where they remained undisturbed until the next day. Beginning on post natal day (PND) 9, all litters were left undisturbed, except for weekly cage changes. On PND 21, all pups were weaned from the mother and pair housed by same sex until behavioral testing began on PND 60.

Behavioral Testing

A 70cm X 30cm X 30cm chamber equipped with three separate compartments was used to assess conditioned place preference (CPP). Each side compartment measured 29.7 x 28.7 x 11.8 cm³, had a distinct wall color (black or white), and distinct floor

texture (bars or grid). Side compartments had a door with a removable sliding panel leading into the center compartment. The middle compartment measured 9.8 x 28.7 x 11.8 cm³ and was gray with a smooth floor. The chamber was equipped with a fan for ventilation and noise cover, an adjustable LED light, and a 7 x 15 photobeam grid tracking system located 2.5 cm above the floor of the chamber. Data from photobeam breaks were transferred into a Dell computer running a program (Motor Monitor by Kinder Scientific) that measured time spent in each compartment and activity level by total amubulations. One ambulation was defined as two adjacent, consecutive beam breaks.

Methamphetamine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in sterile 0.9% saline at a concentration of 1.0 mg/ml (calculated based on salt weight). Injections were given subcutaneously in a volume of 1.0 ml/kg of body weight, resulting in a methamphetamine dose of 1.0 mg/kg. This dose was selected based on previous studies demonstrating robust locomotor activation and conditioned place preference in rats at a similar dose (Shimosato & Ohkuma, 2000; Zakharova, Leoni, Kichko, & Izenwasser, 2009)

Testing began between PND 58-64 and lasted 10 days. Subjects were brought to the testing room to habituate for 30 minutes before testing each day. Subjects were placed in the center compartment of the conditioned place preference chamber with both doors open and allowed to explore the entire chamber for 30 minutes. Animals were randomly assigned as saline or methamphetamine group. The saline group consisted of both separated and handled animals and received saline injections paired with both chambers. An initial chamber preference was determined for both groups by analyzing the amount

of time spent in each chamber during the habituation day. If a preference existed, methamphetamine was paired with the non-preferred chamber and saline was paired with the preferred chamber. For the saline group, the non-preferred chamber randomly designated as the 'drug' chamber and used for comparison against experimental animals. If no preference existed, the chambers were randomly assigned as drug or saline paired chambers.

All injections occurred 15 minutes prior to being placed in the chamber. The day after chamber habituation, subjects were injected with saline and placed in the previously assigned saline-paired chamber for 30 minutes. On the following day, animals were transported to the testing room and given an injection of saline or methamphetamine (1.0mg/kg) and placed in the drug-paired chamber for 30 minutes. Saline and methamphetamine injections occurred on alternating days until four pairings with each compartment (8 days total) occurred. Control animals received saline injections paired with alternating chambers every day. Ambulation data was also collected on these days.

The day after the last pairing subjects were given no injection and placed in the CPP chamber with both doors open for 30 minutes. Ambulatory data was collected during this time and examined using three different zones (saline paired chamber, center chamber, and meth paired chamber). After 30 minutes, animals were returned to their cages and left undisturbed in the animal colony for 24 hours until sacrifice, dissection, and tissue collection. For every session, each rat was tested in the same apparatus and at approximately the same time of day. Dependent variables for each session included total ambulations (locomotor activity) and time spent in each compartment. A place

preference was determined if the animal spent significantly more time in the drug paired chamber compared to the saline paired chamber.

Tissue Collection

Twenty four hours after the final test day, animals were euthanized by an overdose of Euthasol (pentobarbital sodium and phenytoin sodium) and decapitated. Brains were immediately removed and chilled in 0.9% saline for five minutes. With the aid of a rodent brain matrix, the following structures were dissected out: prefrontal cortex, nucleus accumbens, and ventral midbrain (a single block containing ventral tegmental area and substantia nigra).

Tissue was placed in individual plastic tubes, immediately frozen on dry ice, and kept in -80˚ C storage until mRNA quantification. Total RNA was isolated by manufacturers instructions using an E.Z.N.A. Total RNA Kit (OMEGA). Briefly, tissue samples were homogenized in 1 mL of RNA Solv and 20 µl 2-mercaptoethanol (30 mg of tissue) and incubated for 5 minutes at room temperature. 200µl of chloroform was added and homogenate was incubated at room temperature for 2-3 minutes followed by centrifugation at 12,000 x g at 4°C for 15 minutes. The upper phase was transferred and combined with equal volume of 70% ethanol. Sample was applied to a spin column and centrifuged at room temperature at 10,000 x g for 1 minute. Samples were washed with 500µl of RNA Wash Buffer I, centrifuged at room temperature at 10,000 x g for 1 minute, and flow through was discarded. Samples were washed twice more with 500µl of RNA wash buffer II, centrifuged at room temperature at 10,000 x g for 1 minute. Sample columns were centrifuged at maximum speed for 2 minutes at room temperature.

RNA was eluted in 55µl of nuclease free water by centrifuging at 10,000 x g for 2 minutes. RNA was kept at -80°C until RT-PCR.

Sample total RNA concentration and integrity was determined by A_{260} measurement. mRNA was reverse-transcribed and amplified using a qScript One-Step SYBR Green qRT-PCR kit (Quanta). Briefly, samples were thawed on ice and diluted to equal concentrations (50ng/µl). Reaction mix contained the following volumes per well: 25µl One-Step SYBR Green Master Mix, 3 µl forward primer, 3 µl reverse primer, 8 µl Nuclease free water, 1 µl qScript One-Step RT, and 10 µl RNA template. Samples were assayed in duplicate in 96-well plates using an iCycler (Biosciences). Quantification was achieved by normalizing the target mRNA to the house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A standard curve was calculated using seven serial dilutions (800ng-12.5ng for DAT, CART, and GAPDH, 400ng-6.25ng for NET) of total RNA. The target quantity in experimental samples was normalized to GAPDH and compared against the handled, saline group. Final values were analyzed using a between subjects ANOVA.

CHAPTER 4

RESULTS

Locomotor Activity and Conditioned Place Preference

To determine a chamber preference, a ratio score was calculated by subtracting the

amount of time spent in the drug paired chamber on day ten from the drug paired

chamber on day one and dividing by time spent in drug paired chamber on day 10 ((Time

day10 – Time day1)/TimeDay10). Data was analyzed using a 2 sex (male x female) x 2

condition (control x separated) x 2 drug (saline x methamphetamine) between subjects

ANOVA. There was a significant effect of drug $(F(1,72) = 14.45, p<0.001)$ with

methamphetamine animals spending more time in the drug paired chamber. There was no

significant effect of condition $(F(1,72) = 1.56, p>0.05)$ or sex $(F(1,72) = 0.803, p>0.05)$

or significant interactions for time spent in the drug paired chamber (Supplementary

figure 1).

Two mixed model ANOVAs were used to analyze total ambultions on drug paired

days and saline paired days for subjects in the methamphetamine group. Day was the

within subjects factor (day 2, 4, 6, 8 for saline pairings and 3,5,7,9 for drug pairings) with

condition (control x separated) and sex (male x female) as between subjects factors.

Results for the drug paired days indicated no significant effect of days $(F(3,30) = 2.75,$

$p>0.05)$ and a significant effect of sex $(F(1,32) = 11.43, p<0.001)$ with females exhibiting

higher locomotor activity. Condition was approaching significance $(F(3,32) = 3.57,$

$p=0.068)$ with separated animals exhibiting higher locomotor activity. There was no sex x

condition interaction $(F(1,32) = 0.054, p>0.05)$. Results for the saline paired days

indicated a similar trend with no effect of day $(F(3,30) = 1.12, p>0.05)$ and a significant

effect of sex (F(3,32) = 13.62, p<0.001) with females exhibiting higher locomotor activity. There was no significant effect of condition (F(3,32) = .84, p>.05) and no significant sex x condition interaction (F(1,32 = .61, p>.05). (Supplementary figures 2 & 3).

RT-PCR

There was no significant effect in the ventral midbrain (CART) of condition (F(1,54) = 1.41, p>.05) or drug (F(1,54) = 0.06, p>.05) or sex (F(1,54) = 1.60, p>.05). There were no significant interactions. There was no significant effect in the prefrontal cortex (NET) of condition (F(1,72) = 0.06, p>.05) or drug (F(1,72) = 0.32, p>.05) or sex (F(1,72) = 1.63, p>.05). There were no significant interactions. There was no significant effect in the nucleus accumbens (DAT) of condition (F(1,35) = 1.28, p>.05) or drug (F(1,35) = 0.43, p>.05) or sex (F(1,35) = 0.27, p>.05). There were no significant interactions. (Table 2).

CHAPTER 5

DISCUSSION

Adverse early childhood experiences, or early life stress, increases the risk for human substance use and abuse. Self report data indicate the degree of adverse childhood experiences correlates with risk for drug abuse. A high degree of reported adverse childhood experiences correlates with increased likelihood of beginning drug use (Dube et al., 2003). Animal models furthermore indicate early life stress alters drug related behaviors in adulthood. Compared to handled controls, separated animals exhibit increased sensitivity to cocaine (Brake et al., 2004). Separation and other early life stressors also potentiate the rewarding properties of alcohol, morphine, and cocaine in rodents (Vazquez, Giros, Daugé, 2006; Moffett, Vicentic, Kozel, Plotsky, Francis, Kuhar, 2007). This suggests early life stress alters neurological stress systems leading to long lasting susceptibility to drugs of abuse (Caldji et al., 1998; Liu et al., 1997; Meaney and Szyf, 2005). Alterations of these stress systems likely interact differently with varying drug of abuse. This study sought to determine the effect of early life stress on methamphetamine reward and locomotor activity, as well as to determine contributing neurological factors.

The results of this study indicated subjects exposed to methamphetamine formed a conditioned place preference for the drug paired chamber. Increased preference for the drug paired chamber is a measure of the reinforcing value of a drug and correlates with abuse liability in humans (Bardo & Bevins, 2000). Early drug exposures result in a pleasurable state and these pleasurable feelings reinforce drug taking behavior. Repeated exposure to a drug results in prolonged neurological changes perpetuating far beyond

drug cessation. These neurological changes lead to drug seeking behavior long after

recovery in both humans and animals (Nestler, 2001; Robbinson & Berridge, 2001).

Time spent in the drug paired chamber is conceptualized as an indirect measure of drug

seeking, suggesting rats that received repeated methamphetamine pairings exhibited a

place preference for the drug paired chamber.

Separated and control rats did not differ in the magnitude of methamphetamine place

preference. Previous findings indicate maternally separated rats show increased

vulnerability for rewarding properties of cocaine, alcohol, and morphine using self

administration paradigms (Vazquez, Giros, Daugé, 2006; Moffett, Vicentic, Kozel,

Plotsky, Francis, Kuhar, 2007). However, conditioned place preference reward remains

less characterized in maternal separation. Similar to the results of this study, Faure,

Stein, and Daniels (2010) reported no effect of maternal separation for methamphetamine

conditioned place preference (CPP). Several procedural differences exist between the

current study and that of Faure, Stein, and Daniels (2010). In both studies male and

female pups were separated for three hours per day. The current study separated pups

individually during post natal days (PND) 2-8 and tested male and female adult rats.

Faure, Stein, and Daniels however, separated pups as a group during PND 2-14 and only

tested male adult rats. The procedural discrepancies however still resulted in the same

behavioral outcome for methamphetamine CPP. This suggests early life stress does not

alter methamphetamine CPP in adulthood.

These results however, do not generalize to other drugs of abuse. Campbell and

Spear (1999) showed fifteen minute daily isolations decreased amphetamine conditioned

place preference compared to non handled controls. This suggests early brief isolations

decrease the rewarding properties of amphetamine in adulthood. The current study compared fifteen minute handled controls against three hours of separation and did not find an effect on conditioned place preference. Based on Campbell and Spear's results, the addition of a nonhandled condition may lead to significant differences in methamphetamine conditioned place preference. Additionally, animals separated for three hours per day during post natal day 1-14 exhibited greater and prolonged morphine conditioned place preference relative to standard factory reared animals (Vazquez, Penit-Soria, Durand, Besson, Giros & Daugé, 2005; Vazquez, Weiss, Giros, Martes, & Dauge, 2007). These results suggest early stress increases the rewarding properties of opiates.

The disparity between the aforementioned studies and the current findings may be a result of several factors. The effect of stress on drug reward varies depending on drug class, type of stress, and drug reward measure (Lu, Shepard, Hall, & Shaham, 2003). This suggests the disparity between this study and previous significant findings may be a result of pharmacological differences. Methamphetamine is reported as being more potent in human users and exerts greater effects on the dopamine transporter in vitro and in vivo (National Institute on Drug Abuse, 2006; Goodwin et al., 2009). Higher potency of methamphetamine likely leads to greater aversive properties, which may explain why a difference in methamphetamine place preference was not seen in separated, compared to control animals.

Additionally, procedural differences across early life stress paradigms may account for the disparities among studies. The length of neonatal separation as well as the group used as comparison controls may underlie these differences. Two weeks of neonatal separation may have a greater impact on conditioned drug reward, as observed with

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morphine (Vazquez et al., 2005), compared to one week of neonatal separation employed in the present study. However, Faure, Stein, and Daniels (2010) separated pups for two weeks and did not find an effect on methamphetamine conditioned place preference, suggesting length of separation may not mediate these effects.

Along with drug reward, discrepancies in psychostimulant-induced locomotor sensitivity also occur among neonatal separation paradigms. Kikusui, Faccidomo, and Miczek (2005) found increased cocaine locomotor sensitivity in maternally separated rats compared to handled controls. Conversely, Mathews et al. (2004) reported maternally separated animals had a decreased locomotor response to an acute injection of amphetamine. The current study indicated a trend towards increased locomotor activity in response to methamphetamine injections for separated male animals. Previous studies measured locomotor activity in an open field whereas this study measured locomotor activity in the context of conditioned place preference. The association of the drug and a particular environment (as in conditioned place preference) may differently affect locomotor activity compared to an open field. Future studies should examine the effects of early life stress on methamphetamine locomotor activity in the context of an open field. Additionally, locomotor activity should be examined over a broad range of doses of methamphetamine and with prolonged exposure to methamphetamine. From these types of studies, it can be distinguished whether early life stress affects locomotor sensitivity in a dose dependent manner and whether methamphetamine sensitization is altered by early life stress.

Maternal behavior likely mediates the effects of early maternal separation as dams spend more time licking and grooming when pups are returned after separation (Kosten

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& Kehoe, 2010). Most studies use artificially-created test all-male litters and remove all the pups from the dam for the entire length of separation (Mathews et al., 1996; Ladd et al., 1996; Brake et al., 2004). The current study left two to four pups with the dam during separation to reduce the stress experienced by the dam. This may have decreased the amount of time the dam spent licking and grooming the pups after separation leading to a blunted effect on drug reward in adulthood. Future studies should determine the extent to which the dams behavior mediates the effects of early separation.

Furthermore, varying patterns of maternal care likely contribute to alterations in neurological systems leading to variations in adult drug related behaviors. Drugs of abuse act on dopamine systems; in particular psychostimulants exert their mechanism of action on the dopamine transporter (DAT). Previous studies found that maternally separated animals exhibit decreased DAT levels in the nucleus accumbens using quantitative receptor autoradiography (Brake et al., 2004; Meaney et al., 2002). The decreased levels observed in Brake et al. (2004) may not be reflected in DAT mRNA levels in separated animals, as occurred in the current study. DAT levels appear to mediate the effects of drug reward whereas dopamine receptors may mediate locomotor activity. Dopamine D1, D2, and D3 receptors mediate psychostimulant locomotor activity (Depoortere, 1999), and these receptors are also altered by maternal separation (Brake et al., 2004; Vazquez et al., 2007). The current study found no indications of differences in methamphetamine reward but did find an effect of increased methamphetamine locomotor activity in separated males. Therefore, dopamine receptors would likely be altered rather than the dopamine transporter.

No significant changes were found in norepinephrine transporter (NET) expression in the prefrontal cortex. Lui et al. (2000) found decreased α2 adrenergic receptor density in the locus coeruleus of separated animals relative to handled controls. Additionally, decreased norepinephrine levels were found in the cingulate cortex of separated animals relative to non handled controls (Arborelius & Eklund , 2007). These studies suggest early separation alters brain norepinephrine systems but likely through mechanisms other than prefrontal NET expression. Additionally, decreased norepinephrine levels in the frontal cortex were found 24 hours after eight days of escalating methamphetamine self administration but returned to normal after 24 hours of cessation of methamphetamine (Krasnova et al., 2010). Because of the short-lasting effects of acute methamphetamine on norepinephrine systems, alterations in NET would not likely occur without prolonged administration of methamphetamine. The moderate (1.0mg/kg), intermittent doses of methamphetamine administered in the current study were likely not sufficient to cause alterations in NET expression. The current results suggest early life stress and acute methamphetamine administration in adulthood do not impact prefrontal cortex NET expression long-term.

Finally, no significant changes were observed in the ventral midbrain for expression of cocaine-amphetamine regulated transcript (CART). Few studies have examined CART in relation to methamphetamine (Morio, Ujike, Nomura, et al., 2006) and, to date no studies have reported CART expression after methamphetamine treatment. Previous studies have examined CART in relation to cocaine and, to a lesser extent, amphetamine administration (Douglass, McKinzie, & Couceyro, 1995; Vrang, Larsen, & Kristensen, 2002; Marie-Claire, Laurendeau, Canestrelli, Courtin, Vidaud, Roques, Noble, 2003).

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Variations in the pharmacological effects between amphetamine and methamphetamine may account for variations in gene expression (Goodwin et al., 2009). Furthermore, the effects of other psychostimulants on CART expression remain controversial. Originally, increased expression of CART was found after high dose administration of cocaine and amphetamine (Douglass, McKinzie, & Couceyro, 1995). Additional studies found CART expression does not always increase after cocaine or amphetamine administration, even at similar doses used by Douglass, McKinzie, and Couceyro (Vrang, Larsen, & Kristensen, 2002; Marie-Claire, Laurendeau, Canestrelli, Courtin, Vidaud, Roques, Noble, 2003). The discrepancies among these findings may further be due to dosing patterns. High-dose binge patterns of cocaine administration leads to increased expression of CART (Brenz Verca, Widmer, Wagner, Dreyer, 2001;Hunter, Vicentic, Rogge, & Kuhar, 2005). The low dose used in the current study was likely not enough to cause a change in CART expression. Future studies should look at CART expression in the context of a dose-binge pattern of methamphetamine administration.

Results of the current study suggest early life stress does not affect methamphetamine conditioned place preference but may alter methamphetamine locomotor sensitivity in males. Overlapping, but different brain systems likely mediate the effects of drug reward and drug-induced locomotor activity (Koob & Le Moal, 1997; Kalivas & Stewart, 1991). Conflicting findings among studies suggest the effects of early life stress on drug reward and sensitivity are variable (Kikusui, Faccidomo, & Miczek, 2005; Matthews et al., 2004; Vazquez et al., 2005). These inconsistent findings likely result from differences in drug class, variations among early life stress procedures, and paradigm used to measure drug reward and sensitivity.

Based on the results of this study, it is suggested that early life stress alters methamphetamine induced locomotor activity and does not influence methamphetamine conditioned place preference reward. Additionally, this effect is more robust in males compared to females.

Studies should further characterize the effects of methamphetamine sensitivity in relations to early life stress and the possible mediating effects of sex. Additionally, it will be important to model methamphetamine abuse patterns observed in the human population, including binge dosing. Maternal separation has previously been shown to alter neurotransmitter systems that are vulnerable to methamphetamine neurotoxicity caused by binge dosing. Therefore, future studies should further examine the effect of early life stress on sensitivity to methamphetamine neurotoxicity. These proposed future studies will further characterize the effects of early life stress on methamphetamine sensitivity. Clinically, this research will aid in targeting at risk populations and lead to improved interventions and treatments.

APPENDIX 1

SUPPLEMENTAL DATA

Table 1 Primer Sequences

Target	Forward Primer	Reverse Primer
Rat GAPDH	5'-CTCAACTACATGGTCTACATGTTCCA-3'	5'-CTTCCCATTCTCAGCCTTGACT-3'
Rat DAT	5'- TGACGCAGGAGTCAGTCGAAGAAGAA-3'	5'-TTTAGCCGGGGCCACCACTGA-3'
Rat NET	5'-CAGCACCATCAACTGTGTTACC-3'	5'-AGGACCTGGAAGTCATCAGC-3'
Rat CART	5'-AGTCCCCATGTGTGACGTGGA-3'	5'- GGAAATATGGGAACCGAAGGAGGC-3'

Table 2 RT-PCR Results

	Female				Male			
	CMeth	SMeth	SSaline	CSaline	CMeth	SMeth	SSaline	CSaline
PFC	9.55	10.34	10.43	9.80	10.25	9.58	10.56	9.47
N.Acc.	8.52	10.69	9.20	8.24	9.57	10.86	10.07	8.71
VTA	8.49	8.32	7.87	8.07	7.77	9.04	7.62	8.12

All genes were normalized to GAPDH and compared against the control group (non separated, saline). No significant differences were found for NET ($F_{(1, 36)}$ = 0.55, $p > 0.05$), DAT ($F_{(1, 35)}$ = 0.70, $p > 0.05$), or CART ($F_{(1, 37)}$ = 0.548, $p > 0.05$). Abbreviations: PFC (Prefrontal Cortex), N.Acc. (Nucleus Accumbens), VTA (Ventral Tegmental Area), C (Control), S (Separated).
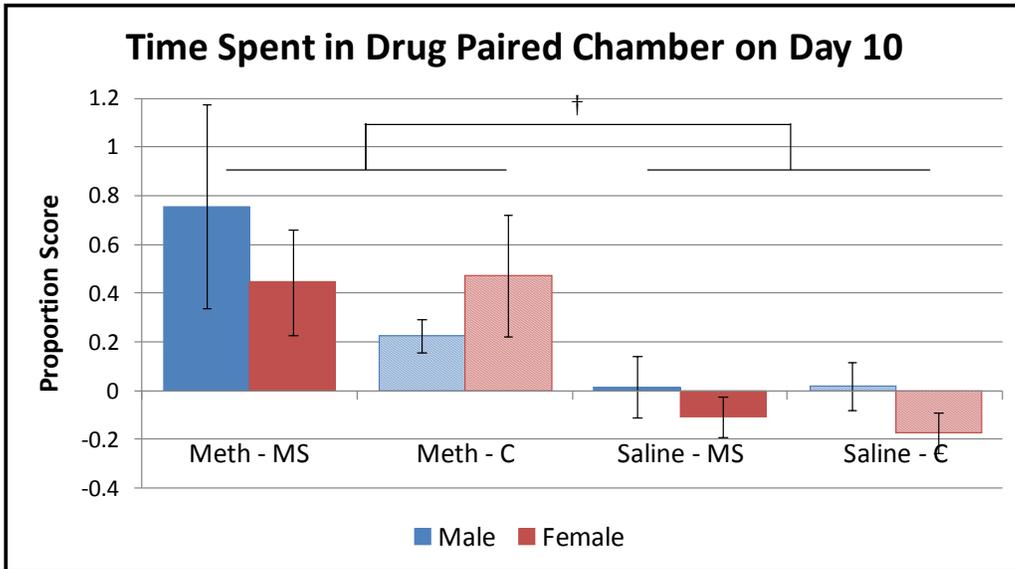
Figure 1

There was a significant effect for drug ($F(1,72) = 14.45$, $p<0.001$†) with methamphetamine animals spending more time in the drug paired chamber. There was not a significant effect condition ($F(1,72) = 1.56$, $p>0.05$) or sex ($F(1,72) = 0.830$, $p>0.05$). There were no significant interactions for the time spent in the drug paired chamber.
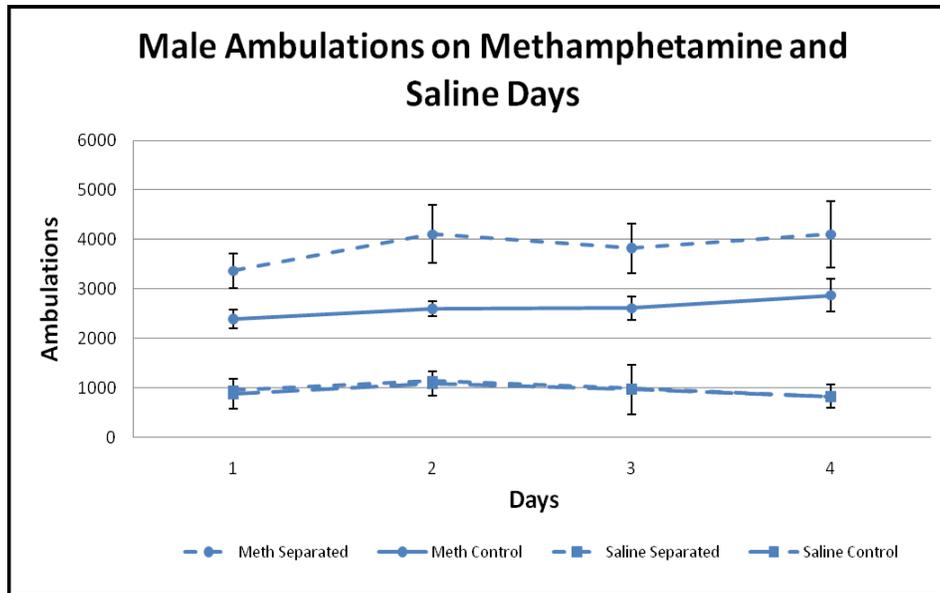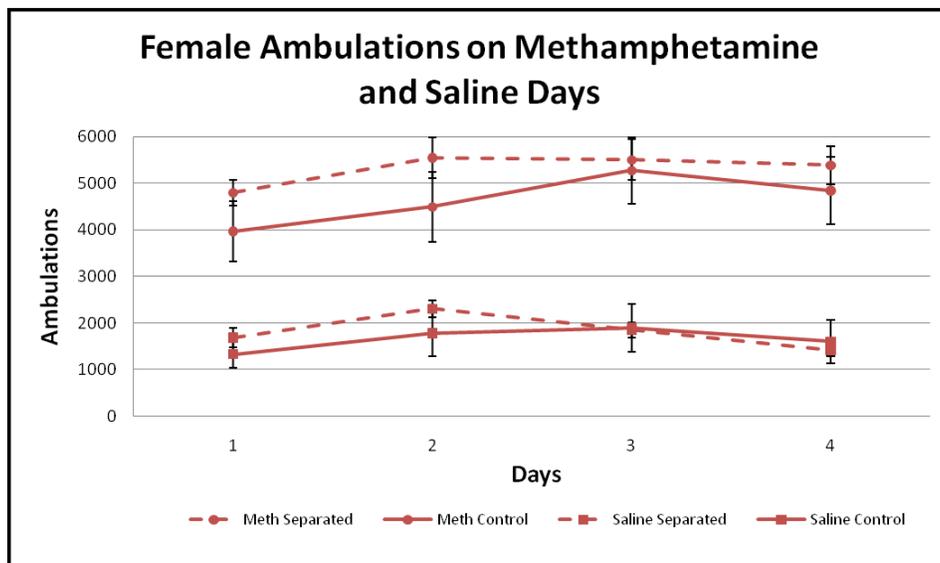
Figure 2

Figure 3

Total ambulations for male (Figure 2) and female (Figure 3) methamphetamine group across drug and saline days. For methamphetamine days (circles) there was a significant effect of sex $(F_{(1, 33)} = 11.43, p<0.001)$ with males (figure 2) exhibiting lower locomotor activity compared to females (figure 3). Condition was approaching significance $(F_{(3, 33)} = 3.57, p=0.068)$ with separated animals (dashed line) exhibiting higher locomotor activity. There was no sex x condition interaction $(F_{(1, 33)} = 0.054, p>0.05)$. For saline days (squares) there was a significant effect of sex $(F_{(1, 30)} = 13.62, p<0.01)$ with females exhibiting higher locomotor activity. There was no effect of condition $(F_{(1, 30)} = 0.84, p>0.05)$. There were no significant interactions.

IACUC APPROVAL

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PROTOCOL FOR ANIMAL CARE AND USE – PROTOCOL NUMBER: R0909-245

TITLE: The Effect of Early Environmental Manipulation on Locomotor Sensitivity and Conditioned Place Preference Reward for Methamphetamine

PRINCIPAL INVESTIGATOR: Laurel Pritchard

COMMITTEE ACTION:

X RECOMMENDED FOR APPROVAL AS SUBMITTED, DATE 10/8/09

☐ MODIFICATIONS NEEDED FOR FINAL APPROVAL, DATE

☐ RETURNED TO INVESTIGATOR FOR REVISION, DATE

☐ DISAPPROVED, DATE
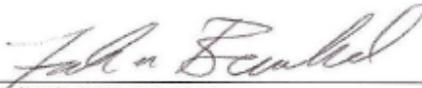
_____
IACUC CHAIRMAN
(or other designated IACUC member)

10/7/9
DATE

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