UNLV | UNIVERSITY LIBRARIES

UNLV Theses, Dissertations, Professional Papers, and Capstones

5-1-2014

The Effect of Early Life Stress on Methamphetamine Damage in the Striatum

Emily Hensleigh
University of Nevada, Las Vegas

Follow this and additional works at: https://digitalscholarship.unlv.edu/thesesdissertations

 Part of the Biological Psychology Commons

Repository Citation

Hensleigh, Emily, "The Effect of Early Life Stress on Methamphetamine Damage in the Striatum" (2014). *UNLV Theses, Dissertations, Professional Papers, and Capstones*. 2092.
http://dx.doi.org/10.34917/5836111

This Dissertation is protected by copyright and/or related rights. It has been brought to you by Digital Scholarship@UNLV with permission from the rights-holder(s). You are free to use this Dissertation in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you need to obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself.

This Dissertation has been accepted for inclusion in UNLV Theses, Dissertations, Professional Papers, and Capstones by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.

THE EFFECT OF EARLY LIFE STRESS ON METHAMPHETAMINE INDUCED DAMAGE IN THE STRIATUM

By

Emily Hensleigh

Bachelor of Science -- Psychology
Montana State University
2008

Master of Arts -- Experimental Psychology
University of Nevada, Las Vegas
2011

A dissertation submitted in partial fulfillment
of the requirements for the

Doctor of Philosophy -- Psychology

Department of Psychology
College of Liberal Arts
The Graduate College

University of Nevada, Las Vegas
May 2014

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

THE GRADUATE COLLEGE

We recommend the dissertation prepared under our supervision by

Emily Hensleigh

entitled

The Effect of Early Life Stress on Methamphetamine Induced Damage in the Striatum

is approved in partial fulfillment of the requirements for the degree of

Doctor of Philosophy - Psychology

Department of Psychology

Laurel Pritchard, Ph.D., Committee Chair

Jefferson Kinney, Ph.D., Committee Member

Daniel Allen, Ph.D., Committee Member

Merrill Landers, Ph.D., Graduate College Representative

Kathryn Hausbeck Korgan, Ph.D., Interim Dean of the Graduate College

May 2014

ABSTRACT

Methamphetamine (METH) abuse impacts the global economy through costs associated with drug enforcement, emergency room visits, and treatment. Hyperthermia is a leading cause of METH induced emergency room visits and may lead to neural damage. Previous research has demonstrated early life stress, such as childhood abuse, increases the likelihood of developing a substance abuse disorder. However, the effects of early life stress on neuronal damage induced by chronic METH administration are unknown. We aimed to elucidate the effects of early life stress on METH induced dopamine damage in the striatum. Animals were separated three hours per day during the first two weeks of development or 15 minutes for control. In adulthood, rats received either a subcutaneous 0.9% saline or 5.0 mg/kg METH injection every two hours for a total of four injections. Rectal temperatures were taken before the first injection and one hour after each subsequent injection. Seven days after testing, rats were euthanized and striatum was collected for quantification of tyrosine hydroxylase (TH) and dopamine transporter (DAT) content by Western blot. METH significantly elevated core body temperature in males and decreased striatal DAT and TH content and this effect was potentiated by early life stress. Females did not exhibit an effect of METH except in the elevated heat condition in the preliminary study, which significantly decreased DAT levels. We further ran a preliminary study looking at early life stress, METH dosing in adulthood during ambient (22-23°C) or elevated (27-30°C) temperature .Preliminary results indicated a replication of experiment one with no effect of elevated temperatures. These studies indicate maternal separation increases METH induced damage in males, and females are less susceptible to METH induced damage.

Acknowledgments: The author would like to acknowledge the following for their help in the projects: Kelly AbuAli, Matthew Eby, John Egan, Aisha Fowler, Ana Reyes, and the UNLV animal care staff. The author would also like to acknowledge the National Center for Research Resources (5P20RR016464-11) and the National Institute of General Medical Sciences (8 P20 GM103440-11) for funding the projects as well as the Graduate and Professional Student Association for funding conference travel. The author would like to thank her committee members: Dr. Merrill Landers, Dr. Daniel Allen, Dr. Jefferson Kinney, and lastly, her mentor, Dr. Laurel Pritchard, for her guidance on the projects and her experience and leadership throughout the author's graduate and future career.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF FIGURES vii

CHAPTER I INTRODUCTION 1

CHAPTER II CLINICAL DRUG USE AND METHAMPHETAMINE INDUCED DAMAGE 3
Methamphetamine Use and Abuse in Human Populations 3
Neural Changes Associated with Methamphetamine Use and Abuse in Humans 7

CHAPTER III MECHANISMS OF METHAMPETAMINE INDUCED NEURAL DAMAGE 11
Mechanisms of Action of Methamphetamine 11
Animal Models of Methamphetamine Use 13
Markers of Neural Damage in Animals 16
Contributing Mechanisms 20

CHAPTER IV STRESS AND DRUGS OF ABUSE 24
Neural Systems of Stress 24
Clinical Evidence of Stress and Drugs of Abuse 25
Preclinical Evidence of Stress and Drugs of Abuse 26

CHAPTER V ANIMAL MODELS OF EARLY LIFE STRESS 30
Paradigms 30
Behavioral Effects of Maternal Separation 32
Neural Changes 34

CHAPTER VI RATIONALE AND HYPOTHESES 37

CHAPTER VII METHODS 39
Experiment 1 39
Breeding 39
Separation 39
Methamphetamine Dosing 40
Experiment 2 41
Western Blots 42
Data Analysis 43

CHAPTER VIII RESULTS 44
Experiment 1 44
Body Weight 44
Rectal Temperature 45
Western Blotting 46
Experiment 2 49
Body Weight 49
Rectal Temperature 50
Western Blotting 52

CHAPTER IX DISCUSSION....56
Limitations & Future Directions....66

APPENDIX....69

REFERENCES....70

CURRICULUM VITA....86

LIST OF FIGURES

Figure 1	Body Weights Exp. 1	44
Figure 2	Temperature Males Exp. 1	45
Figure 3	Temperature Females Exp. 1	46
Figure 4	DAT in Striatum Males Exp. 1	47
Figure 5	DAT in Striatum Females Exp. 1	48
Figure 6	TH in Striatum Males Exp. 1	48
Figure 7	TH in Striatum Females Exp. 1	49
Figure 8	Body Weights Exp. 2	50
Figure 9	Temperature Males Exp. 2	51
Figure 10	Temperature Females Exp. 2	52
Figure 11	DAT in Striatum Males Exp. 2	53
Figure 12	DAT in Striatum Females Exp. 2	54
Figure 13	TH in Striatum Males Exp. 2	54
Figure 14	TH in Striatum Females Exp. 2	55

CHAPTER I

INTRODUCTION

METH use and abuse leads to daily societal issues. In subsequent pages the effects of METH will be described on an economic, societal, organismal, and molecular level. METH affects not only individuals using or abusing the drug but also families, law enforcement, and society. Over the past ten years many steps have been taken to alleviate these issues, but many problems persist today. Persistent problems associated with METH and other illegal drug use warrants a great deal of research to improve societal and individual problems. Identifying early warning signs and assisting at-risk populations remains a beneficial realm of study.

The need to understand the molecular and behavioral factors behind METH use and its effects has escalated as awareness of such problems has increased. As the METH problem propagates, more research is required to adequately address the variety of issues associated with acute and chronic METH use. Chronic METH use results in long-term neurological and behavioral deficits. These deficits occur primarily in dopaminergic and serotonergic neurons and in specific regions of the brain and are associated with several other markers of neuronal damage. The following will explain the biological and behavioral deficits associated with chronic METH use in clinical and preclinical studies.

This research aimed to characterize the effects of early life stress on METH induced neural damage. Study one examined the effects of repeated maternal separation, a model of early life stress, on METH induced dopaminergic neural damage in adulthood. Since elevated hyperthermia drastically affects the extent of neural

damage, study two examined the effects of early life stress on methamphetamine induced dopamine damage at elevated environmental temperatures.

CHAPTER II

CLINICAL DRUG USE AND METHAMPHETAMINE INDUCED DAMAGE

Methamphetamine Use and Abuse in Human Populations

Many amphetamines, such as d-amphetamine, methamphetamine (METH), and 3,4-methylenedioxymethamphetamine (MDMA), are used and abused as recreational drugs. Although dangers are associated with the overuse and abuse of all these substances, there is an increased concern for methamphetamine. METH is typically smoked in a dose-binge pattern, making the usage more dangerous relative to d-amphetamine, and more instances of neural damage have been observed in individuals who abused METH, rather than d-amphetamine or MDMA (reviewed in Gouzoulis-May & Daumann, 2009).

The typical route and pattern of administration for METH are unique among the amphetamines, leading to higher risk of cellular damage with METH use. D-amphetamine and MDMA are typically taken orally as pills whereas METH is typically smoked, leading to faster absorption and distribution and, consequently, rapid effects on the body and brain. Individuals who abuse METH take the drug in a dose-binge pattern, comprised of multiple doses over the course of 4 to 24 hours. This pattern of use leads to elevated blood plasma levels of METH which can lead to subsequent damage observed in human METH abusers (Cho, Melega, Kuczenski, & Segal, 2001). When this pattern of administration is modeled in animals, it produces significant reductions in the density of striatal dopamine terminals (see section II. C.).

Methamphetamine (METH) use and abuse remain continual problems in the United States and around the world. In 2010, the worldwide rate of amphetamines abuse was estimated between 0.3-1.2percent, second only to cannabis abuse (United Nations Office of Drugs and Crime, 2012). Over the past decade, the United States and several countries throughout the European Union increased penalties for making, selling, and distributing METH. Due to these increased penalties and subsequent man power directed towards METH trafficking, rates of METH use have begun to stabilize and, in some cases, slightly decrease throughout the U.S. and the world. However, rates of METH distribution and use have risen in other Asian, South American, and African countries. Although some areas of the world have been successful in diminishing distribution and use of METH, METH use persists at problematic levels throughout the world and United States.

METH is a growing problem in several countries, likely due to its ease of production relative to other amphetamines. METH is easily synthesized from pseudoephedrine by using common household items and readily obtained recipes from online sources. The United States, as well as countries in the EU, recently started enforcing laws limiting the sale of pseudoephedrine. However, pseudoephedrine is now smuggled into the United States in bulk for later distribution to METH laboratories. Additionally, the amounts of illegal METH coming into the United States from Mexico and other countries have increased over the past decade, which leads to increased funding required for drug enforcement (Cunningham et al., 2010).

The elevated use of METH over the past twenty years has contributed to increased economic burdens associated with illegal METH production, sales, and abuse. The production of METH occurs in homemade laboratories, which contain many harmful chemicals. These chemicals may lead to explosions and destruction of property. Additionally, the costs associated with cleaning and disposal of seized METH labs accounts for several millions of dollars spent yearly. Illegal imports, mainly from Mexico, of both METH and pseudoephedrine account for millions of dollars of border enforcement and DEA funding. Finally, the costs associated with emergency room visits, drug associated crimes, and drug treatment therapy accounts for billions of dollars annually (NIDA, 2010; Bureau of Justice, 2011). The economic burden associated with illegal METH production, distribution, and abuse remains a main issue in the United States.

Aside from the economic burdens, METH creates multiple problems on an individual level. Acutely, METH elevates body temperature resulting in increased risk of hyperthermia. Over half of all stimulant induced emergency room visits occur due to complications associated with elevated body temperature (NIDA, 2010). Furthermore, METH use often accompanies risky sexual behavior which puts individuals at an increased risk for contracting a sexually transmitted disease. This is particularly true for risk of HIV infection among homosexual men, a group in which METH use is more prevalent than in the general population (NIDA, 2008). Several health risks are associated with acute use of METH, adding to negative consequences already associated with the drug.

METH can also lead to several chronic physical health problems. Elevated heart rate during METH intoxication increases the risk for stroke due to hypertension (Ho et al., 2009). METH abuse of more than five years can lead to dopamine depletions (discussed in section B), resulting in symptoms similar to Parkinson's disease. These symptoms may be reversible, but recovery of normal motor activity can take several years (Callaghan, Cunningham, Sajeev, & Kish, 2010). Finally, long term use of METH can lead to dental decay (Hamamoto & Rhodus, 2009). Acutely, METH use causes a decrease in saliva production, similar to the dry mouth side effect of ADHD medications with amphetamine. Decreased salivary production increases the chance of plaque development. METH use also causes dehydration, and sugary drinks are commonly drunk by people who use METH. Individuals on a METH "run" or "binge" typically stay up for several hours and exhibit poor oral hygiene; METH also leads to bruxism and trismus which augment the oral deficits associated with METH use (Hamamoto & Rhodus, 2009). These factors combined lead to an increased rate of dental problems in individuals who abuse METH. Although direct evidence that METH causes dental decay is lacking, a higher rate of dental decay is observed in individuals who abuse METH.

Chronic mental health problems also persist in individuals who have abused METH for several years. Acute use of METH is associated with the onset of a first psychotic episode in individuals with family histories of schizophrenia. Long-term abuse of METH, anywhere from one to seven plus years, can lead to METH-induced psychosis, similar to that of schizophrenia including hallucinations, delusions, disorganized thought, and social withdrawal (Grelotti et al., 2010). Although these effects appear to

dissipate with time, the symptoms may reappear several years after abstinence from METH (Sato, Chen, Akiyama, & Otsuki, 1983).

Numerous deficits in cognitive function have also been demonstrated in individuals who have met the criteria for METH dependence for at least five years (Scott et al., 2007). Problems with working memory and recall were observed in patients who met the criteria for METH dependence and had been abstinent for 30 days (Johnason et al., 2006). Individuals also exhibit deficits in attention and working memory tasks for up to six months after maintaining abstinence (McKetin & Mattick, 1997). Some cognitive deficits, such as poor performance in the Wisconsin-card sorting task, appear to recover with long-term abstinence (over a year) but the extent of impairment is often correlated with the duration and frequency of use (Volkow et al., 2001).

Neural Changes Associated with Methamphetamine Use and Abuse in Humans

Multiple studies indicate several neural differences in the brains of chronic methamphetamine users and controls. Human studies show deficits in post mortem tissue samples as well as living patients via various imaging techniques. These differences remain fairly stable even after several months of abstinence.

Post mortem studies indicate decreased dopamine functioning in the brains of METH abusers. Wilson et al. (1996) examined the brains of deceased patients who previously met the criteria for substance dependence and died from causes unrelated to METH or other drug use. METH use was associated with significantly decreased levels of the dopamine transporter (DAT), tyrosine hydroxylase (TH), and dopamine in the

caudate putamen and nucleus accumbens relative controls that did not use METH or other drugs of abuse. DOPA decarboxylase and vesicular monoamine transporter (VMAT) protein levels did not vary between brains of METH users and age-matched controls, suggesting, METH may not lead to terminal damage but a decrease in expression of DAT and TH, depending on the duration and pattern of use. Other markers of neural damage have also been identified. Relative to matched controls, increased markers of oxidation were identified in the caudate nucleus and prefrontal cortex of individuals who met the criteria for METH dependence and had METH in their system at the time of death, suggesting oxidation occurs in humans which may be a result of METH administration (Fitzmaurice et al., 2006). Finally, looking at METH users who previously met criteria for substance dependence and had METH in their system at time of death, Kish et al. found decreased serotonin transporters in the prefrontal cortex and occipital cortex (Kish et al., 2009). These studies suggest METH may decrease dopamine terminals in areas of the striatum and serotonin terminals in areas of the cortex. However, chronic METH use may simply decrease expression of these proteins rather than leading to terminal damage.

Magnetic resonance spectroscopy (MRS) studies indicate cellular damage and decreased function in METH users. Long term METH users (met criteria for substance abuse) showed decreased markers of cellular integrity and function one year after abstinence. Specifically, lower ratios of N-acetyl aspartate (NAA) to creatine (CR) were observed in the frontal cortex and basal ganglia of recovering METH users relative to control patients (Ernst et al., 2000). Small NAA/CR ratios and low NAA are associated

with neuronal loss, decreased neuronal function, and disease states. A follow up study showed similar decreased NAA/CR ratios correlated with cognitive impairments and extent and frequency of METH use (Sekine et al., 2002). Finally, decreased NAA was observed in recovering METH patients diagnosed with HIV, and this pattern did not occur in matched HIV positive controls (Chang et al., 2005). These studies indicated reduced cellular function in abstinent METH abusers.

Positron emission tomography (PET) imaging studies indicate similar findings to the previously mentioned structural imaging in the brains of individuals who met the criteria for METH dependence relative to controls. PET studies indicate decreased dopamine transporters and D2 receptors in the striatum of one-month abstinent METH abusers. The extent of these deficits correlated with cognitive performance in that the greater the dopaminergic deficits, the worse individuals performed on cognitive tasks (Volkow et al., 2001a; Volkow et al., 2001b). METH abusers also show decreased metabolism in the prefrontal cortex at rest relative to control subjects. Decreased resting metabolism correlated with decreased performance in the Wisconsin Card Sorting task, a task highly dependent on prefrontal function (Volkow et al., 2001a). These studies indicate decreased markers of dopamine terminals associated with decreased performance in cognitive tasks. Based on these observations, it has been hypothesized that chronic METH use causes deficits in dopamine systems, which, in turn affect cognitive ability. The exact mechanisms of how dopamine deficits lead to decreased cognitive abilities remain widely unknown and need further study.

As with most human studies, several confounds exist. One glaring issue remains in determining a cause and effect relationship. Little clinical evidence exists indicating the structure and function of the brain of individuals before beginning drug use. Some of these findings may have been present before initiation of drug use and contributed to developing an addiction. Altered dopamine functioning occurs in many other disorders, such as attention deficit hyperactivity disorder (ADHD). Low doses of psychostimulants reduce symptoms of ADHD, such as cognitive deficits and inattention. Individuals with ADHD, or other dopamine associated disorders, may start self medicating with stimulants, eventually leading to the development of an addiction (Lakhan, Shaheen, Kirchgessner, & Annette, 2012). However, never-medicated patients with ADHD exhibit increased dopamine transporters in the caudate nucleus, suggesting the underlying dopaminergic variations in these disorders do not account for METH-induced changes. Although preclinical studies have made great strides in determining mechanisms for the observed deficits, we cannot examine *in vivo* effects of chronic METH use in drug-naïve humans. Therefore, it remains a challenge to determine the extent of neuronal damage caused by METH abuse.

CHAPTER III

MECHANISMS OF METHAMPHETAMINE INDUCED NEURAL DAMAGE

Mechanisms of Action of Methamphetamine

Researchers categorize methamphetamine as an amphetamine with two main isomeric forms. The l-enantiomer exhibits relatively low efficacy and is commonly mixed with the more efficacious d-isomer in therapeutic compounds. The d-enantiomer exhibits the highest efficacy and appears as a colorless powder or crystal (Mendelson et al., 2006). Illicit METH may appear dirty or yellowish due to its impurities and the process by which it was made (Cho, 1990). METH is sold legally in Europe as a mix of the l- and d-enantiomers for its therapeutic effects to treat ADHD. METH is also sold as a treatment for obesity and is an effective weight loss drug, likely due to its appetite suppressant effects (Schifano, Corkery, & Cuffolo, 2007). METH is readily soluble in water and may be consumed orally, intravenously, through mucus membranes, or smoked. METH reaches peak plasma levels around an hour after administration and has a half life of 7-8 hours (Cook, et al., 1993). METH is broken down hepatically and is excreted mainly in its original form. About 5-15% of METH is converted into amphetamine which acts as an active metabolite (Caldwell, Dring, & Williams, 1972).

Physiologically, METH stimulates the central and sympathetic nervous systems, which contribute to its therapeutic effects (Schneider, 1972). Acutely, METH elevates heart rate and blood pressure, increases respiration, dilates bronchia, decreases gut motility, and decreases salivary production (Kiyatakin et al., 2007). Acute administration

of METH also increases release of cortisol, the main stress hormone in humans, and elevated levels are observed several hours after administration of the drug (Harris et al., 2003).

METH exerts its biochemical effects on the monoamines: dopamine, serotonin, and norepinephrine. METH is lipophilic and readily crosses the blood brain barrier to enter the brain and reach its target proteins. *In vitro* studies indicate METH affects membrane bound dopamine transporters by binding to the same site as dopamine and reverses the proton gradient to pump dopamine into the synaptic cleft (Sulzer et al., 2005). Although METH likely interacts with norepinephrine and serotonin transporters in the same way, this has yet to be demonstrated. Moderate concentrations of METH can also lead to the internalization of the dopamine transporter leading to a reduction in the cell's ability to remove dopamine from the synaptic cleft (Li et al., 2010). Elevated levels of norepinephrine and serotonin are also observed at the synapse level after the administration of METH, however, the role of METH in dopamine release has been the neurotransmitter studied extensively. Interesting, METH releases norepinephrine to the greatest degree, followed by dopamine, and finally serotonin to the least degree (Han and Gu, 2006). Although METH has the greatest effect on norepinephrine, long-term effects on dopamine systems are more commonly observed relative to norepinephrine systems (Kish, 2008). Possible reasons for this effect will be discussed in section III C.

The chemical structure of METH, allowing for its high lipophilicity, allows METH to enter presynaptic terminal buttons through monoamine transport proteins or directly across the cell membrane (Mack & Bonisch, 1979). Intracellularly, METH interacts with

the vesicular monoamine transporters (VMATs), transport proteins which normally sequester cytosolic monoamines into synaptic vesicles. METH interacts with this protein to decrease the amount of monoamines taken into synaptic vesicles, thereby increasing the amount of monoamines in the cytosol (Fleckenstein, Volz, & Hanson, 2009). It is also thought that METH may reverse the proton gradient of the vesicular monoamine transporter and spill monoamines into the intracellular space (Partilla et al., 2006). However, the effect of reverse transport of VMATs by METH occurs at very high concentrations and has yet to be demonstrated at doses taken by typical METH abusers.

Animal Models of Methamphetamine Use

Clinical populations indicate several long lasting pharmacodynamic and cognitive changes associated with METH use. Clinical self-report data, as well as imaging and behavioral studies, have revealed several effects of acute and chronic METH use. However, human studies remain limited in fully understanding the cellular mechanisms which contribute to the effects of short term and long term METH use. Much of the research regarding molecular mechanisms of METH has been clarified with the aid of animal studies in primates and rodents. Rodents are easier to obtain and have shorter life spans relative to primates; therefore, the majority of studies have been performed on rodent models. This section focuses mainly on results from rodent studies. Rodent models of METH use fall into two main categories: acute effects of METH administration and chronic effects of METH administration. These areas of study examine behavioral,

neurochemical, and cognitive effects predisposing animals to self-administer METH and the effects after METH administration.

Studies of acute METH administration largely examine the neurobiological mechanisms of drug reward and factors contributing to METH self-administration. Several studies have observed the immediate increase in extracellular monoamines throughout the brain following METH administration. Dopamine release in the nucleus accumbens occurs once METH has entered the brain, rapidly after administration. This release is associated with the rewarding properties of METH as measured by self-administration and conditioned place preference. Blocking dopamine release in the nucleus accumbens diminishes METH induced drug reward indicating the involvement of this circuit in the rewarding effects of METH (reviewed in Sulzer, Sonders, Poulson, & Galli, 2005).

Since METH is a widely abused substance and may cause cognitive and neuronal deficits, much of the preclinical research has focused on effects of long term administration of METH rather than acute effects. Both primate and rodent studies, mainly rodent, have attempted to determine what neural and cognitive deficits occur due to repeated METH administration, whether these deficits are long-term or if they are recoverable, and more recently, what are the factors contributing to the observed deficits. Many of these early studies directly looked at behavior after high doses not resembling human equivalents. More recently, acute effects of METH are being determined using self administration studies rather than researcher administration or conditioned place preference measurements (Kraemer & Maurer, 2002).

Early studies focused on neural systems and neural deficits associated with METH administration. Many of these studies found damage to serotonin and dopamine systems. The damage observed lasted for several years and never fully recovered. However, one major issue with these studies was the large doses of METH used over short periods of time. The pattern of dosage and volume administered was not representative of METH use in humans. Early studies gave large bolus injections, such as two injections of 25.0 mg/kg METH (a very high dose), and looked at dopamine and serotonin markers days to weeks after administration (Seiden, Fischman, & Schuster, 1976). This approach did not allow for adequate comparisons to human populations.

Early research in animal models used high doses of METH as a model of Parkinson's disease. Parkinson's disease is a neurodegenerative disorder characterized by extensive loss of dopamine neurons in the substantia nigra that project into the striatum. High doses of METH, well beyond those observed in human populations, lead to extensive and irreversible neurotoxic damage to dopamine neurons (Perez-Reyes et al., 1991). Early research utilized this knowledge and used METH as a model of Parkinson's disease by injecting rodents or primates with large doses of METH, either subcutaneously (S.C.) or directly into the brain. These bolus doses lead to extensive dopamine cell death and specific Parkinson-like motor behavioral phenotypes. Motor deficits and neuronal damage have been very well documented, however, METH is no longer accepted as an appropriate model of Parkinson's disease for one key reason. METH use may lead to Parkinson's like motor deficits in human subjects and animal models because METH affects dopamine neurons. However, extensive METH use leads

to degeneration of dopamine axon terminals that may be somewhat reversible after several years of abstinence (Sonsalla, Jochnowitz, Zeevalk, Oostveen, & Hall, 1996). Parkinson's disease destroys entire dopamine neurons, rather than the terminal damage observed after long term METH administration. Although similar behavioral phenotypes exist between Parkinson's disease and extensive METH dosing, the cellular mechanisms appear to affect dopamine neurons differently. Importantly, several markers of METH induced damage are observed regardless of development of Parkinson's disease symptomologies.

More recently, studies focus on dose regimens similar to those observed in human users. As stated earlier, human uses administer METH in a dose-binge pattern. This usage pattern allows for elevated blood plasma levels of METH due to continual administration before METH is fully metabolized. The elevated METH plasma levels are thought to contribute to long-term neuronal effects. Animal models attempt to mimic the dosage pattern of humans by giving small to moderate doses of METH (1.0mg/kg-7.5mg/kg) during short windows of time (every two hours), roughly equivalent to those which occur in humans.

Markers of Neural Damage in Animals

Several METH induced neural changes occur in clinical populations of those individuals who have met the criteria for METH dependence. Similar deficits are observed in animal models following four to five doses of 3.0mg/kg-10.0mg/kg METH administration. Researchers began to examine the effects of METH on the central

nervous system in the 1970s, mainly in rodents and primates. With advanced imaging technology, human subjects corroborated many of the observed deficits. METH affects dopamine, norepinephrine, serotonin, glutamate, neuronal metabolism, and neuronal signaling.

Early studies on primates indicated decreased markers of monoamines and overall neuronal function after METH administration. After several high doses of METH (25.0-50.0mg/kg), rhesus monkeys and rodents exhibit decreased dopamine and serotonin levels in areas of the cortex (Seiden, Fischman, Schuster, 1976; Wagner et al., 1980). Decreased serotonin and dopamine synthesis rates were also observed in areas of the cortex and striatum. Synthesis rates were determined by examining the amount of rate-limiting enzymes (tryptophan hydroxylase and tyrosine hydroxylase), decreased serotonin and dopamine content in the cortex and striatum, and overall decreased dopamine turnover in the striatum and prefrontal cortex (Gibb & Kogan, 1979; Hotchkins, Morgan, & Gibb, 1979; Seiden, Commins, Vosmer, Axt, & Marek, 1988; Wagner et al., 1980). Decreased dopamine transporters and serotonin transporters were also observed after acute administration of METH (Ricaurte et al., 1982; Seiden, Commins, Vosmer, Axt, & Marek, 1988). Studies which looked at the lasting effects of METH indicate many of these deficits occur several months, even years (at least 7), after cessation from METH (Ricaurte et al., 1982; Johnson, Hanson, & Gibb, 1989; Brunswick, Benmansour, Tejani-Butt, & Hauptmann, 1992). These studies indicate METH results in significant decreases in markers of serotonin and dopamine systems, which can persist long term.

Although decreased markers of serotonin and dopamine systems are observed after METH dosing, it is difficult to conclude that METH leads to neurotoxic effects on dopamine terminals. Acutely, METH can cause intracellular sequestration of dopamine transporters for 24-72 hours (Kahlig & Galli, 2003; Fleckenstein, Metzger, Wilkins, Gibb, & Hanson, 1997). This effect stems from METH's direct effect on dopamine transporters in that the transporters are briefly sequestered in the cytoplasm to counteract the METH-induced over activity. Chronic METH dosing can additionally lead to decreased gene expression of the dopamine transporter. Decreased dopamine transporters likely lead to an inability of the neuron to remove dopamine from the synapse after increased dopamine release. This effect likely potentiates METH's effect on synaptic terminals (Xie et al., 2002). The effect on serotonin is largely uncharacterized, but likely is affected by similar mechanisms (Haughey, Brown, Wilkins, Hanson, & Fleckenstein, 2000).

Additional markers of synaptic integrity and synaptic structure support the idea that METH causes neuronal damage. Administration of both low (4.0mg/kg) and high doses (10.0mg/kg) of METH results in the degenerations of axon terminals in the nigrostriatal pathway. Silver staining techniques indicate terminal degeneration in the striatum of rodents after 4 doses of 4.0mg/kg METH (Ellison & Switzer, 1993). This effect is observed in a range of doses (3.0mg/kg-10.0mg/kg), is accompanied by elevated microglia activation, and is potentiated in elevated temperatures (Bowyer et al., 1994; Broening, Pu, & Vorhees, 1997; O'Callaghan & Miller, 1994). Different than the degeneration observed in Parkinson's disease, METH affects dopaminergic nerve terminals and leaves midbrain cell bodies intact, except in the case of extremely high

doses (Sonsalla, Jochnowitz, Zeevalk, Oostveen, & Hall, 1996). These studies suggest decreased dopamine markers are likely a result of terminal degeneration rather than decreased rate of protein synthesis or intracellular sequestration.

Early studies mainly focused on the damaging effects to dopamine systems in the striatum, but, more recently similar effects on terminal degeneration are observed in non-dopaminergic cortical regions. Degeneration of serotonin terminals is observed in areas of the frontal cortex after two administrations of 10.0mg/kg METH (Commins, Axt, Vosmer & Seiden, 1987). Aside from the observed serotonin degeneration, other cell types and areas of the cortex undergo terminal degeneration after METH administration. Using Fluoro-Jade staining, Eisch, Schmued, and Marshall (1998) observed decreased neuronal integrity in the somatosensory cortex after 4 injections of 4.0mg/kg every two hours. Additionally, several studies went on to demonstrate damage in the somatosensory cortex is observed several months after an increasing METH regimen and is potentiated during times of elevated temperatures and the production of reactive oxygen species (O'Dell & Marshall, 2000; O'Dell & Marshall, 2002; O'Dell & Marshall, 2005). The degeneration in cortical regions not only affects serotonin and dopamine terminals but occurs in several other cortical neurotransmitter systems including glutamatergic and GABAergic neurons (Eisch, O'Dell, & Marshall, 1996; Eisch & Marshall, 1998).

These studies indicate METH administration has profound effects on several neurotransmitter systems. METH not only alters dopamine signaling but has altered norepinephrine, serotonin, glutamate, and general cell signaling. The extensive effects

of acute and chronic METH administration indicate a vast array of cellular effects which alter brain activity both short and long-term.

Contributing Mechanisms

METH administration leads to several acute and chronic effects. Extensive administration (more than 6 months of use in humans, 4 moderate injections in animals) of METH results in decreased markers of dopamine systems which persist for months to years after abstinence. Morphological and neurochemical studies indicate these deficits occur at the cellular level and are not only changes in transcription or translation. Several mechanisms contribute to the deficits observed after METH administration. Several studies support the following mechanisms' contribution to METH induced damage: hyperthermia, oxidative stress, excitotoxicity, and, more recently, DNA damage, apoptosis, and microglia activation (see appendix for a summary of these effects).

Elevated body temperature, or hyperthermia, contributes greatly to the deficits observed after METH dosing. Alone, hyperthermia does not cause damage to dopamine systems. Rather, hyperthermia occurs as a result of multiple injections of METH. Elevated core body temperatures correlates with decreased dopamine transporters in the striatum of animals that received 5.0 mg/kg METH every 2 hours for 8 hours (Bowyer et al., 1994). Further elevating body temperatures during 4.0 mg/kg x 4 doses METH leads to enhanced damage to terminals in the striatum (Tata, Raudensky, & Yamamoto, 2007). Inhibiting hyperthermia during METH dosing attenuates damage to

dopamine and serotonin systems (Bowyer et al., 1994; Ali, Newport, & Slikker, 1996; Bowyer & Ali, 2006). Finally, compounds which exhibit protective effects against METH lower body temperature, but when core body temperature is elevated through environmental manipulations, these substances do not have beneficial effects. This suggests the effects of these compounds are likely due to their ability to attenuate a hyperthermic response rather than directly alter the effects of METH (Bowyer et al., 1994). These studies indicate core body temperature is a main mediator of METH induced neural damage. Controlling for hyperthermia attenuates METH induced damage but does not completely save these deficits. Some studies demonstrated neuronal deficits independent of hyperthermia, suggesting other factors aside from hyperthermia contribute to the long term effects of METH.

METH leads to increased dopamine release in the cytoplasm and synaptic terminal. Excessive amounts of dopamine leads to auto oxidation and the formation of reactive oxygen species. The excessive amount of reactive oxygen species produced burdens scavenger systems which lead to oxidative stress. Depleting dopamine before METH administration attenuates dopamine deficits in the striatum (Axt, Commins, Vosmer, Seiben, 1990; Seiden, Commins, Vosmer, Axt & Marek, 1988). Increasing dopamine availability before METH administration greatly increases damage to dopamine and serotonin systems in the striatum and cortex (Schmidt et al., 1991). Dopamine availability plays an important role in METH induced dopamine and serotonin damage which is likely due to auto-oxidation of dopamine leading to the overproduction of free radicals and oxidative stress.

METH not only leads to elevated monoamine release but increased firing of glutamate neurons (Abekawa, Ohmori, & Koyama, 1994; Stephens & Yamamoto, 1994). High glutamatergic activity, or extensive firing of one type of neuron, leads to increased intracellular calcium from extracellular space and mitochondria. Elevated intracellular calcium levels have several damaging effects to neurons and may lead to excitotoxicity and eventual death of the cell through several mechanisms. Blocking glutamate release after METH leads to protective effects to dopamine terminals in the striatum but not complete attenuation of damage to dopamine systems (Sonsalla, Albers, & Zeevalk, 1998; Sonsalla, Nicklas, & Heikkila, 1989). The effects of glutamate release on METH induced damage also occur independently of temperature and oxidative effects (Bowyer et al., 1994; Chipana, Torres, Camarasa, Pubill, Escubedo, 2008; Sonsalla, Riordan, & Heikkila, 1991). Extensive glutamate release likely contributes to the long term effects of METH on dopamine and serotonin systems but is not the sole mechanism of neuronal damage.

Evidence also suggests METH administration causes several other effects which contribute to cellular damage. METH administrations leads to microglia activation immediately following a dose, and microglia remain active for up to three days after METH cessation (Asanuma, Tsuji, Black, & Itzhak, 1999; LaVoie, Card, & Hastings, 2004). Microglia are part of the brain's immune response and become active after damage occurs in the brain. Thus, it is likely that METH administration induces cellular damage. The role of microglia in METH induced terminal damage remains unclear, but likely aids in cellular repair and damage immediately after METH administration (Thomas & Kuhn,

2005; Sriram, Miller, O'Callaghan, 2006). However, it has also been suggested that microglia contribute to neuronal damage. Other mechanisms by which METH-induced damage may occur include intracellular DNA damage are observed in dopamine neurons one week after METH administration (Jayanthi, Deng, Noailles, Ladenheim, & Cadet, 2004). Additionally, activation of several apoptotic pathways occurs directly after METH administration (Deng et al., 2002). Activation of these pathways likely results from damage to DNA, dysfunction of the endoplasmic reticulum due to reactive oxygen species, and dysfunction of the mitochondria due to elevated intracellular calcium levels (Cadet, Jayanthi, & Deng, 2003; Cadet, Jayanthi, & Deng, 2005; McCullough, Martindale, Klotz, Aw, & Holbrook, 2001). Additionally, hyperthermia likely potentiates these effects (Brown & Yamamoto, 2003). These emerging mechanisms likely play important roles in the basis of METH induced cellular damage.

METH induces cellular damage to dopamine and serotonin neurons through the interactions of several mechanisms. DNA damage, activation of apoptotic pathways, and microglia activation hours to days after METH administration indicates cellular distress. Markers of this damage include decreased dopamine and serotonin terminals, compromised neural integrity via degraded structural components, and lower dopamine and serotonin content. This damage remains stable for at least seven days after METH cessation. This damage occurs through the contributions of METH induced hyperthermia, oxidative stress, and exitotoxicity. However, the degree and role these mechanisms play in METH induced neuronal damage is not fully understood.

CHAPTER IV

STRESS AND DRUGS OF ABUSE

Neural Systems of Stress

External and internal stressors activate biological stress systems including the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. Various stressors activate these systems to equip the organism to handle subsequent acute and chronic stressors (McEwen, 2007; Tsigos & Chrousos, 2002). The extent of activation of these systems depends on the frequency and duration of the stressor (Levine, 2000). The effects of stress vary on an individual basis based on prior history, fitness, and extent of the stressor. In general acute, brief stressors tend to show mild benefit for the organism whereas chronic, prolonged stress or multiple stressors tend to show detrimental effects.

The physiological stress response consists of two main aspects, sympathetic nervous system activation and HPA-axis activation. In mammals, the presence of one or many stressors immediately activates the sympathetic nervous system. This activation happens within seconds and prepares the body to face the stressor or readies it to flee (Selye, 1936). The body releases epinephrine from the core of the adrenal glands which elevates heart rate, dilates pupils and air ways, and increases breathing rate. Endocrine signals are released shortly after activation of the sympathetic nervous system (Habib, Gold, & Chrousos, 2001). The hypothalamus releases corticotrophin releasing factor (CRF) in the anterior pituitary which releases adrenocorticotropin hormone (ACTH) into

the peripheral circulation. ACTH further signals the cortex of the adrenal glands to secrete the body's main stress hormone, cortisol in humans and corticosterone in rodents. Cortisol, or corticosterone, further prepares the body to deal with the stressor, chiefly by mobilizing glucose. Once released, cortisol binds to glucocorticoid receptors in the hippocampus and hypothalamus to signal the termination of this response. These two responses occur after the onset of a stressor and the duration and frequency of the activation depends on the duration of the stressor, the number of stressors, and prior histories (Selye, 1936).

Clinical Evidence of Stress and Drugs of Abuse

A physiological system tries to maintain homeostasis (or set point), however, with prolonged exposure to specific stimuli, this set point may reset. The physiological stress response aids in maintaining homeostasis and proves to be beneficial for the organism unless activated chronically or at multiple time points throughout the life history of the organism. Exposure to minor stressors for short periods of time aids in the regulation of the stress response and may be beneficial. Exposure to chronic or cumulative stressors alters the homeostatic set point and may be detrimental to the organism. A history of multiple, major life stressors is associated with several detrimental health effects. These deficits include decreased immune functioning, elevated heart rate, and insulin resistance. Research in humans indicates individuals in high stress careers and those who report higher amounts of cumulative life stressors show an increased risk of cardiac disease, type II diabetes, depression, and immune

disorders (McEwen, 2007). Elevated heart rate and respiration likely contribute to the elevated risk of heart disease and stroke, whereas increased glucose mobilization contributes to increased risk of type II diabetes.

Stress additionally increases an individual's sensitivity to drugs of abuse, increases the susceptibility of developing a substance use disorder, and contributes to maintaining an addiction. Higher rates of alcohol use and abuse occur in high stress rated careers relative to less stressful careers, while controlling for socio economic status (Brown, Vik, Patterson, Grant, & Schuckit, 1995). Individuals with histories of early life stress, such as childhood abuse or a diagnosis of PTSD, have a nineteen times higher likelihood of developing a substance abuse disorder (Dube et al, 2003; Saxon et al., 2001). These studies indicate life histories of stress are a risk factor for drug abuse. Stress further contributes to the maintenance of substance use disorders. Individuals diagnosed with a substance use disorder report relapsing to drugs of abuse during times of high stress (Kreek & Koob, 1998; Sinha, 2001). After an acute stressor, recovering cocaine addicts report more drug cravings relative to non-stressed controls (Volkow et al., 2002). These findings suggest stress influences the susceptibility, development, and maintenance of substance abuse.

Preclinical Evidence of Stress and Drugs of Abuse

Both acute and chronic stress interact with mechanisms of drugs of abuse. Although clinical evidence supports this claim, much of the knowledge elucidating neural mechanisms has occurred in animal models. Models of drug sensitivity, drug

reward, and drug craving indicate an effect of both acute and chronic stressors in relation to drugs of abuse.

Animal models indicate mild stress may be moderately beneficial by decreasing the rewarding properties of drugs of abuse, but chronic stress is detrimental. Relative to controls which received no manipulations, rats previously exposed to mild stressors, such as exercise, showed decreased conditioned place preference scores for amphetamine and morphine, suggesting mild stress may attenuate the rewarding properties of these drugs (Papp, Muscat, & Wilner, 1993; Valverde, Smadja, Roques, Maldonado, 1997). On the other hand, chronic, noxious stressors, such as foot shock, increased conditioned place preference scores for amphetamine and morphine relative to rats which did not receive any stress, suggesting chronic stress enhances the rewarding properties of these drugs (Will, Watkins, & Maier, 1998).

Rodents exhibit increased sensitivity to the initial rewarding properties of drugs of abuse after being exposed to a chronic stressor. Drug self administration is a common measure of drug reward in that animals are trained to press a lever to receive a drug infusion. Animals will self administer almost all drugs of abuse, including nicotine, opiates, cocaine, amphetamine, and methamphetamine. Rats acquire drug self administration more quickly when exposed to an acute stressor prior to training, suggesting an increased sensitivity to the rewarding and addictive properties of drugs of abuse (Kitanaka & Takemura, 2003; Robinson & Berridge, 2001). Rats also acquire amphetamine self administration more quickly after previous exposure to chronic stressors (Maccari et al., 1991). After exposed to an acute stressor, such as foot shock,

rats self administer more cocaine relative to those not exposed to stress (Ahmed & Koob, 1997). These studies indicate both acute and chronic stressors decrease acquisition time and elevate the amount of drug self administered.

Acute and chronic stressors also lead to reinstatement of drug seeking behavior. Drug self administration can be extinguished after multiple pairings of the original lever press with saline. Drug seeking behavior may be reinstated through injections of the original drug or exposure to stressors. Rats resume lever pressing for cocaine after extinction if given an acute stressor, suggesting stress increases drug seeking behavior in animal models (Ahmed & Koob, 1997). Chronic stressors, including intermittent random stressors and food deprivation, also lead to reinstatement of lever pressing and conditioned place preference for cocaine (Erb, Shaham, & Stewart, 1996; Lu, Liu, & Ceng, 2001). Finally, pharmacological chronic stress which induces immune system activation leads to reinstatement of methamphetamine lever pressing (Shepard, Shaham, & Morales, 2011). These studies indicate acute and chronic stress plays a role in drug seeking behaviors.

HPA-axis activation appears to play a critical role in the effects of stress on drug reward. Acute use of drugs leads to activation of the HPA-axis and several stress hormones are associated with drug seeking behavior. Pretesting corticosterone levels correlate with the amount of cocaine self administered (Carroll, 1985). Injecting glucocorticoids into adrenalectomized rats leads an enhanced sensitivity to the rewarding properties and locomotor activation properties of amphetamine (Deroche et

al., 1995). Blocking the release of corticotrophin releasing factor attenuates stress induced cocaine reinstatement (Shaham, Erb, Leung, Buczek, & Stewart, 1998).

The above studies indicate both behavioral and physiological cross sensitivity between stress systems and drugs of abuse. Additionally, stress early in life alters regulatory stress and reward systems leading to increased drug sensitivity.

CHAPTER V

ANIMAL MODELS OF EARLY LIFE STRESS

Paradigms

Early life stress greatly increases an individual's susceptibility to developing a substance use disorder. Children who are physically abused are nineteen times more likely to develop an addiction relative to non-abused children of the same socioeconomic status (Dube et al., 2004). Although this pattern emerges within clinical data, the underlying biological mechanisms remain unknown. Development of the HPA-axis occurs during the first two weeks of post natal development in rodents, analogous to the third trimester of fetal development in humans. The presence of the dam inhibits the stress response and her absence activates the HPA-axis (De Kloet, Rosenfeld, Van Eekelen, Sutanto, & Levine, 1988). Because of the mediating activity of the dam during this critical period, her prolonged absence results in continual activation of the HPA-axis. This prolonged activation leads to long term biological alterations which persist into adulthood (Vazquez, 1998). Three categorical animal paradigms used this concept to model early life stress in humans: maternal deprivation, maternal separation, and social isolation.

Maternal deprivation was one of the first procedures to attempt to emulate early life stress. Maternal deprivation removes the pups from the dam for a 24 hour period during the first week of development (Levine, 1988). Control litters are left with the dam. This paradigm was among the first developed and tested cognitive tasks and

sensitivity to opiates in adult animals. Maternal deprivation impedes physical growth, leading to deficits in weight gain in deprived groups. This model is no longer widely used, as it is impossible to attribute behavioral outcomes to the early life stress itself, rather than delayed physical development.

Maternal separation has replaced maternal deprivation as the more common paradigm. Using the same principles, these procedures separate pups from the dam for anywhere from one to six hours a day during the first week or two weeks during post-natal development. Separated litters do not exhibit stunted growth, but do exhibit distinct behavioral phenotypes in adulthood (Meaney, Brake, & Gratton, 2002). These effects are described in depth in the following section.

Stress does not have to occur during the first two weeks of post natal development to have long term effects. Social isolation studies the effects of stress during adolescence. Immediately after weaning from the dam, around post natal day 23, rats are housed in individual cages or pair housed as a control group. Isolated rats remain individually house throughout adolescent development until adulthood, around post natal day 60. Adult rats exhibit similar behavioral characteristics as maternally separated rats in that they exhibit anxiety-like behaviors (Lapiz et al., 1999). However, socially isolated also exhibit minor deficits in working memory and prepulse inhibition and therefore is typically used as a model for schizophrenia (Varty et al., 2000; Wilkinson et al., 1994).

Physiological, behavioral, and cognitive deficits occur after extensive lengths and durations (more than 1 hour per day for at least one week) of early life stress. This

indicates prolonged activation of the HPA-axis during critical stages of development leads to long term effects which persist into adulthood. These models allow us to better elucidate the effects of early life on long term development.

Behavioral Effects of Maternal Separation

The effects of prolonged stress during development lead to distinct behavioral phenotypes in adulthood, similar to problems observed in the clinical population. Human studies indicate early childhood stress is associated with several disorders, including depression, anxiety disorders, and substance use disorders. Animal studies indicate similar behaviors in that animals that have undergone early life stress show behaviors associated with despair, anxiety, and increased vulnerability to drugs of abuse.

Animals who underwent early life stress exhibit signs of anxiety and despair. Relative to controls, maternally separated animals exhibit decreased struggle time in the forced swim task and the tail suspension task, two model of despair (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2007; Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2008). Separated animals also fail to escape in a learned helplessness task, indicative of depressive-like symptoms (Vollymayr & Henn, 2001; Chen et al., 2001). Separated rats also exhibit anxiety-like behaviors in adulthood. Separated animals display increased freezing behavior and hyperactivity in response to a novel environment (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000; Kalinichev et al., 2002). In an elevated plus maze task, separated rats spend more time in the closed arms compared to controls (Aisa et al.,

2007). These depressive- and anxiety- like responses are similar to human studies which suggest early life stress is a risk factor for developing several disorders, including depression and anxiety disorders (Sadowski, Ugarte, Kolvin, Kaplan, & Barnes, 1999).

Aside from depressive- and anxiety- like symptoms, early life stress leads to sensitivity to the behavioral effects of drugs of abuse. Separated animals exhibit increased sensitivity to analgesic effects of morphine along with increased pain sensitivity after withdrawal from morphine (Vazquez, Giros, & Dauge, 2006). Separated animals show increased sensitivity to the locomotor activating effects of acute cocaine and amphetamine (Kikusui et al., 2005; Matthews, Hall, Wilkinson, & Robbins, 1996). However, separated animals do not exhibit long term locomotor sensitization to amphetamine across a range of doses (Brake et al., 2004; Weiss et al., 2001). This effect may have been due to procedural differences or suggest early life stress may affect the acute locomotor sensitivity but does not affect sensitivity long term. Additionally, separated animals may be maximally sensitized due to the effects of early life stress, such that they are unable to develop further sensitization to multiple amphetamine injections. Interestingly, early life stress sensitizes animals to the acute locomotor effects of METH at low and medium doses, which additionally may indicate an acute sensitivity rather than a long term effect (Pritchard, Hensleigh, & Lynch, 2012). These studies suggest early life stress has varying effects on sensitivity to behavioral effects of several drug classes.

A large field of evidence additionally supports the role that early life stress increases sensitivity to the rewarding properties of drugs of abuse. Separated animals

will self administer more morphine and spend more time in a chamber paired with morphine relative to controls (Herman & Panksepp, 1978). The acute rewarding properties of cocaine reward are additionally affected by early life stress. Cocaine self-administration is acquired more rapidly and at lower doses in separated animals relative to controls (Kosten, Miserendino, & Kehoe, 2000; Moffett et al., 2006). Separated animals further show sensitivity to the rewarding properties to an acute injection of amphetamine by increased electrical activity in the nucleus accumbens (Der-Avakian & Markou, 2010). However, early life stress did not alter METH reward in a conditioned place preference paradigm (Faure, Stein, & Daniels, 2011). One reason we might see increased sensitivity to the locomotor, but not the rewarding effects of METH may be that stress differentially affects systems underlying these two behaviors. However, several studies demonstrate early life stress sensitizes animals to the rewarding properties of other drug classes.

Neural Changes

Several neural alterations occur as a result of early life stress. These changes occur in several regions of HPA-axis function as well as dopamine systems. These neuronal alterations likely underlie the behavioral deficits observed in these animals.

Early life stress alters the physiological response to stress. Separated animals do not differ from controls in terms of basal circulating levels of the stress hormones: adrenocorticotropin hormone (ACTH) and corticosterone. After an acute stressor, separated rats show higher release of ACTH and corticosterone relative to control

animals, indicating a potentiated stress response (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2008; Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007). Separated animals also show prolonged elevated levels of stress hormones after an acute stressor indicating deficits with negative feedback shut-off of the HPA-axis (Aisa et al., 2007; Aisa et al., 2008). Additional studies indicate decreased glucocorticoid receptor density in the hippocampus of separated animals. These receptors are important for mediating the negative feedback signal (Plotsky et al., 2005). This decrease was likely an effect of prolonged activation of these receptors by heightened levels of corticosterone during the maternal separation procedure. In adulthood, separated rats exhibited increased mRNA CRF levels in the paraventricular nucleus indicating a possible compensatory mechanism due to the elevated stress hormone levels (Plotsky et al., 2005). Early life stress modifies many aspects of the HPA-axis including activation, release, and deactivation. These effects likely underlie many of the behavioral deficits observed in adult rats, such as depressive like symptoms and anxiety.

Dopamine systems are heavily tied to the rewarding and sensitizing effects of drugs of abuse and early separation greatly alters these systems. Separated animals show decreased dopamine transporter and D3 receptor mRNA and protein levels, as well as increased stress-induced dopamine release, in the nucleus accumbens (Brake et al., 2004; Meaney et al., 2005). Furthermore, increased dopamine release was observed in the nucleus accumbens after cocaine administration. These combined effects likely underlie the enhanced rewarding properties of drugs due to increased dopamine in the synaptic cleft and a decreased reuptake capacity. Post synaptic changes also occur.

Separated animals display increased D1 and D2 receptors in the nucleus accumbens, likely as a compensatory mechanism due to elevated dopamine levels (Moffett et al., 2006). Although it is clear early life stress alters reward circuits, it remains unclear exactly how this occurs. It likely results from interactions with the over activation of the HPA-axis during development, but is not well characterized.

A large body of research supports the role of early life stress in altering stress and reward circuits. Several behavioral alterations are observed in these animals which are likely due to these long term biological changes. However, to fully understand early life stress' function in behavior changes, further research is required. The alterations in dopamine systems may affect susceptibility to METH induced damage, however, this effect has yet to be examined in the maternal separation model.

CHAPTER VI

RATIONALE AND HYPOTHESES

METH use may result in long-term neuronal and cognitive deficits which may be potentiated by stress. In the same regard, early life stress is a major risk factor for developing a substance abuse disorder. Animal models indicate early life stress leads to sensitivity to drugs of abuse, including METH. It is likely early life stress alters several neurological circuits which may result in an increased susceptibility to METH induced neuronal damage. The studies aimed to characterize the effect of early life stress on METH induced dopamine damage in the striatum.

In the first study, separated or control rats received four injections of 5.0mg/kg METH over a period of eight hours in adulthood. Temperature was monitored before the first injection and one hour after every METH injection. The striatum was dissected out one week after the METH dosing for protein quantification of dopamine transporters (DAT) and tyrosine hydroxylase (TH). A low to moderate dose of METH was chosen to detect between group effects and avoiding a ceiling effect. It was hypothesized METH would elevate core body temperature but not above stroke-inducing levels (40°C). Two markers of dopamine neurons were selected to determine the effects of METH on dopamine function. METH leads to down-regulation of DAT, which contributes to terminal damage due to the decreased ability to remove dopamine from the synaptic cleft. It was hypothesized that in METH treated rats, early life stress would lead to decreased levels of DAT and TH relative to control litters.

The second preliminary study examined the effects of early life stress on METH-induced dopamine damage at elevated temperatures. Study two was similar to study one except another group of elevated temperature rats were tested (see methods for more in-depth description). METH use commonly occurs in settings with elevated temperatures, such as raves, and METH abusers typically do not monitor their temperature unless they require medical attention. Additionally, early life stress may lead to problems with thermoregulation. This may occur due to impaired function of HPA-axis and immune system function. Since hyperthermia is commonly associated with METH use and is a main contributor to METH induced neuronal damage, it is vital to understand early life stress' impact on these effects. In study two, it was hypothesized that early life stress would decrease DAT and TH in the striatum and this effect would be potentiated by elevated temperatures.

CHAPTER VII

METHODS

Experiment 1

Breeding

A total of eighteen Long-Evans breeding rats (Harlan) were pair housed by same sex and allowed a one week habituation to the facility before breeding. Breeding pairs were used for both studies. Breeding pairs were housed together in hanging wire cages and monitored for the presence of a vaginal plug for up to five days. Pairs were separated upon visualization of a vaginal plug and returned to plastic tub cages. Males were returned to their same sex cage mate and females were moved to a single cage and monitored for pregnancy. If no plug was present after five days, pairs were separated and later paired with a different mate. Pregnant females remained untouched except for cage changes until birth of the litter. A total of 148 rats (experiment 1 = 90; experiment 2=56) derived from 14 litters of 10-12 pups, were used in the studies.

Separation

Separation and control procedures began after birth. The day of birth was designated post natal day (PND) 0 and litters remained untouched until the next day. On PND 1, the pups were sexed and either fostered or culled to roughly equal litter sizes. Fostered pups were rolled in the cage bedding of a litter born within one day of the original birth litter and monitored for aggressive behavior from the foster dam. Litters

underwent pseudo-random assignment into a control or separated groups to achieve roughly equal numbers for each group. During PND 2-14, control litters were removed from the dam and weighed, with this procedure lasting approximately fifteen minutes each day. Separated litters were removed from the dam, weighed, and individually separated in plastic containers for three hours per day during PND 2-14. Separated pups stayed on a heating pad, maintained at a constant temperature of 32°C, and were checked every 30 minutes for signs of hyperthermia or hypothermia. After the three hour separation period, pups were returned to the dam. Identification of individual pups occurred by use of a non-toxic pen until PND 6-7, at which point ear punches were used for identification. Pups remained untouched, except for weekly cage changes, with the dam during PND 15-20. Weaning of pups occurred on PND 21, at which time pups were weighed and housed in same sex pairs. Starting on PND 40, pups were handled daily until the start of testing between PND 60-70. A total of 56 rats were used in the preliminary study.

Methamphetamine Dosing

Methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile 0.9% saline at a concentration of 5.0mg/ml (expressed as salt weight). Between PND 60-70 animals received saline or methamphetamine (METH) injections over an eight hour period. Core body temperature measurements were taken every two hours starting between 8:00am-8:30am. A thermocouple probe (Fluke) was inserted approximately five to six millimeters into the rectum for five to ten seconds to obtain baseline temperature. Rats then received one subcutaneous injection of 5.0mg/kg

METH or 0.9% saline (1.0ml/kg) every two hours for a total of four injections. Rectal temperatures were taken one hour after every injection of saline or METH. If the core body temperature exceeded 40°C, animals were cooled by use of a washcloth and ice until core body temperature dropped below 39°C, at which point no further injections were given and rats were monitored for the remainder of the day. After the final temperature measurement, rats were returned to their home cages and left undisturbed for seven days. After seven days, rats underwent euthanasia by anesthetic overdose of *Somnasol* (390 mg pentobarbital and 50 mg phenytoin). The brain was dissected out and the striatum was collected by use of a rodent brain matrix and a 0.3mm micropunch. Tissue samples were placed in *Eppendorf* tubes and immediately placed on dry ice, followed by storage at -80°C until quantification by Western Blot. Sacrifice seven days after METH administration was selected to determine the effects of MS on terminal damage as opposed to acute regulation of dopamine systems. METH acutely leads to the sequestration of DAT in the cytosol 24-72 hours after METH administration, but levels return to normal after this time period (Kristopher, Kahlig & Galli, 2003). Taking brain tissue seven days after METH administration gave an accurate picture of METH's enduring effects on dopamine systems.

Experiment 2

All procedures occurred as described above except with an elevated temperature condition. Animals were pseudo-randomly assigned to one of two temperature conditions: ambient or heat. Animals in the ambient condition stayed at a

constant ambient temperature of 22-23°C throughout the injection time. Animals in the heat condition stayed at an elevated temperature of 27-30°C throughout all injections by use of a heating pad placed under the cage monitored by a thermometer placed in the cage.

Western Blots

Tissue samples thawed on ice and were homogenized in RIPA buffer using a rotor and pestles. Samples were centrifuged at 12,000g for 20 minutes. The supernantant was aliquoted into *Eppendorf* tubes and stored at -80°C. Each sample underwent a bicinchoninic acid protein concentration assay (Pierce, Rockford, IL) followed by western blot. Following heat denaturing, samples were loaded into 12% polyacrylamide gels with 10µg protein per lane. Samples were transferred to PVDF membranes and blocked for 3 hours in 5% milk in Tris-buffered saline (TBS). Membranes were washed 3 x 5 minutes and incubated in primary antibody for β-actin anti-mouse (1:30,000; Proteintech), dopamine transporter anti-goat – DAT (1: 1,000; Santa Cruz), and tyrosine hydroxylase anti-mouse – TH (1:1,000; Millipore) in 5% milk in Tris-buffered saline with Tween 20 (TBST) over night. Membranes were washed 3 x 10 minutes in TBST followed by IR-Dye secondary anti-bodies, goat or mouse (1:5,000; Li-Cor) incubation in 5% milk in TBST for 1 hour. Membranes were washed 3 x 10 minutes in TBS and imaged by use of an Odyssey imager. Fluorescent signals are directly proportional to the amount of the target protein. These values were normalized to β-actin and reported as percentage of control group (control-saline animals).

Data Analysis

For experiment one rectal temperature data were analyzed using two mixed model ANOVAs with between subjects variables as condition (separated x control) and drug (saline x meth) and within subjects variable as time (baseline, 1 hour, 3 hours, 5 hours, 7 hours). Separate ANOVAs were run for males and females. For preliminary experiment two, rectal temperature data were analyzed using two-mixed model ANOVAs with between subjects variables as condition (separated x control), drug (saline x meth), and temperature (ambient x heat) and within subjects variable as time (baseline, 1 hour, 3 hours, 5 hours, 7 hours). Western blotting data were analyzed by use of the Odyssey imaging program. Fluorescent intensity for each band was normalized to β-actin and reported as percentage of control (control-saline males or control-saline females). For experiment one, data were run through a 2x2 between-subjects ANOVA with between-subjects factors as: condition (separated x control) and drug (saline x METH). Two separate ANOVAs were run for dopamine transporter and tyrosine hydroxylase. For the preliminary experiment two, data were analyzed using the Aligned Rank Transform (Wobbrock et al. 2011). This approach allows for factorial analysis of nonparametric data, including interactions between factors, by first applying a transform that aligns data for each effect, then ranking the data points. The aligned rank data are then subjected to a factorial ANOVA. Significant interaction effects were followed up with simple effects analysis, and significant dose effects were followed by Fisher's least significant difference post hoc tests. Significance was set at $\alpha=0.05$.

CHAPTER VIII

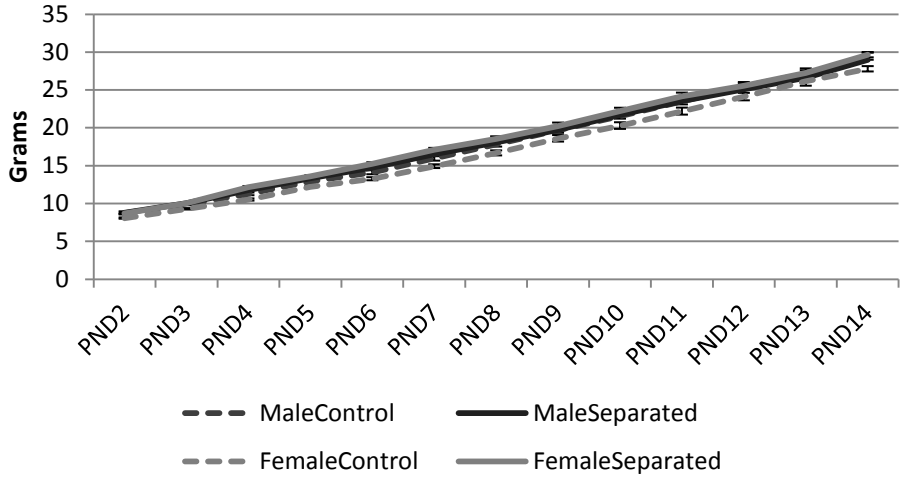
RESULTS

Experiment 1

Body Weight

A repeated-measures ANOVA was run for body weights during the first two weeks with the within subjects factor as day and the between subjects factors as condition (control x separated) and sex (male x female). There was a significant effect of day ($F_{12,360}$=1,568, p<0.001) with weight increasing over the first two weeks, and no significant effects of condition, sex, or significant interaction (figure 1). Error bars represent standard error.

Figure 1: Body Weights Exp. 1



Rectal Temperature

Two repeated-measures ANOVAs were run for males and females for temperature during drug administration. The within-subjects factor was time (1, 2, 3, 4, 5) and the between-subjects factors were condition (control x separated) and drug (saline x meth). For males, there was a significant effect of time ($F_{4,80} = 27.13$, $p<0.001$), a significant time x drug interaction ($F_{4,80} = 5.48$, $p<0.001$) and no other significant within-subjects effects. There was a significant effect of drug ($F_{1,83} = 15.09$, $p<0.001$) with methamphetamine increasing rectal temperature. There were no significant effects of condition or a condition x drug interaction (figure 2). For females, there was a significant effect of time ($F_{4,66} = 17.86$, $p<0.001$) and no other significant within-subjects interactions. There were no significant between-subjects effects of condition or drug (figure 3). All error bars represent standard error.

Figure 2: Temperature Males Exp. 1

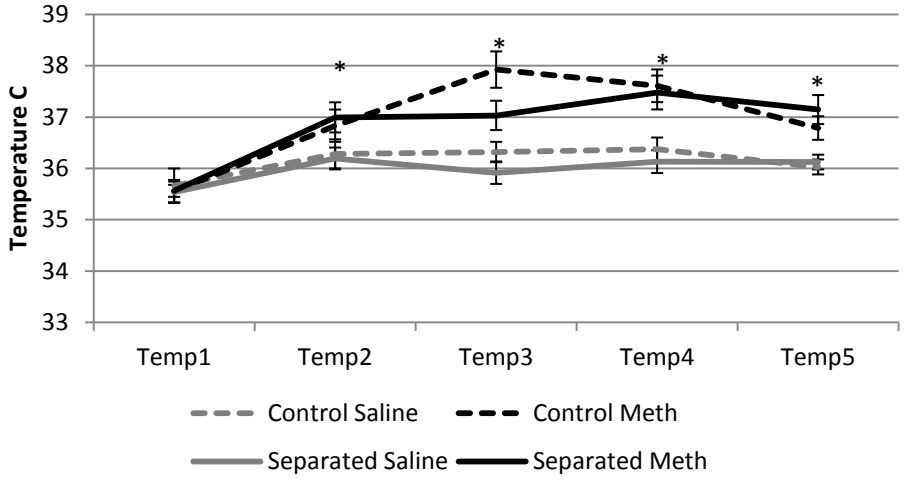
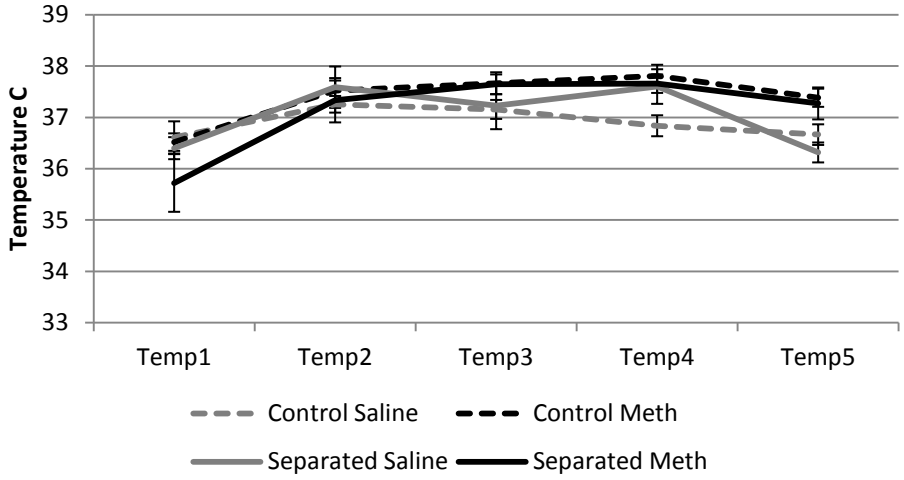


Figure 3: Temperature Females Exp. 1

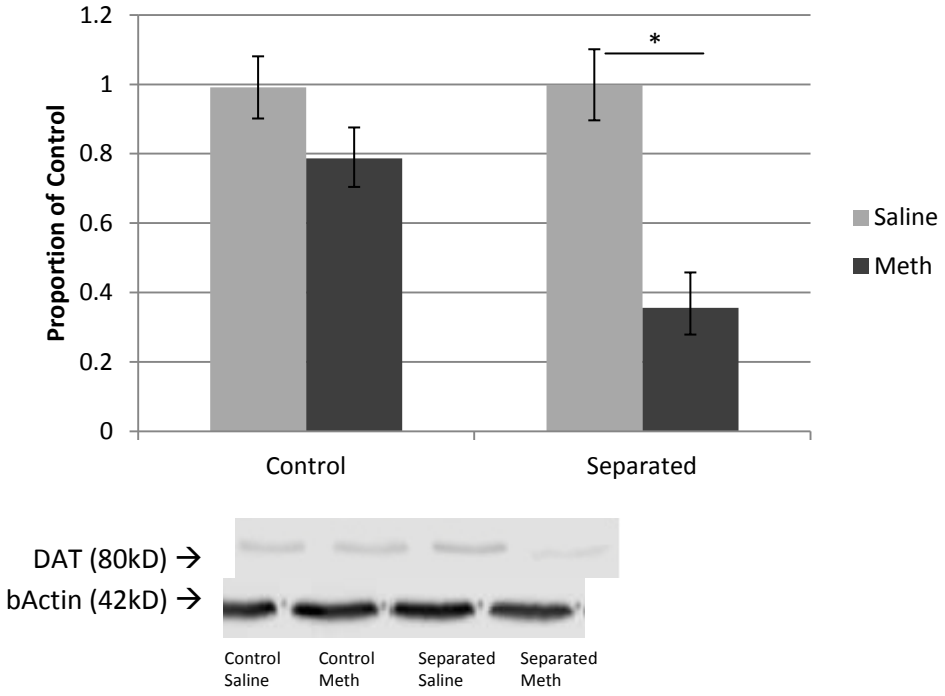


Western Blotting

For DAT levels, two separate 2 x 2 between-subjects ANOVAs were run for males and females, and the same was run for TH levels. Between-subjects factors were condition (control x saline) and drug (saline x meth). For DAT in males, there was a significant effect of drug ($F_{1,40} = 32$, $p<0.01$), condition ($F_{1,40} = 9.08$, $p<0.01$), and condition x drug interaction ($F_{1,40} = 8.4$, $p<0.01$). A test of simple effects revealed that the effect of drug on DAT levels was significant for separated animals ($F_{1,37} = 36.8$, $p<0.01$) but not for control animals ($F_{1,37} = 3.63$, $p>0.05$) (figure 4). For females, there was no significant effect of drug, condition, or a drug x condition interaction (figure 5). For TH in males, there was a significant effect of drug ($F_{1,44} = 7.18$, $p<0.05$) with methamphetamine decreasing TH. There were no significant effects of condition or a drug x condition interaction (figure 6). For TH in females, there were no significant

effects of drug, condition, or drug x condition interaction (figure 7). All error bars represent standard error.

Figure 4: DAT in Striatum Males Exp. 1



Note: all future western data will be presented in this order.

Figure 5: DAT in Striatum Females Exp. 1

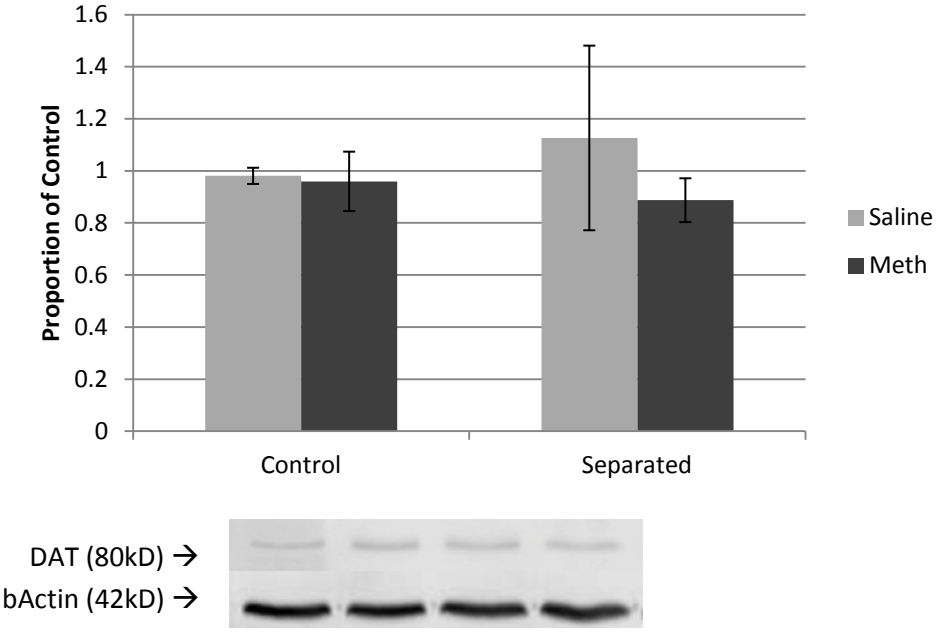


Figure 6: TH in Striatum Males Exp. 1

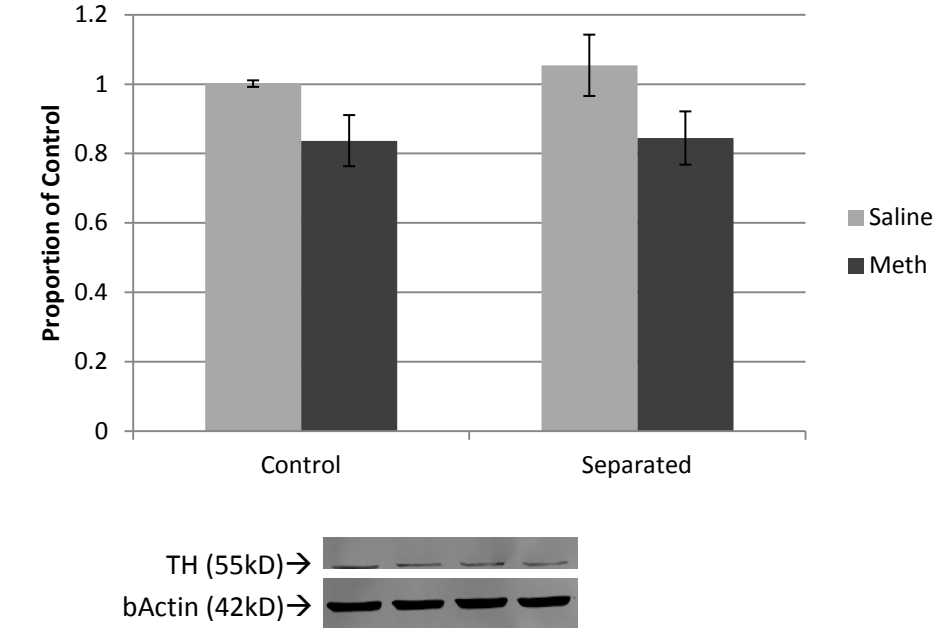
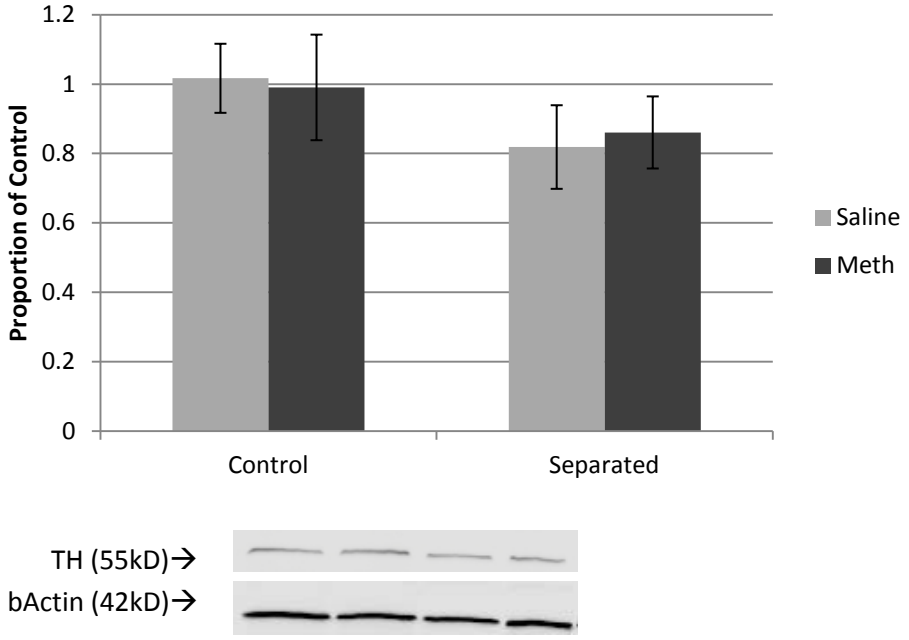


Figure 7: TH in Striatum Females Exp. 1

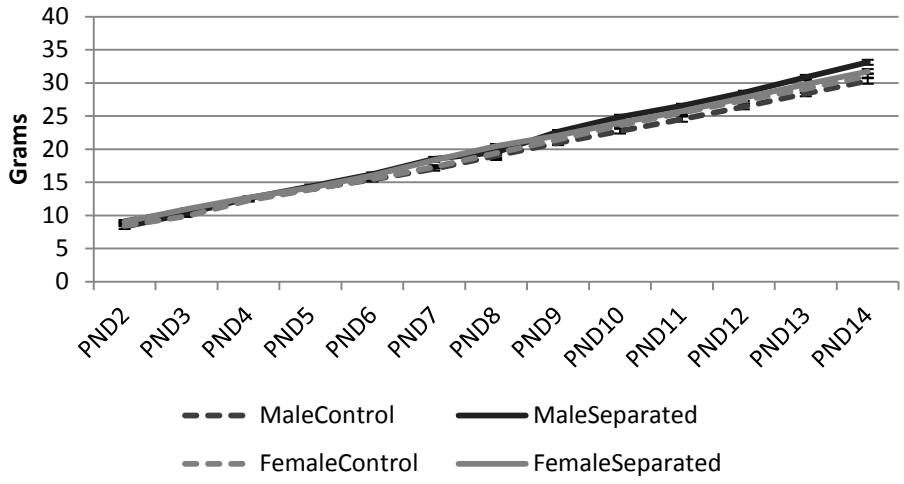


Experiment 2

Body Weight

A repeated-measures ANOVA was run for body weights during the first two weeks with the within subjects factor as day and the between subjects factors as condition (control x separated) and sex (male x female). There was a significant effect of condition, with weight being higher in separated animals ($F_{1,65}$=8.9, p<.01) (figure 8). Body weights did not differ at time of testing. Error bars represent standard error.

Figure 8: Body Weights Exp.2



Rectal Temperature

Two repeated-measures ANOVAs were run for males and females for rectal temperature during drug administration. The within-subjects factor was time (1, 2, 3, 4, 5) and the between-subjects factors were condition (control x separated), drug (saline x meth), and temperature (heat x ambient). For males, there was a significant effect of time ($F_{4,17} = 6.35$, $p<0.01$) and a significant time x drug interaction ($F_{4,17} = 4.9$, $p<0.01$) and no other significant within-subjects interactions. For between-subjects effects, there was a significant effect of drug ($F_{1,20} = 9.66$, $p<0.01$), with meth increasing core temperature and a significant condition x drug interaction ($F_{1,20} = 4.51$, $p<0.05$). There were no significant effects of condition, temperature, or other significant interactions (figure 9). For females, there was a significant effect of time ($F_{4,23} = 12.01$, $p<0.01$), a significant time x drug interaction ($F_{4,23} = 4.08$, $p<0.01$), a significant time x temperature interaction ($F_{4,23} = 3.72$, $p<0.05$), and a significant time x drug x temperature interaction

($F_{4,23}$ = 2.91, p<0.05). Only females maintained at elevated temperature (heat) showed elevated core body temperature at times 3, 4, and 5. There were no other significant within-subjects interactions. For between-subjects effects, there was a significant effect of drug ($F_{1,27}$ = 11.63, p<0.01), with meth increasing core temperature, a significant effect of temperature ($F_{1,27}$ = 14.77, p<0.01) and a significant drug x temperature interaction ($F_{1,27}$ = 5.56, p<0.05). There were no significant effects of condition or other significant interactions (Figure 10). All error bars represent standard error.

Figure 9: Temperature Males Exp. 2

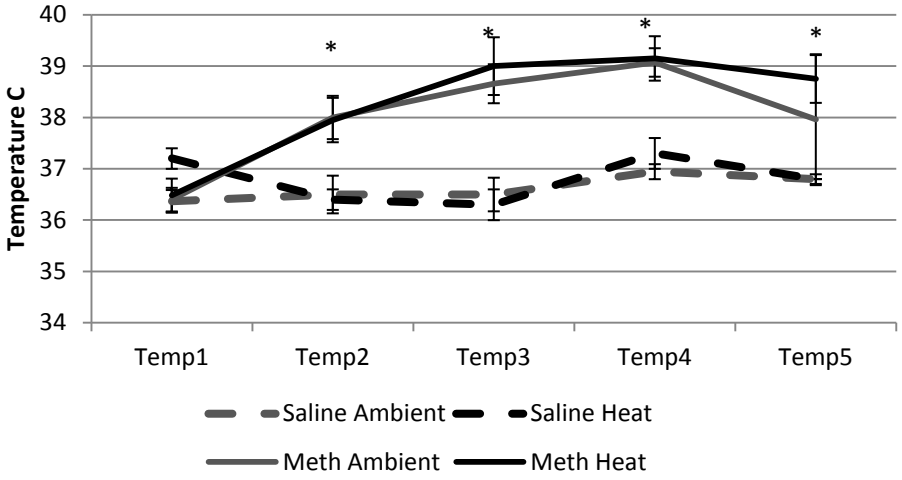
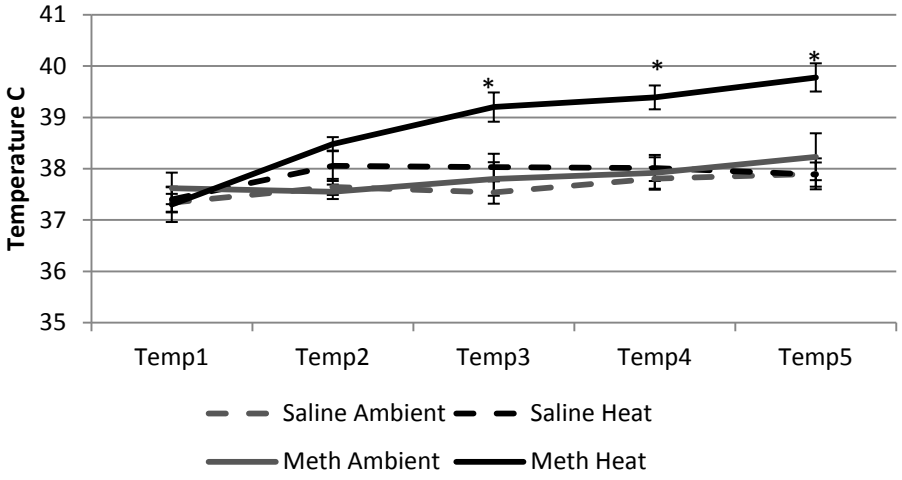


Figure 10: Temperature Females Exp. 2



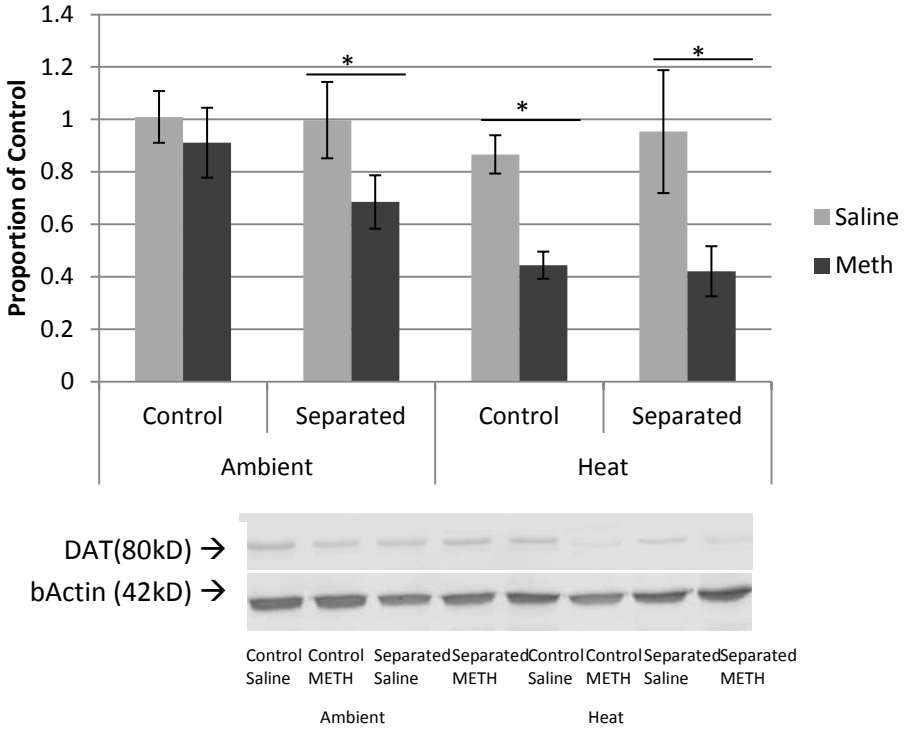
Note: Values are collapsed across condition

Western Blotting

For DAT levels, values were transformed using the aligned-rank approach followed by two separate 2 x 2 x 2 between-subjects ANOVAs for males and females, and the same was run for TH levels. Between-subjects factors were condition (control x saline), drug (saline x meth), and temperature (ambient x heat). For DAT in males, there was a significant effect of drug ($F_{1,24}$ = 12.11, p<0.01) with meth decreasing DAT levels and a significant effect of temperature ($F_{1,24}$ = 10.48, p<0.01) there was no significant effect of condition or significant interactions (figure 11). For DAT in females, there was a significant effect of drug ($F_{1,23}$ =15.1, p<0.01) and temperature ($F_{1,23}$ =10.88, p<0.01) and a drug x temperature interaction ($F_{1,23}$ =16.16, p<0.05) with meth decreasing DAT levels in the heat condition. There was no significant effect of condition or other

significant interactions (figure 12). For TH in males, there was a significant effect of condition ($F_{1,24}$ = 4.85, p<0.01) and drug ($F_{1,20}$ = 13.8, p<0.01) with methamphetamine decreasing TH levels mainly in separated animals and no other significant effects or interactions (figure 13). For TH in females, there were no significant main effects or interactions (figure 14). All error bars represent standard error.

Figure 11: DAT in Striatum Males Exp. 2



Note: all future western data will be presented in the above order.

Figure 12: DAT in Striatum Females Exp. 2

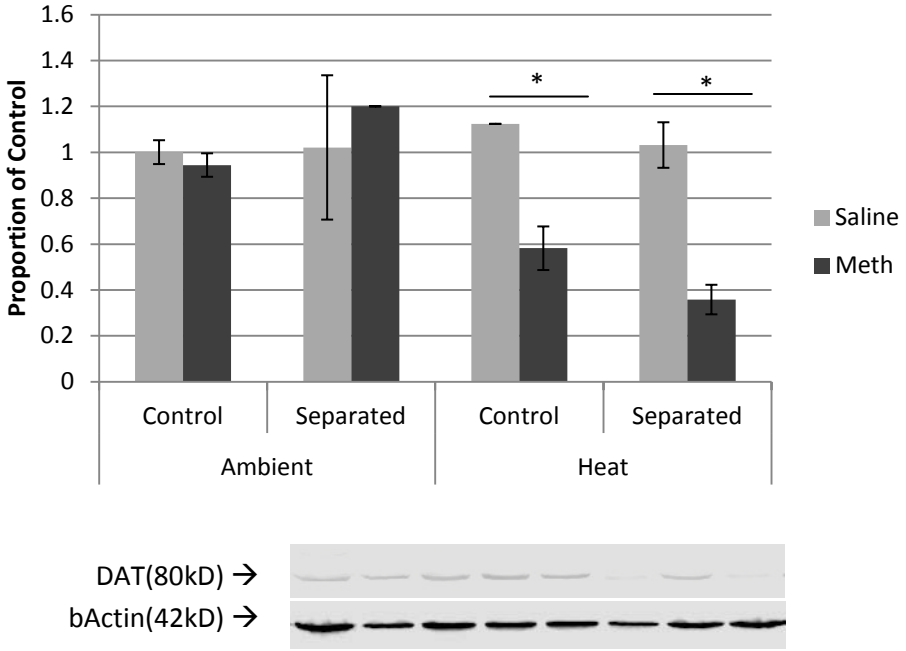


Figure 13: TH in Striatum Males Exp. 2

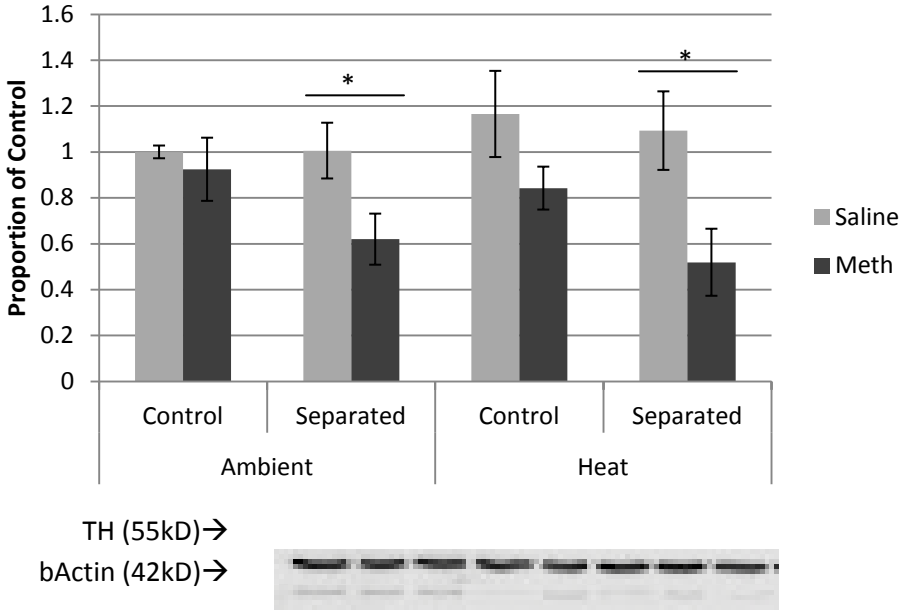
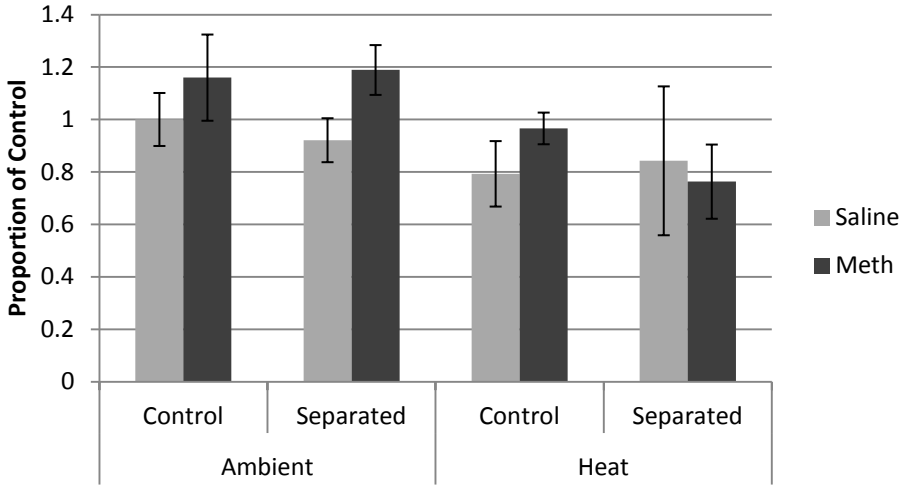
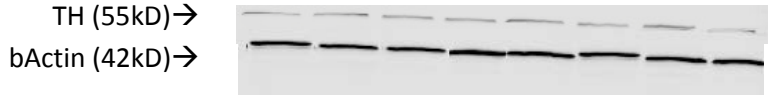


Figure 14: TH in Striatum Females Exp. 2





CHAPTER IX

DISCUSSION

The current research examined the effects of early life stress on methamphetamine induced damage in the striatum. The key findings were as follows: 1. Decreased dopamine markers occurred in males after methamphetamine administration. 2. Overall male separated animals exhibited a greater reduction in methamphetamine induced dopamine transporters relative to controls 3. Females exhibited decreased markers of dopamine when exposed to both methamphetamine and elevated temperatures, relative to methamphetamine alone.

The most prominent effects occurred in males. Males exhibited the greatest reduction in dopamine transporters seven days after methamphetamine administration, and heat potentiated this reduction in control males. DAT levels in separated males that received methamphetamine stayed at the same reduced level regardless of temperature. A similar pattern was observed in TH levels. The largest reduction in TH levels occurred in separated males, but control males had to be in the elevated temperature condition to reach about a twenty percent change in TH levels. Tissue was taken seven days after methamphetamine dosing, suggesting these levels remain stable and are not a result of acute down-regulation or sequestration after the methamphetamine binge. Because both DAT and TH levels exhibited the largest decline in separated animals, these animals may be more susceptible to the effects of methamphetamine striatal damage relative to control animals.

Damage to striatal dopamine neurons leads to several behavioral effects. Decreased striatal dopamine leads to motor deficits, cognitive impairments, and possible psychosis. However, these effects occur within disease states such as Parkinson's disease and schizophrenia. Few animal studies indicate a direct effect of methamphetamine binge dosing on behavior. Binges of methamphetamine decrease recognition memory performance in rats one week, but not seven weeks after dosing (Belcher, Feinstein, O'Dell, & Marshall, 2008; Clemens, Cornish, Hunt, & McGregor, 2006). Methamphetamine binge-dosing also results in elevated stereotypy and decreased social interactions, but no alterations in the forced swim task (Clemens, Cornish, Hunt, & McGregor, 2006). Although few animal studies have addressed the behavioral and cognitive deficits associated with methamphetamine-induced dopamine damage, human studies do indicate multiple deficits associated with long-term methamphetamine abuse (Volkow et al., 2001a; Volkow et al., 2001b). Future studies need to address the behavioral effects of methamphetamine dosing regimens that reduce dopaminergic terminal markers. Additionally, many methamphetamine-induced deficits in humans occur in complex cognitive measurements, whereas there are not adequate ways to quantify these deficits in animal research.

Although behavior was not quantified in these studies, our results indicate early life stress may increase vulnerability to methamphetamine induced striatal dopamine damage in males. Differences in dopamine systems between separated and non-separated rats may underlie this effect. Separated males exhibit decreased dopamine transporters in the nucleus accumbens core and caudate-putamen (striatum) relative to

controls (Brake et al., 2004). Neonatally isolated rats also exhibit increased dopamine release in the nucleus accumbens after an acute amphetamine injection (Kehoe et al., 2004). Methamphetamine reverses transport of the dopamine transporter, leading to elevated extracellular dopamine. Excess dopamine can auto-oxidize, contributing to the production of reactive oxygen species and terminal damage. Separated animals release more dopamine, leading to elevated levels in the extracellular space. In addition to fewer reuptake sites, dopamine likely accumulates in the synaptic cleft leading to increased methamphetamine induced damage in separated males. However, other variables cannot be ruled out at this point in time. Overall, the data presented here suggests early life stress likely potentiates methamphetamine-induced decreases in dopamine markers.

Previous studies additionally indicate early life stress alters behavioral effects of psychostimulants. Maternally separated males obtain cocaine, amphetamine, and methamphetamine self-administration more readily compared to controls, indicating an increased sensitivity to drug reward (Der-Avakian & Markou, 2010; Lewis et al., 2013; Moffet et al., 2006). Additionally, separated animals self-administer more cocaine and methamphetamine relative to controls, suggesting early life stress escalates drug taking behavior (Lewis et al., 2013; Moffet et al., 2006). Separated animals exhibit increased locomotor activating effects of methamphetamine compared to control animals (Pritchard, Hensleigh, & Lynch, 2012). However, separation may (Dimatelis et al., 2012) or may not (Faure et al., 2005) influence methamphetamine conditioned place preference reward. Although there are no direct studies, it does appear sensitivity to

psychostimulant locomotor activity likely predicts psychostimulant induced monoaminergic damage. Neurotoxic dosing of amphetamines causes mice to become more sensitive to the locomotor activating effects of amphetamines (Itzhak, Achat-Mendes, Ali, & Anderson, 2004). Additionally, repeated doses of d-amphetamine sensitize mice to the locomotor activating effects of various amphetamines and decreases brain serotonin levels, however, methamphetamine was not tested (McMillen, Scott, & Williams, 1991). These findings suggest repeated psychostimulant exposure increases locomotor sensitization and, at high enough doses these drugs can cause monoaminergic damages. Finally, early life stress increases sensitivity to psychostimulant reward and locomotor sensitivity. These effects likely share common mechanisms with systems susceptible to psychostimulant neural damage.

Clinical evidence further supports the role of stress affecting sensitivity to psychostimulants. The current studies indicated early life stress increases methamphetamine-induced dopamine damage in the striatum in males. Although there are no direct clinical studies indicating early life stressors directly relate to increased drug-induced damage, several studies indicate stress systems highly overlap with systems affected by methamphetamine. Individuals with a history of life stress are more likely to develop a substance use disorder (Dube et al., 2004). In chronic methamphetamine users, stressors increase craving and increase risk of relapse (King et al., 2010; Soderpalm, Nikolayev, & de Wit, 2003). In addition to behavioral relationships, several stress pathways and mechanisms of methamphetamine damage overlap. Methamphetamine dosing can lead to oxidative stress, increased glutamate release, and

mitochondrial dysfunction. Similarly, chronic stress can lead to the production of reactive oxygen species, increased glutamate release in areas of the striatum and cortex, and may contribute to dysfunction of mitochondria. Chronic stress also potentiates methamphetamine induced hyperthermia (reviewed in Tata & Yamamoto, 2007). Although these effects have not been directly observed in humans, the large amount of animal research suggests stress alters sensitivity to psychostimulants and chronic stress contributes to methamphetamine induced dopamine damage.

The effects of chronic methamphetamine use on dopaminergic terminals have also been documented in human subjects. Humans who met criteria for methamphetamine substance dependence exhibit decreased dopamine transporters, dopamine, and tyrosine hydroxylase in the caudate-putamen and nucleus accumbens (Wilson et al., 1996). Individuals who met the criteria for methamphetamine dependence additionally show decreased D2 receptors, DAT levels, and decreased metabolism in the frontal cortex. The extent of damage additionally correlates with declines in cognitive functioning, indicating methamphetamine causes long-term decreases in brain functioning, and these effects correlate with cognition (Volkow et al., 2001a; Volkow et al., 2001b). Humans also exhibit several markers of methamphetamine-induced cellular damage including increased oxidation levels in the caudate nucleus and prefrontal cortex (Fitzmaurice et al., 2006). Additionally, methamphetamine substance abusers exhibit low cellular integrity in the frontal cortex and basal ganglia as measured by MRS which correlates to cognitive decline (Ernst et al., 2000; Sekine et al., 2002). These findings indicate methamphetamine causes several

long-term impairments to neural systems and these impairments highly relate to cognitive impairments.

Unlike males, separation did not affect methamphetamine-induced depletions in females. Several studies show early life stress increases sensitivity to psychostimulant drugs, however, the majority of these studies were only performed in males (Der-Avakian & Markou, 2010; Lewis et al., 2013; Moffet et al., 2006). Separated females do show some potentiated effects of psychostimulants such as increased locomotor response to an acute injection of methamphetamine relative to males (Pritchard, Hensleigh, & Lynch, 2012). However, the effects of maternal separation on neural responses to psychostimulants in females remain largely uncharacterized.

In the current studies, females did not exhibit methamphetamine-induced reductions in DAT or TH levels, this being a different pattern than that observed in males. This may be a result of relatively long-lasting effects of methamphetamine in females. Females exhibit decreased striatal dopamine and dopamine transporters one day after a bolus (40mg/kg) dose of methamphetamine, the same as their male counterparts. However, three days after the same dose, males continue to exhibit decreased striatal DAT levels whereas female levels begin to recover (Bourque, Dluzen, & Di Paolo, 2012; Dluzen et. al, 2010). Additionally, a greater DAT depletion occurs in male mice relative to females seven days after 20 or 40 mg/kg methamphetamine injection (Bourque et al., 2010). Unlike our studies, these studies still found a decrease in DAT in females seven days after methamphetamine dosing, however, mice were given methamphetamine in one bolus dose, rather than over the course of eight hours

which may have caused a greater decrease in dopamine markers. Importantly, these studies indicate females exhibit an acute decrease in dopamine transporters, but these levels recover after three days, which does not occur in males. These findings indicate the effects of methamphetamine on dopamine transporters in females are relatively short-lived whereas the effects in males remain fairly stable. This may result from a sequestration or down-regulation of DAT in females but a degeneration effect on dopamine terminals in males.

However, evidence from the current studies and others suggest that methamphetamine might affect females more chronically under high temperatures. It is well documented that elevated temperature potentiates striatal damage caused by methamphetamine. Males exhibit methamphetamine-induced core body temperature elevation when given a binge regimen (current studies) or a bolus dose of methamphetamine (Dluzen, McDermott, & Darvesh, 2010). Further evidence comes from experiment two, which indicates females core body temperature increased after methamphetamine injections but only under elevated temperature conditions. Females in the elevated temperature conditions also exhibited decreased DAT levels, whereas those in the ambient temperature condition did not. This suggests that females' ability to maintain a lower core temperature after methamphetamine injections relative to males may aid in preventing the long-term decreases in striatal DAT levels. However, given the small sample size and preliminary nature of these data, further studies are needed to confirm.

Variables other than temperature likely contribute to the differences between males and females. Females show several differences in dopamine systems relative to males. Males also show decreased dopamine transporters in the striatum relative to females, and males show a decreased rate of dopamine reuptake relative to females (Bhatt & Dluzen, 2005; Morissette & Paolo, 1993; Walker et al., 2000). This suggests females exhibit an increased ability to sequester dopamine. Additionally, males exhibit heightened dopamine release in the striatum after methamphetamine administration (Kunnathur et al., 2006). This indicates, relative to females, males have heightened dopamine release in response to methamphetamine and an impaired ability to decrease the extracellular amount of dopamine. Elevated dopamine levels lead to reactive oxygen species and sequestrations of DAT which likely explains the initial decrease in DAT. Sequestration of DAT only lasts for one to three days after methamphetamine dosing, suggesting the decreased DAT observed in males are chronic reductions rather than acute (Fleckenstein et al., 1997).

Other differences may also play a role in the variations between males and females. Gonadal hormones likely play a role in methamphetamine-induced dopamine damage. Females exhibit about a 40% reduction in dopamine compared to about a 75% reduction in males when given 10 mg/kg x 4 dosing pattern of methamphetamine. These levels remained constant regardless of intact ovaries, indicating hormones might not play a role (Yu & Wagner, 1994). However, estrogens protect against dopamine damage in female mice when administered exogenously and females exhibit greater methamphetamine-induced dopamine depletions when estrogen levels are low during

diestrus (D'Astrous et al., 2005; Yu & Liao, 2000). These studies indicate estrogen may play a protective role in methamphetamine induced dopamine damage. Hormones and estrous cycle were not quantified in the two studies presented here, however, so we cannot draw any conclusions on the role of hormones.

Human populations exhibit similar sex effects as those observed in animal models. Similar to animal studies, women exhibit increased methamphetamine-induced behavioral effects relative to males (Liu & Dluzen, 2007). Women additionally show a decreased amphetamine-induced dopamine release in the striatum relative to males (Munro et al., 2006). Although no clinical studies directly indicated methamphetamine damage is greater in males relative to females, preclinical studies suggest this is highly likely given the increased dopamine released caused by amphetamines. Additionally, disorders involving deficits in dopamine functioning, such as Parkinson's disease, Huntington's disease, and schizophrenia, occur more frequently in men than in women (Scott et al., 2000; Tamir et al., 1969; Aleman et al., 2003). Although we cannot be certain methamphetamine causes a higher likelihood of dopamine damage in men relative to women, the preclinical data tells a rather compelling story. Preclinical evidence will hopefully lead the way for future human studies and possible gender-specific therapeutic approaches.

Clinical research regarding the effects of early life stress or chronic stress on dopamine terminal damage remains nonexistent. However, human studies indicate several effects of stress on dopamine system functioning. Children who have been neglected or abused show lower fMRI activity in areas of the basal ganglia in response

to natural reward (Dillon et al., 2009; Mehta et al., 2010). Furthermore, individuals with lower maternal care as children exhibit increased stress-induced dopamine release in the striatum (Pruessner et al., 2004). Finally, adults with histories of childhood stress exhibit a greater release of dopamine in the striatum after given an injection of amphetamine (Oswald et al., 2013). These studies suggest early life stress effects dopamine functioning and increase amphetamine-induced dopamine release. However, future studies will need to further evaluate the role of early life stress in methamphetamine-induced neural damage.

The current studies have many implications for clinical research. The results of the current studies indicate early life stress may potentiate methamphetamine-induced neurodegenerative effects, specifically in males. Particularly, individuals exposed to childhood abuse or neglect have an increased likelihood of developing a substance abuse disorder and, based on the current results, also have an increased likelihood of psychostimulant-induced damage to dopamine systems. Future studies will need to further elucidate these effects in human subjects. Additionally, these findings emphasize the importance of identifying at-risk populations to develop early interventions.

In conclusion, these were the first studies of the effects of early life stress and sex on methamphetamine-induced striatal dopamine damage. The results suggest early life stress may increase methamphetamine induced dopamine transporter loss in males. Additionally, females do not exhibit an effect of early life stress but did exhibit methamphetamine induced dopamine transporter decreases but only under elevated temperature conditions.

Limitations & Future Directions

The two studies used dopamine transporters and tyrosine hydroxylase levels as a measure of methamphetamine-induced damage. Binge-dosing patterns of methamphetamine, such as the regimen used in these two studies, result in damage to dopamine terminals and cell structure. Similar dosing patterns result in decreased levels of terminal markers such as: dopamine transporters, tyrosine hydroxylase, and dopamine receptors, which remain decreased for seven days up to seven years (Gibb & Korgan, 1979; Seiden, Commins, Vosmer, Axt, & Marek, 1988; Wagner et al., 1980). These markers may briefly change during the first 1-3 days after methamphetamine cessation; however, after seven days these depletions remain fairly stable (Ricaurte et al., 1982). Other studies indicate these reductions occur in conjunction with several other markers of damaged terminals. Rats given four doses of 4.0mg/kg methamphetamine over eight hours exhibit axon terminal degeneration in the striatum by use of silver staining techniques, indicating even low doses of methamphetamine can lead to terminal damage (Ellison & Switzer, 1993). Additionally, dosage patterns as low as fours doses of 3.0mg/kg to 10.0mg/kg every two hours cause microglial activation and increased striatal terminal damage at elevated temperatures (Bowyer et al., 1994; Broening, Pu, & Vorhees, 1997). Clinical studies also support these findings. Individuals with a history of chronic methamphetamine use exhibit decreased gray matter in areas of the limbic system, cingulate cortex, and hippocampus as well as decreased integrity of white matter tracts throughout areas of the corpus calossum and striatum. Importantly, these deficits correlated highly with the frequency and duration of drug

use and performance in several cognitive tasks (Ernst et al., 2000; Thompson et al., 2004). Although we cannot fully conclude from these studies the presence of methamphetamine-induced striatal damage, previous studies strongly suggest that decreased dopamine transporters seven days after methamphetamine cessation are a hallmark for long-lasting terminal damage caused by a dose-binge pattern of methamphetamine.

Future studies should address these issues in depth by looking more closely at markers in human populations and animal studies. Specifically, it will be important to examine markers of synaptic integrity by utilizing techniques such as fluoro-jade staining to determine the long-lasting cellular effects of methamphetamine administration and the correlation with dopamine transporter and tyrosine hydroxylase levels. Other markers should additionally be characterized such as DOPA decarboxylase or vesicular monoamine transporters to help determine whether the alterations observed in the current studies were a result of increased terminal degeneration or a long-term down-regulation of transporters. Additionally, several behavioral tasks should be considered to investigate whether these changes result in long-term behavioral alterations. Specifically, there are few studies that examine the behavioral effects of chronic methamphetamine administration in animals. Of these studies, the largest behavioral deficit is decreased social interaction. One problem likely lies with behavioral assays which are not sensitive enough to detect cognitive changes. Deficits are observed in human subjects, but we do not have rodent equivalents to complex measurements such

as the Wisconsin card sorting task. Future studies should look at developing more sensitive measurements of cognitive functioning in animals.

Although these studies indicate methamphetamine-induced deficits in dopamine systems in separated males and females at elevated temperatures, they lack a mechanism to explain the results. The above discussion posed many reasons for the early life stress effect and the sex differences. However, future studies should better elucidate how early life stress alters vulnerability to methamphetamine damage and why this tends to be specific to males.

APPENDIX

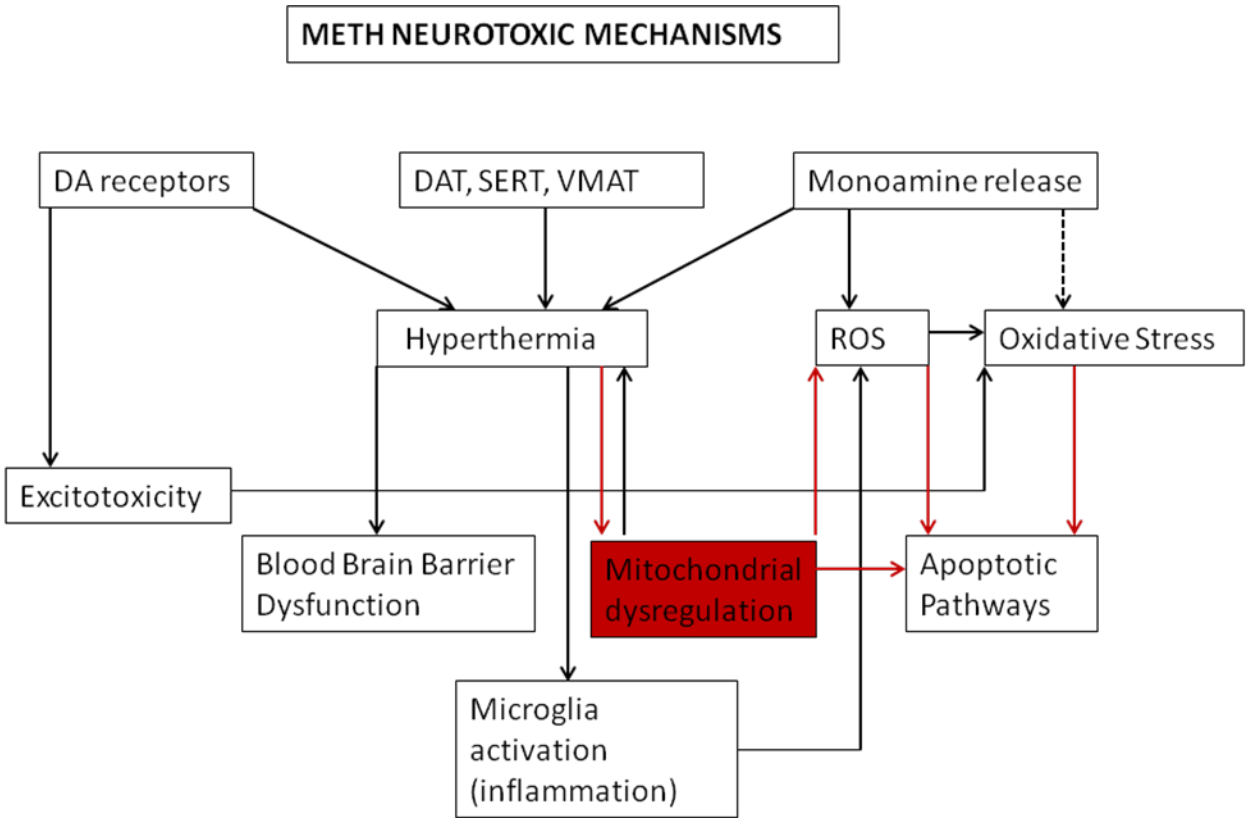


Diagram of neurotoxic mechanisms of methamphetamine. Black arrows indicate a causal relationship; red arrows indicate there may not be a direct relationship.

REFERENCES

Abekawa, T., Ohmori, T., & Koyama, T. (1994). Effects of repeated administration of a high dose of methamphetamine on dopamine and glutamate release in rat striatum and nucleus accumbens. *Brain Research, 643*, 276-281.

Ahmed, S. H., & Koob, G. F. (1997). Cocaine- but not food-seeking behavior is reinstated by stress after extinction. *Psychopharmacology, 132*(3), 289-295.

Ali, S.F., Martin, J.L., Black, M.D., & Itzhak, Y., (1999). Neuroprotective role of melatonin in methamphetamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity. *Annuals of the New York Academy of Sciences, 890*, 119.

Ali, S.F., Newport, G.D. & Slikker, W. Jr. (1996). Methamphetamine-induced dopaminergic toxicity in mice. Role of environmental temperature and pharmacological agents. *Annual New York Academy of Science, 801*, 187-198.

Al Sweidi, S., Sanchez, M.G., Bourque, M., Morissette, M. Dluzen, D., & Di Paolo, T. (2010). Oestrogen receptors and signaling pathways: implications for neuroprotective effects of sex steroids in Parkinson's disease. *Journal of Neuroendocrinology, 1*, 48-61. Review.

Asanuma, M., Tsuji, T., Miyazaki, I., Miyoshi, K., & Ogawa, N. (2003) Methamphetamine-induced neurotoxicity in mouse brain is attenuated by ketoprofen, a non-steroidal anti-inflammatory drug. *Neuroscience Letters, 352*, 13-16.

Aisa, B., Tordera, R., Lasheras, B., Del Rio, J., & Ramirez, M. J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology, 32*(3), 256-266.

Aisa, B., Tordera, R., Lasheras, B., Del Rio, J., & Ramirez, M. J. (2008). Effects of maternal separation on hypothalamic-pituitary-adrenal responses, cognition and vulnerability to stress in adult female rats. *Neuroscience, 154*(4), 1218-1226.

Aleman, A., Kahn, R.S., Selten, J.T. (2003). Sex differences in the risk of schizophrenia: Evidence from meta-analysis. *Archives of General Psychiatry, 60*, 565-571.

Axt, K.J., Commins, D.L., Vosmer, G. & Seiden, L.S. (1990). Alpha-Methyl-p-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation. *Brain Research, 515* (1-2), 269-279.

Barr A.M., Panenka W.J., MacEwan G.W., Thornton A.E., Lang D.J., Honer W.G., Lecomte T. (2006). The need for speed: an update on methamphetamine addiction. *Journal of Psychiatry Neuroscience, 31*(5), 301-313. Review.

Belcher, A.M., Feinstein, E.M., O'Dell, S.J., & Marshall, J.F. (2008). Methamphetamine influences on recognition memory: comparison of escalating and single-day dosing regimens. *Neuorpsychopharmacology, 33*(6), 1453-1463.

Bhatt, S.D. & Dluzen, D.E. (2005). Dopamine transporter function differences between male and female CD-1 mice. *Brain Research, 1035*, 188-195.

Bourque, M., Dluzen, D., & Di Paolo, T. (2012). Sex and temporally-dependent effects of methamphetamine toxicity on dopamine markers and signaling pathways. *Neuropharmacology, 62*, 2363-2372.

Bourque, M., Liu, B., Dluzen, D., & Di Paolo, T. (2010). Sex differences in methamphetamine toxicity in mice: Effect on brain dopamine signaling pathways. *Psychoneuroendrocrinology, 36*, 955-969.

Bowyer, J.F. & Ali, S., (2006). High doses of methamphetamine that cause disruption of the blood–brain barrier in limbic regions produce extensive neuronal degeneration in mouse hippocampus*. Synapse, 60*, 521–532.

Bowyer, J.F., Davies, D.L., Schmued, L., Broening, H.W., Newport, G.D., Slikker, Jr,, W,, & Holson, R.R., (1994). Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics, 268*, 1571-1580.

Brady, K.T. & Randall, C.L. (1999): Gender differences in substance use disorders-.*Psychiatry of Clinical North America, 22*, 241–252.

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *The European Journal of Neuroscience, 19*(7), 1863-1874.

Brecht ML, O'Brien A, von Mayrhauser C, Anglin MD (2004). Methamphetamine use behaviors and gender differences. *Addiction Behavior, 29*, 89 –106.

Broening, H.W., Pu, C., & Vorhees, C.V. (1997). Methamphetamine selectively damages dopaminergic innervations to the nucleus accumbens core while sparing the shell. *Synapse, 27*(2).

Brown, S.A., Vik, P.W., Patterson, T.L., Grant, I., & Schuckit, M.A. (1995). Stress, vulnerability and adult alcohol relapse. *Journal Stud Alcohol, 56*, 538–545.

Brown, J.M., & Yamamoto, B.K. (2003). Effects of amphetamines on mitochondrial function, role of free radicals and oxidative stress. *Pharmacological Therapeutics, 99*, 45–53.

Brunswick, D.J., Benmansour, S., Tejani-Butt, S.M., & Hauptmann, M. (1992). Effects of high-dose methamphetamine on monoamine uptake sites in rat brain measured by quantitative autoradiography. *Synapse, 11*(4), 287-293.

Bureau of Justice (2011). Drug Use and Dependence Crime Statistics 2010 report. Bjs.gov

Cadet, J.L., Jayanthi, S., & Deng, X., (2003). Speed kills: cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. *FASEB J., 17,* 1775-1788.

Cadet, J.L., Jayanthi, S., & Deng, X., (2005). Methamphetamine induced neuronal apoptosis involves the activation of multiple death pathways. Review. *Neurotoxic Research, 8*, 199-208.

Caldji, C., Francis, D., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 22*(3), 219-229.

Caldwell, J., Dring, L.G., & Williams, R.T. (1972). Metabolism of (14C) methamphetamine in man, the guinea pig and the rat. *Biochemical Journal, 129*, 11-22.

Callaghan, R.C., Cunningham, J.K., Sajeev, G., & Kish, S.J. (2010). Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders. *Movement Disorders, 25*(14), 2333-2339.

Carroll, M.E. (1985). The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. *Drug Alcohol Dependence, 16*, 95–109.

Chang, L., Cloak, C., Patterson, K., Grob, C., Miller,E.N., Ernst, T. (2005). Enlarged striatum in abstinent methamphetamine abusers: a possible compensatory response. *Biological Psychiatry, 57*(9), 967-974.

Chen, J. C., Chen, P. C., & Chiang, Y. C. (2009). Molecular mechanisms of psychostimulant addiction. *Chang Gung Medical Journal, 32*(2), 148-154.

Chipana, C. Torres, I., Camarasa, J. Pubill, D., & Escubedo, E. (2008). Memantine protects against amphetamine derivatives-induced neurotoxic damage in rodents. *Neuropharmacology, 54*(8). 1254-1263.

Cho, A. K. (1990). Ice: a new dosage form of an old drug. *Science, 249*, 631-634.

Cho, A.K., Melega, W.P., Kuczenski, R., & Segal, D.S. (2001). Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. *Synapse, 39*(2), 161-166. Review.

Clemens, K.J., Cornish, J.L., Hunt, G.E., & McGregor, I.S. (2007). Repeated weekly exposure to MDMA, methamphetamine or combination: long-term behavioural and neurochemical effects in rats. *Drug and Alcohol Dependence, 86*(2-3), 183-190.

Commins, D.L., Axt, K.J., Vosmer, G., & Seiden, L.S. (1987). 5,6-Dihydroxytryptamine, a serotonergic neurotoxin, is formed endogenously in the rat brain. *Brain Research, 403*(1).

Cook, C.E., Jeffcoat, A.R., Hill, J.M., Pugh, D.E., Patetta, P.K., & Sadler, B.M. (1993).Pharmakokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metabolic Disposition, 21*, 717-723.

Cunningham, J.K., Maxwell, J.C., Campollo, O., Liu, L.M., Lattyak, W.J., & Callaghan, R.C. (2012). Mexico's precursor chemical controls: emergence of less potent types of methamphetamine in the United States. *Drug Alcohol Dependence, 129*(1-2), 125-136.

D'Astous, M., Mickley, K.R., Dluzen, D.E., & Di Paolo, T. (2005). Differential protective properties of estradiol and tamoxifen against methamphetamine induced nigrostriatal dopaminergic toxicity in mice. *Neuroendocrinology, 82*, 111-120.

Darke, S., Kaye, S., McKetin, R., & Duflou, J. (2008). Major physical and psychological harms of methamphetamine use. *Drug Alcohol Review, 27*(3), 253-262.

De Kloet, E. R., Rosenfeld, P., Van Eekelen, J. A., Sutanto, W., & Levine, S. (1988). Stress, glucocorticoids and development. *Progress in Brain Research, 73*, 101-120.

Der-Avakian, A., & Markou, A. (2010). Neonatal maternal separation exacerbates the reward-enhancing effect of acute amphetamine administration and the anhedonic effect of repeated social defeat in adult rats. *Neuroscience, 170*(4), 1189-1198.

Deroche, V., Marinelli, M., Maccari, S., Le Moal, M., Simon, H., & Piazza, P. V. (1995). Stress-induced sensitization and glucocorticoids. I. sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 15*(11), 7181-7188.

Dillon, D.G., Holmes, A.J., Birk, J.L., Brooks, N., Lyons-Ruth, K., & Pizzagali, D.A. (2009). Childhood adversity is associated with left basal ganglia dysfunction during reward anticipation in adulthood. *Biological Psychiatry, 66*, 206-213.

Donovan, B., Padin-Rivera, E., & Kowaliw, S. (2001). "Transcend" initial outcomes from a posttraumatic stress disorder/substance abuse treatment program. *Journal of Trauma Stress, 14*(4), 757-772.

Dube, S. R., Felitti, V. J., Dong, M., Chapman, D. P., Giles, W. H., & Anda, R. F. (2003). Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: The adverse childhood experiences study. *Pediatrics, 111*(3), 564-572.

Dluzen, D., McDermott, J.L., Darvesh, A.S. (2010). Relationship among gender, age, time, and temperature in methamphetamine-induced striatal dopaminergic neurotxicity. *Neuroscience, 167*(4), 985-993.

Eisch, A.J. & Marshall, J.F. (1998). Methamphetamine neurotoxicity: dissociation of striatal dopamine terminal damage from parietal cortical cell body injury. *Synapse, 30*(4).

Eisch, A.J., O'Dell, S.J., & Marshall, J.F. (1996).Striatal and cortical NMDA receptors are altered by neurotoxic regimen of methamphetamine. *Synapse, 22*(3).

Eisch, A.J., Schmued, L.C., & Marshall, J.F. (1998). Characterizing cortical neuron injury with Fuoro-Jade labeling after a neurotoxic regimen of methamphetamine. *Synapse, 30*(3).

Ellison, G. & Switzer, R.C. III (1993). Dissimilar patterns of degeneration in brain following four different addictive stimulants. *Neuroreport, 5*(1), 17-20.

Erb, S., Shaham, Y., Stewart, J. (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and drug-free periods. *Psychopharmacology, 128*, 408–412.

Ernst, T., Chang, L., Leonido-Yee, M., & Speck, O. (2000). Evidence for long-term neurotoxicity associated with methamphetamine abuse: A 1H MRS study. *Neurology, 54*(6), 1344-1349.

Faure, J., Stein, D. J., & Daniels, W. (2009). Maternal separation fails to render animals more susceptible to methamphetamine-induced conditioned place preference. *Metabolic Brain Disease, 24*(4), 541-559.

Fitzmaurice, P.S., Tong, J., Yazdanpanah, M., Liu, P.P., Kalasinsky, K.S., & Kish, S.J. (2006). Levels of 4-hydroxynonenal and malondialdehyde are increased in brain of human chronic users of methamphetamine. *Journal of Pharmacology and Experimental Therapeutics, 319*(2), 703-709.

Fleckenstein, A.E., Metzger, R.R., Wilkins, D.G., Gibb, J.W., & Hanson, G.R. (1997). Rapid and reversible effects of methamphetamine on dopamine transporters*. Journal of Pharmacology and Experimental Therapeutics, 282*, 834-838.

Fleckenstein, A.E., Volz, T.J., & Hanson, G.R. (2009). Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: neurotoxic and therapeutic implications. *Neuropharmacology, 56* (1),133-138.

Gibb, J.W. & Kogan, F.J.(1979). Influence of dopamine synthesis on methamphetamine-induced changes in striatal and adrenal tyrosine hydroxylase activity.*Naunyn Schmiedebergs Archives in Pharmacology, 310*(2), 185-187.

Gouzoulis-Mayfrank, E., & Daumann, J. (2009). Neurotoxicity of drugs of abuse--the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues in Clinical Neuroscience, 11*(3), 305-317. Review.

Grelotti, D.J., Kanayama, G., Pope, H.G. Jr. (2010). Remission of persistent methamphetamine-induced psychosis after electroconvulsive therapy: presentation of a case and review of the literature. *American Journal of Psychiatry, 167*(1), 17-23.

Habib, K.E., Gold, P.W., & Chrousos, G.P (2001). Neuroendocrinology of stress*. Endocrinology and Metabolic Clinic North America, 30*, 695-728.

Hamamoto, D.T. & Rhodus, N.L. (2009). Methamphetamine abuse and dentistry. *Oral Disease, 15*(1), 27-37.

Han, D.D. & Gu, H.H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacology, 3*(6).

Harris, D.S., Boxenbaum, H., Everhart, E.T., Sequeira, G., Mendelson, J.E., & Jones, R.T. (2003). The bioavailability of intranasal and smoked methamphetamine.*Clinical Pharmacology and Therapeutics, 74*(5), 475-86.

Haughey, H.M., Brown, J.M., Wilkins, D.G. Hanson, G.R., & Fleckenstein, A.E. (2000). Differential effects of methamphetamine on Na(+)/Cl(-) dependent transporters. *Brain Research, 863*, 59-63.

Hensleigh, E., Smedley, L., & Pritchard, L. M. (2011). Sex, but not repeated maternal separation during the first postnatal week, influences novel object exploration and amphetamine sensitivity. *Developmental Psychobiology, 53*(2), 132-140.

Herman, J.P., Stinus, L., & LeMoal, M. (1984). Repeated stress increases locomotor response to amphetamine. *Psychopharmacology (Berlin), 84*(3), 431-435.

Herman, B.H. & Panksepp, J. (1978). Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect. *Pharmacology and Biochemical Behavior, 9*(2), 213-220.

Ho, E.L., Josephson, S.A., Lee, H.S.,& Smith, W.S. (2009). Cerebrovascular complications of methamphetamine abuse. *Neurocortical Care, 10*(3), 295-305.

Hotchkiss, A.J., Morgan, M.E., & Gibb, J.W. (1979)..The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life Science, 25*(16), 1373-1278.

Itzhak, Y., Achat-Mendes, C.N., Ali, S.F., & Anderson, K.L. (2004). Long-lasting behavioral sensitization to psychostimulants following p-chloroamphetamine-induced neurotoxicity in mice. *Neuropharmacology, 46*(1), 74-84.

Jayanthi, S., Deng, X., Noailles, P.A., Ladenheim, B., & Cadet, J.L., (2004). Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades. *FASEB J., 18*, 238-251.

Johanson, C.E., Frey, K.A., Lundahl, L.H., Keenan, P., Lockhart, N., Roll, J. et al. (2006). Cognitive function and nigrostriatal markers in abstinent methamphetamine abusers. *Psychopharmacology, 185*, 327-338.

Johnson, M., Hanson, G.R., & Gibb, J.W. (1989).Effect of MK-801 on the decrease in tryptophan hydroxylase induced by methamphetamine and its methylenedioxy analog. *European Journal of Pharmacology, 165*(2-3), 315-318.

Kahlig, K.M. & Galli, A. (2003). Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. *European Journal of Pharmacology, 479*(1-3), 153-158.

Kalinichev, M., Easterling, K. W., Plotsky, P. M., & Holtzman, S. G. (2002). Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in long-evans rats. *Pharmacology, Biochemistry, and Behavior, 73*(1), 131-140.

Kikusui, T., Isaka, Y., & Mori, Y. (2005). Early weaning deprives mouse pups of maternal care and decreases their maternal behavior in adulthood. *Behavior Brain Research, 162*(2), 200-206.

King, G., Alicata, D., Cloak, C., & Chang, L. (2010). Psychiatric symptoms and HPA axis function in adolescent methamphetamine users. *Journal of Neuroimmune Pharmacology, 5*(4), 582-591.

Kish, S.J., Fitzmaurice, P. S., Boileau, I., Schmunk, G.A., Ang, L.C., Furukawa, Y., Chang, L.J., Wickham, D.J., Sherwin, A., & Tong, J. (2009). Brain serotonin transporter in human methamphetamine users. *Psychopharmacology (Berlin), 202*(4), 649-661.

Kitanaka, N., Kitanaka, J., & Takemura, M. (2003). Behavioral sensitization and alteration in monoamine metabolism in mice after single versus repeated methamphetamine administration. *European Journal of Pharmacology, 474*(1), 63-70.

Kiyatkin EA. (2007). Brain temperature fluctuations during physiological and pathological conditions. *European Journal of Applied Physiology, 101*(1), 3-17.

Kerr, T., Wood, E., Grafstein, E., Ishida, T., Shannon, K., Lai, C., Montaner, J., & Tyndall, M.W. (2005). High rates of primary care and emergency department use among injection drug users in Vancouver. *Journal of Public Health (Oxford), 27*(1), 62-66.

Kosten, T. A., Miserendino, M. J., & Kehoe, P. (2000). Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. *Brain Research, 875*(1-2), 44-50.

Kraemer, T. & Maurer, H. H. Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drug, amphetamine, methamphetamine, and their N-alkyl derivatives. *Therapeutic Drug Monitoring, 24*, 277-289.

Kreek, M.J. & Koob, G.F. (1998). Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Dependence, 51,* 23–47

Kristopher, Kahlig, & Galli, (2003). Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. *European Journal of Pharmacology, 479*(1-3), 153-158.

Kunnathur, V., Shemisa, K., Liu, B., Salvaterra, T.J., & Dluzen, D.E. (2006). Sex differences in methamphetamine-evoked striatal dopamine of mice are reversed by nomifensine. *Neurotoxicology & Teratology, 28*, 557-562.

Lakhan, Shaheen, E., Kirchgessner, & Annette (2012). Prescription stimulants in individuals with and without attention deficit hyperactivity disorder: misuse, cognitive impact, and adverse effects. *Brain and Behavior, 2*(5), 661-677.

Lapiz, M.D., Parker, T.L., & Marsden, C.A. (1999). Social isolation affects response to novelty and to d-amphetamine. *Proceedings Australian Neuroscience Society, 10*, 200.

LaVoie ,M.J., Card, J.P., & Hastings, T.G., (2004). Microglial activation precedes dopamine terminal pathology in methamphetamine- induced neurotoxicity. *Experimental Neurology, 187*, 47-57.

Lewis, C.R., Staudinger, K., Schedk, L., & Olive, M.F. (2013). The effects of maternal separation on adult methamphetamine self-administration, extinction, reinstatement, and MeCP2 immunoreactivity in the nucleus accumbens. *Frontiers in Psychiatry, 55*(4).

Li, L., Lopez, J.C., Galloway, G.P., Baggott, M.J., Everhart, T., & Mendelson, J. (2010). 54.Estimating the intake of abused methamphetamines using experimenter-administered deuterium labeled R-methamphetamine: selection of the R-methamphetamine dose. *Therapeutic Drug Monitoring, 32*(4), 504-507.

Lippmann, M., Bress, A., Nemeroff, C. B., Plotsky, P. M., & Monteggia, L. M. (2007). Long-term behavioural and molecular alterations associated with maternal separation in rats. *The European Journal of Neuroscience, 25*(10), 3091-3098.

Lu, L., Liu, D., Ceng, X. (2001). Corticotropin-releasing factor receptor type 1 mediates stress-induced relapse to cocaine-conditioned place preference in rats. *European Journal of Pharmacology, 415,* 203–208.

Lynch, W.J., Roth, M.E., & Carroll, M.E. (2002). Biological basis of sex differences in drug abuse: preclinical and clinical studies. *Psychopharmacology (Berlin), 164*(2), 121-137.

Maccari, S., Piazza, P.V., Deminiere, J.M., Lemaire, V., Mormede, P., Simon, H., Angelucci, L., & Le Moal, M. (1991). Life events-induced decrease of corticosteroid type I receptors is associated with reduced corticosterone feedback and enhanced vulnerability to amphetamine self administration. *Brain Research, 547*, 7–12.

Mack, F. & Bonisch, H. (1979). Dissociation constants and lipophilicity of catecholamines and related compounds. *Archival Pharmacology*, 310-319.

Matthews, K., Hall, F. S., Wilkinson, L. S., & Robbins, T. W. (1996). Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: Effects of prefeeding, d-amphetamine, dopamine antagonists and clonidine. *Psychopharmacology, 126*(1), 75-84.

McCullough, K.D., Martindale, J., Klotz, L., Aw, T., & Holbrook, N. (2001). Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Molecular Cell Biology, 21*, 1249-1259.

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

McKetin, R. & Mattick, R.P. (1997). Attention and memory in illicit amphetamine users. *Drug and Alcohol Dependence 48*, 235-242.

McMillen, B.A., Scott, S.M., & Williams, H.L. (1991).Effects of subchronic amphetamine or amofonelic acid on rat brain dopaminergic and sertonergic function. *Journal of Neural Transmission Genetic Section, 83*(1-2), 55-66.

Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: A neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology, 27*(1-2), 127-138.

Mehta, M.A. (2010). Hyporesponsive reward anticipation in the basal ganglia following severe institutional deprivation in life. *Journal of Cognitive Neuroscience, 22*, 2316.

Mendelson, J., Uemura, N., Harris, D., Nath, R.P., Fernandez, E., & Jacob, P. III (2006). Human pharmacology of the methamphetamine stereoisomers. *Clinical Pharmacological Therapeutics, 80*, 403-420.

Moffett, M. C., Harley, J., Francis, D., Sanghani, S. P., Davis, W. I., & Kuhar, M. J. (2006). Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. *The Journal of Pharmacology and Experimental Therapeutics, 317*(3), 1210-1218.

Morissette, M. & Di Paolo, T. (1993). Sex and estrous cycle variations of rat striatal dopamine uptake sites. *Neuroendocrinology, 58*, 16-22.

Nakama, H., Change, L., Cloak, C., Jiang, C., Alicata, D., & Haning, W. (2008). Association between psychiatric symptoms and craving in methamphetamine users. *American Journal of Addiction, 17*(5), 441-446.

National Institute on Drug Abuse (2008). Monitoring the Future Survey. www.monitoringthefuture.org.

National Institute on Drug Abuse (2010).National Survey on Drug Use and Health. www.samhsa.gov/data/NSDUH.aspx

O'Callaghan, J.P. & Miller. D.B., (1994). Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *Journal of Pharmacology and Experimental Therapeutics, 270,* 741–751.

O'Dell, S.J., & Marshall, J.F., (2000). Repeated administration of methamphetamine damages cells in the somatosensory cortex: overlap with cytochrome oxidase-rich barrels. *Synapse, 37*, 32-37.

O'Dell, S.J. & Marshall, J.F. (2002). Effects of vibrissae removal on methamphetamine-induced damage to rat somatosensory cortical neurons. *Synapse, 43*(2).

O'Dell, S.J. & Marshall, J.F. (2005). Neurotoxic regimens of methamphetamine induce persistent expression of phosphor-c-Jun in somatosensory cortex and substantia nigra. *Synapse, 55*(3).

Oswald, L.M., Wand, G.S., Kuwabana, H., Wong, D.F., Zhu, S., & Brasic J.R. (2014). History of childhood adversity in positively associated with ventral striatal dopamine responses to amphetamine. *Psychopharmacology.*

Panenka, W.J., Procyshyn, R.M., Lecomte, T., Macewan, G.W., Flynn, S.W., Honer, W.G., & Barr, A.M. (2013). Methamphetamine use: A comprehensive review of molecular, preclinical and clinical findings. *Drug Alcohol Dependence, 129*(3), 167-179

Papp, M., Muscat, R., Willner, P. (1993).Subsensitivity to rewarding and locomotor stimulant effects of a dopamine agonist following chronic mild stress. *Psychopharmacology, 110*, 152–158.

Perez-Reyes, M., White, W.R., McDonald, A., Hicks, R.E., Jeffcoat, A.R., & Hill, J.M. (1991). Clinical effects of daily methamphetamine administration. *Clinical Neuropharmacology, 14*, 352-358.

Plotsky, P. M., Thrivikraman, K. V., Nemeroff, C. B., Caldji, C., Sharma, S., & Meaney, M. J. (2005). Long-term consequences of neonatal rearing on central corticotropin-

releasing factor systems in adult male rat offspring. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 30*(12), 2192-2204.

Robinson, T. E., & Berridge, K. C. (2001). Incentive-sensitization and addiction. *Addiction (Abingdon, England), 96*(1), 103-114.

Rhodus, N.L. & Little, J.W. (2008). Methamphetamine abuse and "meth mouth". *Pa Dental Journal, 75*(1), 19-29.

Ricaurte, G.A., Guillery, R.W., Seiden, L.S., Schuster, C.R., & Moore, R.Y. (1982). Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Research, 235*(1), 93-103.

Sadowski, H., Ugarte, B., Kolvin, I., Kaplan, C., & Barnes, J. (1999). Early life family disadvantages and major depression in adulthood. *British Journal of Psychiatry, 174*, 112-120.

Sato, M., Chen, C.C., Akiyama, K., & Otsuki, S. (1983). Acute exacerbation of paranoid psychotic state after long-term abstinence in patients with previous methamphetamine psychosis. *Biological Psychiatry, 18*(4), 429-440.

Saxon, A.J., Davis, T.M., Sloan, K.L., McKnight, K.M., McFall, M.E., & Kivlahan, D.R.(2001). Trauma, symptoms of posttraumatic stress disorder, and associated problems among incarcerated veterans. *Psychiatric Services, 52*(7), 959-964.

Schifano, F., Corkery, J.M., & Cuffolo, G. (2007).Smokable ('ice', 'crystal meth') and non smokable amphetamine-type stimulants: clinical pharmacological and epidemiological issues, with special reference to the UK. *Annual 1st Super Sanita, 43*, 110-115.

Schmidt, C.J., Black, C.K., & Taylor, V.L. (1991). L-DOPA potentiation of serotonergic deficits due to a single administration of 3,4- methylenedioxymethamphetamine, p-chloroamphetamine or methamphetamine to rats. *European Journal of Pharmacology, 203*(1).

Scott, B., Borgman, A., Egler, H., Johnels, B., & Aquilonius, S.M. (2000). Gender differences in Parkinson's disease symptom profile. *Acta Neurologica Scandinavica, 102*, 37-43.

Scott, J.C., Woods, S.P., Matt, G.E., Meyer, R.A., Heaton, R.K., Atkinson, J.H. et al. (2007). Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychiatric Review, 17*, 275-276.

Seiden, L.S., Commins, D.L., Vosmer, G., Axt, K., & Marek, G. (1988). Neurotoxicity in dopamine and 5-hydroxytryptamine terminal fields: a regional analysis in nigrostriatal and mesolimbic projections. *Annual New York Academy of Science, 537*, 161-172.

Seiden, L.S., Fischman, M.W., & Schuster, .C.R., (1976). Long-term methamphetamine induced changes in brain catecholamines in tolerant rhesus monkeys. *Drug Alcohol Dependence, 1,* 215–219.

Sekine, Y., Iyo, M., Ouchi, Y., Matsunaga, T., Tsukada, H., Okada, H., et al., & Mori, N., (2001). Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *American Journal of Psychiatry, 158*, 1206-1214.

Selye, H. (1936). A syndrome produced by the diverse nocuous agents*. Nature, 138*, 32-36.

Semple, S.J., Grant, I., & Patterson, T.L. (2004). Female methamphetamine users: social characteristics and sexual risk behavior. *Women Health, 40*(3), 35-50.

Shepard, J.D., Chuang, D.T., Shaham, Y., & Morales, M. (2006). Effect of methamphetamine self-administration on tyrosine hydroxylase and dopamine transporter levels in the mesolimbic and nigrostriatal dopamine pathwars of the rat. *Pharmacology (Berlin), 185*(4), 505-513.

Shaham, Y., Erb, S., Leung, S., Buczek, Y., & Stewart, J. (1998). CP-154, 526, a selective, non peptide antagonist of the corticotropin-releasing factor type 1 receptor attenuates stress-induced relapse to drug seeking in cocaine-and heroin-trained rats. *Psychopharmacology, 137*, 184–190.

Sinha, R. (2001) How does stress increase risk of drug abuse and relapse? *Psychopharmacology (Berlin), 158,* 343–359.

Söderpalm, A., Nikolayev, L., & de Wit, H. (2003). Effects of stress on responses to methamphetamine in humans. *Psychopharmacology (Berlin), 170*(2), 188-199.

Sonsalla, P.K., Albers, D.S., & Zeevalk, G.D. (1998). Role of glutamate in neurodegeneration of dopamine neurons in several animal models of parkinsonism. *Amino Acids, 14*(1-3), 69-74.

Sonsalla, P.K., Nicklas, W., & Heikkila, R.E., (1989). Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. *Science, 243*, 398-400.

Sonsalla, P.K., Riordan, D.E. & Heikkila, R.E. (1991). Competitive and noncompetitive antagonist at N-methyl-D-aspartate receptors protect against methamphetamine induced dopaminergic damage in mice. *Journal of Experimental Therapeutics, 256*(2).

Sriram, K., Miller, D.B., & O'Callaghan, J.P. (2006). Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-alpha. *Journal of Neurochemistry, 96*(3), 706-718.

Stephans, S.E. & Yamamoto, B.K., (1994). Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. *Synapse, 17***,** 203–209.

Sulzer, D., Sonders, M.S., Poulsen, N.W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Progressive Neurobiology, 75*(6), 406-433.

Tamir, A., Whittier, J., & Korenyi, C. (1969). Huntington's chorea: A sex difference in psychopathological symptoms. *Diseases of the Nervous System, 30*, 103.

Tata, D.A. & Yamamoto, B.K. (2007). Interactions between methamphetamine and environmental stress: role of oxidative stress, glutamate and mitochondrial dysfunction. *Addiction, 1*, 49-60.

Thomas, D.M., & Kuhn, D.M., (2005). MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity. *Brain Research, 1050*, 190-198.

Thompson, P.M., Hayashi, K.M., Simon, S.L., Geaga, J.A., Hong, M.S., Sui, Y., et al. (2004). Structural abnormalities in the brains of human subjects who use methamphetamine. *Journal of Neuroscience, 24*(26), 6028-6036.

United Nations Office on Drugs and Crime (2012). World Drug Report. *United Nations Publications*. www.unodc.org

Valverde, O., Smadja, C., Roques, B.P., Maldonado, R. (1997).The attenuation of morphine-conditioned place preference following chronic mild stress is reversed by a CCKB receptor antagonist. *Psychopharmacology, 131*, 79–85.

Varty, G.B., Paulus M.P., Braff D.L., & Geyer, M.A. (2000). Environmental enrichment and isolation rearing in the rat: effects on locomo tor behavior and startle response plasticity. *Biological Psychiatry, 47*,864–873.

Vazquez, V., Giros, B., & Dauge, V. (2006). Maternal deprivation specifically enhances vulnerability to opiate dependence. *Behavioral Pharmacology, 17*(8), 715-724.

Vazquez, V., Weiss, S., Giros, B., Martres, M. P., & Dauge, V. (2007). Maternal deprivation and handling modify the effect of the dopamine D3 receptor agonist, BP 897 on morphine-conditioned place preference in rats. *Psychopharmacology, 193*(4), 475-486.

Volkow, N.D., Chang, L., Wang, G.J., Fowler, J.S., Franceschi, D., Sedler, M.J., Gatley, S.J., Hitzemann, R., Ding, Y.S., Wong, C., & Logan, J., (2001a). Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. *American Journal of Psychiatry, 158*, 383-389.

Volkow N. D., Chang L.,Wang G. J., Fowler J. S., Franceschi D., Sedler M. *et al.* (2001b). Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience, 21,* 9414–9418.

Volkow N. D., Chang L., Wang G. J., Fowler J. S., Leonido-Yee M., Franceschi D. *et al.* (2001c). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *American Journal of Psychiatry, 158,* 377–382.

Volkow N. D., Chang L.,Wang G. J., Fowler J. S., Ding Y. S., Sedler M. *et al.* (2001d). Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. *American Journal of Psychiatry, 158,* 2015–2021.

Wagner, G.C., Ricaurte, G.A., Seiden, L.S., Schuster, C.R., Miller, R.J., & Westley, J., (1980). Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Research, 181*, 151-160.

Wagner, G.C., Carelli, R.M., & Jarvis, M.F., (1985). Pretreatment with ascorbic acid attenuates the neurotoxic effects of methamphetamine in rats. *Research. Communication of Chemistry Pathology and Pharmacology, 47*, 221-228.

Walker, Q.D., Rooney, M.B., Wightman, R.M., & Kuhn, C.M. (2000). Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. *Neuroscience, 95*, 1061-1070.

Wang, G.J., Smith, L., Volkow, N.D., Telang, F. Logan, J., Tomasi, D., et al. (2012). Decreased dopamine activity predicts relapse in methamphetamine abusers. *Molecular Psychiatry, 9*, 918-925.

Wilkinson, L.S., Killcross, A.S., Humby, T., Hall, F.S., Geyer, M.A., & Robbins, T.W. (1994). Social isolation produces developmental- ly-specific deficits in pre-pulse

inhibition of the acoustic startle response but does not disrupt latent inhibition. *Neuropsychopharmacology, 10*, 61–72.

Will M.J., Watkins, L.R., Maier, S.F. (1998). Uncontrollable stress potentiates morphine's rewarding properties. *Pharmacology and Biochemical Behavior, 60*, 655–664.

Wilson, J.M., Kalasinsky, K.S., Levey, A.I., Bergeron, C., Reiber, G., Anthony, R.M., Schmunk, G.A., Shannak, K., Haycock, J.W., & Kish, S.J., (1996). Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *National Medicine, 2*, 699-703.

Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J. (2011). The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. Conf Hum Factors Comput Syst-Proc 143-146.

Xie, T., Tong, L.Q., Barrett, T., Yuan, J., Hatzidimitriou, G., McCann, U.D. et al. (2002). Changes in gene expression linked to methamphetamine-induced dopaminergic neurotoxicity. *Journal of Neuroscience, 22*(1), 274-283.

Yu, L. & Liao, P.C. (2000). Sexual differences and estrous cycle in methamphetamine induced dopamine and serotonin depletions in the striatum of mice. *Journal of Neural Transmission, 107*, 419-427.

CURRICULUM VITA

Emily Hensleigh
University of Nevada Las Vegas
Department of Psychology

Address

University:
Department of Psychology
University of Nevada Las Vegas
4505 Maryland Pkwy
Las Vegas NV, 89154

Home:
1851 N Green Valley Pkwy
Apt. 712
Henderson NV, 89074

Phone: Cell: (406) 581-0150 Work: (702) 895-1821

Email: Primary: Emily.Hensleigh@unlv.edu Secondary: ehensleigh@yahoo.com

EDUCATION

Ph.D., Experimental Psychology. University of Nevada Las Vegas, NV (expected May 2014)
Emphasis: Neuroscience
Advisor: Laurel M. Pritchard, Ph.D.

M.A., Experimental Psychology. University of Nevada Las Vegas, Las Vegas, NV (Spring 2011)
Emphasis: Neuroscience
Advisor: Laurel M. Pritchard, Ph.D.

B.S., Psychology. Summa cum Laude. Montana State University, Bozeman, Montana. (Spring, 2008).

RESEARCH

Dissertation: The Effect of Early Life Stress on Methamphetamine Induced Damage in the Striatum

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

PUBLICATIONS

Hensleigh, E. & Pritchard, L.M. (2014). The effect of early environmental manipulation on locomotor sensitivity and methamphetamine conditioned place preference reward. *Behavior Brain Research*, in press.

Hensleigh, E. & Pritchard, L.M. (2013). Glucocorticoid receptor expression and sub-cellular localization in dopamine neurons of the rat midbrain. *Neuroscience Letters 556,* 191-195.

Pritchard, L.M., **Hensleigh, E.,** & Lynch, S. (2012). Altered locomotor and stereotyped responses to acute methamphetamine in adolescent, maternally separated rats. *Psychopharmacology 223*(1), 27-35.

Pritchard, L.M. & **Hensleigh, E.** (2012). Preclinical psychopharmacology and neurotoxicology of methamphetamine and 3,4-methylenedioxymethamphetamine. Antoine Rincón (Ed.), *Amphetamines: Neurobiological Mechanisms, Pharmacology and Effects*, (1-44), Nova Publishers.

Hensleigh, E., Smedley, L., & Pritchard, L. M. (2011). Sex, but not repeated maternal separation during the first postnatal week, influences novel object exploration and amphetamine sensitivity. *Developmental Psychobiology 53*(2), 132-140.

CONFERENCE PRESENTATIONS/ABSTRACTS

Hensleigh, E., Abuali, K., Eby, M., Egan, J., Fowler, A.K., & Pritchard, L.M. (2013). The effect of early life stress on methamphetamine induced damage in the striatum. Program No. 157.21. Neuroscience Meeting Planner. San Diego, CA: *Society for Neuroscience*. Online

Dold, K., **Hensleigh, E.,** Pierce, M., Lynch, S., Fowler, A.K., Abuali, K., Jager, A., Egan, J., Orlewicz, M, & Pritchard, L.M. (2013). Early life stress, drug abuse, exercise effects on BDNF and sex-influenced exercise differences. Program No. 475.19. Neuroscience Meeting Planner. San Diego, CA: *Society for Neuroscience*. Online

Pierce, M., Koenig, A., Zevallos, C., Egan, J., Orlewicz, M., Schumacher, T., **Hensleigh, E.,** Pritchard, L.M. (2013). The hormonal correlates of posttraumatic stress disorder in female veterans. Program No. 176.10. Neuroscience Meeting Planner. San Diego, CA: *Society for Neuroscience*. Online

Hensleigh, E., AbuAli, K., Egan, J., Eby, M., Fowler, A., & Pritchard, L.M. (2012) Effects of Restraint Stress and Allopregnanolone Inhibition on Amphetamine Locomotor Sensitivity. Program No. 667.08. Neuroscience Meeting Planner. New Orleans, LA: *Society for Neuroscience*. Online

Pritchard, L.M., **Hensleigh, E**., Pierce, M., Lynch, S., Fowler, A., AbuAli, K., Jager, A., Egan, J., & Orlewicz, M. (2012) Effects of maternal separation on voluntary wheel running and cocaine conditioned place preference. 388.17. 2012 Neuroscience Meeting Planner. New Orleans, LA: *Society for Neuroscience*, Online.

Pierce, M.E., **Hensleigh, E**., Egan, J., Schumacher, T., & Pritchard, L.M. (2012). Hormonal Correlates of Stress and Posttraumatic Stress Disorder in Female Veterans. Proc. of the 110th *American Psychological Association*, Chicago IL.

Fowler, A., Abu Ali, K., Schuetz, J., **Hensleigh, E.**, & Pritchard, L.M. (2011). Can exercise ameliorate increased sensitivity to cocaine in maternally separated rats? Proceedings of the UCLA Psychology Undergraduate Research Conference, Los Angeles, CA

Hensleigh, E., Lynch, S., AbuAli, K., Semmel, M., & Pritchard, L.M. (2011). Neonatal maternal separation alters methamphetamine-induced locomotor activity in a dose- and sex-dependent manner. .Program No. 503.12. 2011 Neuroscience Meeting Planner. San Diego, CA: *Society for Neuroscience*, Online.

Pritchard, L.M., **Hensleigh, E**., Engel, S., Fowler, A., Semmel, M., Zizzo, M., Ridolfi, N., & Thomason, S. (2010). Glucocorticoid receptor expression in dopamine neurons of the rat midbrain. . Program No. 190.13. Neuroscience Meeting Planner. Washington, D.C.: *Society for Neuroscience*, Online.

Hensleigh, E., Lynch, S., Semmel, M., Pierce, M., & Pritchard, L.M. (2010).The effects of early postnatal separation and sex on methamphetamine reward. Program No. 189.3. Neuroscience Meeting Planner. Washington, D.C.: *Society for Neuroscience*, Online.

Hensleigh, E., Lynch, S., Semmel, M., & Pierce, M., & Pritchard, L.M. (2010). Sex and early maternal separation effects on methamphetamine reward and sensitivity. Proc. of the inaugural Meeting of Translational Research in Methamphetamine Addiction, Chico MT.

Pritchard, L.M., **Hensleigh, E.**, Smith, L., Engel, S., Ridolfi, N., Pierce, M. & Dennehy, K. (2009). Maternal separation produces sex-, dose- and time-dependent alterations in amphetamine sensitivity. Program No. 468.7. Neuroscience Meeting Planner. Chicago, IL: *Society for Neuroscience*, Online.

Hensleigh, E., Dennehy, K., Smith, L., Engel, S. & Pritchard, L.M. (2009). Effects of maternal separation on novel object interaction. *Western Psychological Association*, 89th Annual Convention, Portland Oregon.

LAB SKILLS

RT-PCR, Western Blotting, Immunohistochemistry, ELISAs, protein assays, DNA/RNA extraction, tissue mounting, multiple behavioral assays

TEACHING

Instructor - University of Nevada Las Vegas (Fall 2010-present)

- Responsible for entire course from curriculum development and implementation through assessment and grade assignments.

Courses taught:

PSY 101: Introductory to Psychology
Fall 2010 – 2 class sections
Spring 2011 - 1 class section
Fall 2011 – 2 class sections
Spring 2012 – 1 class section
BIOL 223 Lab: Anatomy and Physiology I
Summer 2013 – lab instructor 2 sections
PSY 303(previously 403): Physiological Psychology
Spring 2011 – 1 class section
Fall 2012 – 2 class sections
Spring 2013 – 1 class section
Fall 2013 – 2 class sections
PSY 403: Physiological Psychology – hybrid course
Fall 2013 – 1 class section
PSY 422: Psychopharmacology of Abused Drugs
Spring 2012 – 1 class section
Spring 2013 – 1 class section
Spring 2014 – 2 class sections

HONORS AND AWARDS

2013 (fall) – GPSA Travel Grant
2013 (spring) – Outstanding Graduate Student Teaching Award, second place
2012 (fall) -2013 (spring) - Patricia J. Sastaunik Scholarship
2012 (spring) GPSA Research Fair, First Place in Social Science Platform
2011 Summer Session Scholarship
2011 (spring) GPSA Travel Grant
2011 (fall) GPSA Travel Grant
2010 (spring) GPSA Travel Grant

PROFESSIONAL AFFILIATIONS:

Society for Neuroscience (2009-present)
Teaching of Psychology (2010-2011)
Western Psychological Association (2008-2009)
Psi Chi National Honor Society (life time membership in 2006)

MENTORING

Undergraduate research assistants:
Nicole Ridolfi –spring 2009-spring 2011, admitted to medical school summer 2012

Sarah Lynch – spring 2010-fall 2011, admitted to M.A. health science program summer 2012

Meghan Pierce – fall 2009-present, admitted to UNLV counseling M.S. program summer 2009, admitted to psychology Ph.D. program fall 2011

Aisha Fowler –spring 2011- summer 2012, B.A. Psychology spring 2012, enrolled in biology courses to apply for pharmacy school fall 2014

Kelly AbuAli –spring 2011-present, undergraduate psychology/biology applying to Ph.D. neuroscience graduate programs fall 2013

Overseer and aided in independent research project – Sarah Engel (Spring 2010)

Senior Honor's Thesis Committee Member – Karissa Dold (fall 2012-spring 2013)

Overseer and aided in independent research project – Kelly AbuAli (summer 2013)

Graduate Student Mentor for undergraduate Neuroscience Journal Club