METHAMPHETAMINE SELF-ADMINISTRATION IN RATS

DEVELOPMENTALLY EXPOSED TO LEAD

A Dissertation

by

ANGELICA ROCHA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Psychology

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Approved by:

Co-Chairs of Committee,	Jack R. Nation
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ABSTRACT

Methamphetamine Self-administration in

Rats Developmentally Exposed to Lead. (May 2007)

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Methamphetamine is gaining mainstream popularity across the United States at the same time that lead exposure remains at elevated levels. Perinatal (gestation/lactation) lead exposure has been found to modify the reward efficacy of various drugs of abuse (e.g., cocaine, opiates) across the phases of initial selection, use, and abuse. Lead-induced changes in sensitivity to methamphetamine have not been examined in animals perinatally exposed to lead. Accordingly, four studies were conducted to examine the effects of perinatal lead exposure on adult self-administration of intravenous (i.v.) methamphetamine across all relevant transition points of drug addiction.

Adult female rats were administered a 16-mg lead or a control solution for 30 days prior to breeding with non-exposed males. Exposure continued through pregnancy and lactation and was discontinued at weaning (postnatal day [PND] 21). Animals born to control or lead-exposed dams received indwelling jugular catheters as adults (PND 60 and PND 90) and subsequently were randomly assigned to one of the four studies mentioned above, using only one male rat per litter for each study.

In Experiment 1, an acquisition study revealed that perinatal exposure to environmentally relevant levels of lead resulted in a smaller percentage of rats reaching the criterion for intravenous (i.v.) methamphetamine (.02 mg/kg) acquisition, relative to non-exposed controls. In Experiment 2, a dose-effect curve yielded a biphasic pattern of attenuation of the self-administration of methamphetamine (.04 mg/kg) in lead-exposed animals. In Experiment 3, lead-exposed animals reached lower breaking points for methamphetamine (.04 mg/kg) in a progressive ratio task, in comparison to control animals. Finally in Experiment 4, a reinstatement study revealed that perinatally lead-exposed animals showed a decreased propensity to relapse to methamphetamine (.04 mg/kg) self-administration after a period of forced abstinence. The general attenuation to the rewarding efficacy of methamphetamine observed in animals perinatally exposed to lead may functionally translate into a form of tolerance or counteradaptation. The data collected from these four studies further strengthen the possibility that pollutants in the environment may play a modulatory role in substance abuse.

ACKNOWLEDGEMENTS

I would like to thank Jack Nation, Paul Wellman, Barry Setlow, and Gerald Bratton for their intellectual contributions to this work and for the guidance, direction, and support they provided me throughout my tenure at Texas A&M.

Further, I would like to extend my gratitude to Rodrigo Valles, Nicholas Jones, Diana Joseph, and Avanthi Tayi whose direct assistance and technical expertise were instrumental in the completion of my dissertation.

I dedicate this work to my family and friends who have been loving and indispensable sources of support. Finally, I would like to acknowledge my parents for instilling in me the importance of living a life of conviction and purpose and, in so doing, helping me pursue and attain this academic achievement.

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INTRODUCTION

Lead

Environmental lead emissions plummeted following the phasing-out of leaded paint in 1978, and lead-based gasoline in the early 1980's (Hubbs-Tait *et al*, 2005). However, lead continues to be one of the major toxicants in North America producing widespread health risks to those who come into contact with the heavy metal. According to the National Health and Nutrition Examination Survey (NHANES) III study (1991-1994) there were approximately 900,000 children with blood lead levels that were equal to or exceeded the level considered "safe" by the Centers for Disease Control and Prevention, i.e., 10 µg/dL, compared to about 14 million children in 1978 (Pirkle *et al*, 1998).

A social reality is that economically disadvantaged individuals who lack resources to move out of substandard housing in the inner city are at increased risk for lead exposure. A recent account estimates that 70% of children in inner cities are exposed to lead at much higher levels than the general population (Mielke, 1999). Older homes are more likely to contain lead-based alloys and pipes that pollute the water supply and increase vulnerability to the adverse side effects produced by lead (Ensminger *et al*, 1997). Soil and dust in impoverished areas, in particular, areas in close proximity to highways and deconstruction of old buildings (Manuel, 2003), also

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This thesis follows the style of *Neuropsychopharmacology*.

contain higher concentrations of past emissions from lead-based gasoline and particles from lead-based paint that are readily airborne (Lanphear *et al*, 2002).

In extreme situations, lead poisoning can take the form of encephalopathy, characterized by seizures, coma, and death (Rosen and Mushak, 2001). Over the past 15 to 20 years, severe acute cases of lead poisoning have declined due to stringent governmental regulations (Pirkle *et al*, 1998). However, research suggests that chronic lead exposure at a level that is considered to be acceptable by current Centers for Disease Control and Prevention (CDC) guidelines (i.e., <10 µg/dL) can produce neurophysiological and neurobehavioral deficits (Bellinger and Needleman, 2003; Canfield *et al*, 2003; Needleman *et al*, 1990, 2002; Tong *et al*, 1998), in particular in children as young as 3-5 years of age (Canfield *et al*, 2003).

Lead and Behavior

Disruptions that occur in the frontal cortex (Volkow *et al*, 2002) may be producing lead-induced impairments early in the developmental phase. These disruptions may explain deficits in self-monitoring behavior and inhibitory functioning of higher order thinking. With a decrease in activity in the frontal cortex, cognitive operations that mediate appropriate judgment of behavior are replaced with automatic, non-directed, sensory-driven behaviors, such as is seen in drug addiction. Hypodopaminergic effects impair functioning of the orbitofrontal cortex (OFC) and anterior cingulate gyrus (CG) contributing to impulsive behavior and impaired inhibition (Volkow *et al*, 2002). This is particularly a problem for children because the frontal cortex is late in developing. It is of further concern in lead-exposed children who have

lead-induced developmental delays and hypodopaminergic activity at brain sites imperative for self-monitoring behavior (Cory-Slechta, 1997).

In addition to direct neurochemical changes in pregnant, lead-exposed rats, alterations in maternal care have been observed. In experimental settings, nonexposed and lead-exposed female rats have shown idiosyncratic quantitative and qualitative differences in anal-licking and grooming of respective control and lead-exposed pups. These differences may have an impact on behavioral endpoints that persist into adulthood such as drug self-administration patterns (Cuomo *et al*, 1996). In experimental work, this problem is controlled by using only one pup from each litter to avoid confounds that are sometimes evident in studies involving toxic exposure (Holson and Pearce, 1992).

Absorption and Excretion

Lead is stored in bone for a half-life of up to three decades and can be mobilized and released into blood plasma during periods of stress and high calcium demands, such as pregnancy (Barltrop, 1968; Gulson *et al*, 1997; Roelfzema *et al*, 1987) and lactation (Silbergeld, 1991). Women raised prior to more stringent CDC regulations are at risk for high blood lead levels. Disturbingly, approximately 45-70% of lead in the blood of reproductive age women originates from long-term tissue stores (Gulson *et al*, 1995). Maternal blood lead levels reach a peak during the second trimester, at which point the metal readily crosses the placental barrier and transfers to the fetus (Angell and Lavery, 1982; Barltrop, 1968; Weizsaecker, 2003).

The deleterious effects of lead appear to begin *in utero*. Lead is a particular threat to the fetus due to the ease with which it is absorbed by the placenta, crosses the underdeveloped blood brain barrier, and is stored in soft bone structure (Weizaecker, 2003). Consequently, children born to mothers who are, or have been, exposed to lead may be predisposed in the fetal stage and through lactation to develop lead-induced impairments. Even in older children there is increased brain lead absorption and decreased lead excretion, relative to adults (Godwin, 2001). Whereas children absorb up to 50% of ingested lead, adults absorb only 10-20% into their bloodstream (Weizaecker, 2003).

The primary routes for lead exposure are ingestion and inhalation. In children, ingestion occurs primarily via consumption of lead-based paint chips, or contaminated soil. Approximately 5-15% of lead that is absorbed by the body is not excreted. Of this amount, 95% is concentrated in bone and teeth (Gardella, 2001). When ingested, or absorbed through the skin, lead can be carried in blood plasma and bound to hemoglobin. Lead in blood may have a biological half-life approximating one month, a substantially shorter half-life than that of lead in bone [i.e., 20-30 years] (Weizaecker, 2003).

Ingested lead is less easily absorbed than lead that is inhaled (e.g., via airborne dust or soil), 20% to 40% is absorbed and, of that amount, 10%-60% of particles smaller than 5 μg are deposited in the lower respiratory tract where they are absorbed by the lung (Barltrop, 1979). These small lead particles are more likely to be sequestered by the kidney where they later can be released into the bloodstream. When larger particles

of lead are inhaled, they are more easily expelled by the respiratory tract, or are trapped in mucus secretions and transported by ciliary action to the larynx where the lead particles are ultimately swallowed for more efficient excretion (Barltrop, 1979). Thus, even the smallest residual lead particles from decades ago, such as those produced by recent deconstruction of lead-contaminated buildings, can continue to produce exceedingly deleterious health effects that appear to be persistent and irreversible (Canfield *et al*, 2003).

Dietary Deficiency. Calcium, iron, zinc or protein deficiencies that are more frequently encountered in economically disadvantaged individuals increase lead absorption (Hubbs-Tait *et al*, 2005; Lidsky and Schneider, 2003). Increased lead absorption is due to the ability of lead to substitute for calcium and enter an excitable cell via voltage-sensitive calcium channels (Hubbs-Tait *et al*, 2005). On a molecular level, calcium activates a chain of essential mechanisms involved in necessary stages of cellular development, such as proliferation and differentiation. The substitution of lead ions for calcium ions impedes the natural cascade of calcium-dependent cellular mechanisms, and therein, alters neurotransmitter function (Kerper and Hinkle, 1997). Adequate nutrient-intake by the mother during gestation has been shown to decrease harmful maternal to fetal transfer of lead via blood plasma (Gulson *et al*, 1997; Johnson, 2001; Tellez-Rojo *et al*, 2004). Clearly, the need for nutritional intervention remains at a high level. Low iron levels and elevated blood lead levels both are common in minority and economically disadvantaged populations (Hubbs-Tait *et al*, 2005). Anemia that develops from severe or chronic iron deficiency is present in a higher percentage of

children from low- (29%) rather than high-income (5%) families (Mahaffey, 1995). Dietary deficiencies suffered during crucial developmental years disrupt normal synaptic neurotransmission and exert effects that are long-lasting.

Neurodevelopmental Toxicity

There are two general ways in which lead exerts its neurotoxicant effects. First, lead may alter neuropharmacological mechanisms by interfering with chemical neurotransmission (Lidsky and Schneider, 2003; Silbergeld, 1992). Secondly, lead may act as a neurodevelopmental toxicant by producing changes in the hardwiring of the brain *in utero* (Moreira *et al*, 2001; Silbergeld, 1992). The latter suggestion is a focus of the following section.

Mechanisms of Action. Lead acts as a neurotoxicant by interfering with cellular proliferation, differentiation, and synaptogenesis in the fetus and neonate. Synaptogenesis is a period in development characterized by a growth spurt of nerve cells in the developing brain. For humans, this period spans the sixth month of gestation through the first few years after birth. In rats, synaptogenesis begins one day prior to birth and terminates on postnatal day 14 (Moreira *et al*, 2001). During synaptogenesis, lead as well as other non-competitive and competitive N-methyl-D-aspartate (NMDA) glutamate subtype antagonists may prevent nerve cells from becoming integrated into a neural network. If this occurs, inhibitory neurotransmitter systems that are late to develop can be prematurely deleted by apoptosis, a process whereby nerve cells are genetically programmed to destruct (Moreira *et al*, 2001). Learning impairments, attention deficits, and adult onset of psychiatric disorders are possible results of lead-

induced apoptosis in the developing organism. The consequences may be similar to those of other NMDA receptor antagonists such as alcohol, ketamine and phencyclidine (PCP) that disrupt inhibitory neurons in the cerebral cortex of the fetus following maternal consumption of such substances (Farber and Olney, 2003).

In addition to cellular changes *in utero*, lead can continue to disrupt cellular activity postnatally. The heme biosynthetic pathway is one of the major sites of lead toxicity. By disrupting this pathway, lead impairs the production of hemoglobin, cytochromes, catalases, and peroxidases (Warren *et al*, 1998). One of two variants of δ-aminolevulinic acid dehydratase (ALAD) is genetically present in the human body. ALAD-1 is the most common, and ALAD-2 is the least common of the two variants. When lead binds to either variant, zinc is displaced and heme biosynthesis is inhibited (Warren *et al*, 1998). In severe cases, insufficient amounts of hemoglobin may produce iron-deficient anemia. Perhaps related, the same individuals who suffer from malnutrition also experience the heaviest lead burdens.

ALAD-2, one of the variants of ALAD, may modify the tissue distribution of lead in the body by sequestering lead in soft tissue where it is less accessible to the central nervous system (CNS). Thus, ALAD-2 may protect against ultimate toxicity of the central nervous system where cognitive impairments are most profound, but enhance toxicity of organs, such as kidney (e.g., renal effects), due to the distribution of lead.

Genetics determine which variant of the allele is expressed. ALAD-2 appears to be differentially expressed in ethnic groups, with approximately 11%-20% of Caucasians and virtually no African-Americans examined expressing this allele (Benkman *et al*,

1983). This is of importance as adolescent carriers of ALAD-2 performed better on a battery of neuropsychological tests compared to those homozygous for the more common variant of ALAD [i.e., ALAD-1] (Bellinger *et al*, 1994).

Lead also can interfere with heme biosynthesis by accumulating in and damaging mitochondria (Anderson *et al*, 1996), therein preventing the metabolism of sufficient cellular energy, leading to oxidative stress in the cell. This effect is substantiated by *in vitro* studies of brain capillary endothelial cells showing that lead accumulates in the same areas of mitochondria as does calcium (Silbergeld *et al*, 1980). Lead disruptions of mitochondria also can produce excitotoxicity (i.e., an overactivation of glutamate receptors that causes cell death) in otherwise normal glutamate transmission (Lidsky and Schneider, 2003).

Glial cells, similarly to ALAD-2, may play a role in protecting the CNS against the toxic effects of lead. By sequestering lead, glial cells prevent depletion of oxygen from the blood supply via lead-induced oxidative stress (Tiffany-Castiglioni, 1989). Along these lines, younger astroglia, rather than older, are more efficient at clearing lead from the blood supply. However, after chronic or elevated blood lead burdens, astroglia may become saturated and will gradually release sequestered lead into the brain, further contributing to the extended duration of lead effects (Holtzman *et al*, 1987).

Chemical Neurotransmission. Whereas many lead-induced neurotoxic effects occur *in utero*, lead may continue to produce disruptions to the organism long into adulthood via what appear to be permanent alterations to neurotransmission. Perinatal lead exposure alters the neurotransmission of glutamatergic, dopaminergic and gamma-

amino-butyric acid (GABA) systems that are known to modulate drug sensitivity (Cory-Slechta, 1995; Hu and White, 1994; Lasley *et al*, 2001). Neurotransmitter systems may play a role in the behavioral deficits exhibited in lead-exposed children. Specifically, deficits in abstract thinking, attention span, conceptual reasoning, and visuospatial perception have been documented in children with moderate to high blood lead levels (Rosen and Mushak, 2001). Children with levels of blood lead below those considered safe (i.e., 10 µg/dl) [CDC, 1991] exhibit increased distractibility, hyperactivity, inability to inhibit inappropriate responding, preservation of incorrect responses, poor judgment, impulse control (Bellinger *et al*, 1994; Brockel and Cory-Slechta, 1997; Canfield *et al*, 2003; Hubbs-Tait *et al*, 2005), and delinquent behavior (Needleman *et al*, 1996).

Glutamatergic Systems. Lead is a noncompetitive antagonist at the NMDA receptor. In addition to NMDA, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate (all ionotropic), and g-protein coupled (metabotropic) receptors are the major glutamate ion channel receptor subtypes. After depolarization of the glutamate ion channel, lead takes the place of the newly released magnesium (Mg++) block. Because it is a non-competitive antagonist, lead prevents the influx of ions, in particular calcium, but does not prevent glycine from binding to the glutamate ion channel (Lasley *et al*, 2001). As noted, calcium ions are necessary for a multitude of neurodevelopmental processes to occur, particularly in the immature brain.

MK-801 has been used to assess NMDA (i.e., glutamate) receptors binding in forebrain membrane preparations of adult animals that were exposed to lead during development. Lead appears to exert an antagonistic effect on glutamate receptors,

producing a compensatory upregulation. A 31% increase in forebrain (Guilarte *et al*,

1993) and a 15-41% increase in hippocampal and cortex MK-801 binding sites were

observed in rats exposed to lead, versus controls (Ma *et al*, 1997). However, 15-30%

decreases in NMDA receptors also have been found in multiple brain regions following

postweaning lead-exposure (Cory-Slechta *et al*, 1997). Lead-induced changes in the

sensitivity (or number) of receptors and brain regions affected vary depending on the

length and time of lead exposure (Lasley *et al*, 2001).

Lead and other NMDA receptor antagonists, such as MK-801, have been found

to block the ability of opiates to establish a conditioned place preference [CPP]

(Tzsentke *et al*, 1995; Valles *et al*, 2003) and may inhibit the acquisition of morphine

self-administration (Semenova *et al*, 1999). MK-801 given prior to methamphetamine

administration also has been shown to prevent the development of behavioral

sensitization that accompanies repeated administration of MA. Whereas rats that were

treated with saline prior to a methamphetamine challenge showed augmentation of

locomotor activation effects, rats treated with a variety of doses of MK-801 showed

decreased motor activity at the same methamphetamine challenge (Ohmori *et al*, 1994).

These findings suggest NMDA receptor antagonists, such as lead, modify the reinforcing

properties of various drugs of abuse, and thus, vulnerability/susceptibility to substance

abuse.

Dopaminergic Systems. Dopamine (DA) is the neurotransmitter that is, perhaps,

most strongly implicated in the reward potency of drugs of abuse, particularly in the

mesolimbic pathway system that originates in the ventral tegmental area (VTA) and

projects to the nucleus accumbens (NAcc) and prefrontal cortex. D1- and D2-like dopamine receptors are the most common targets for extracellular dopamine release. The D1-like family includes D1 and D5 receptors. The D2-like family includes D3 and D4 receptors. Whereas D1-like agonists stimulate adenylyl cyclase activity; D2-like dopamine agonists inhibit adenyly cyclase (Waddington *et al*, 2006). Adenylyl cyclase is an enzyme that can be activated or inhibited by G proteins that are coupled to membrane receptors. There are nine known adenylyl cyclase subtypes in mammals. Type V appears to be selectively localized to the corpus striatum and is associated with limbic structures that are important in reward-motivated behavior [e.g., drug-taking, drug-seeking, and relapse] (Self and Nestler, 1998).

Long-term neuroadaptations following chronic drug use may be induced as a result of increases in levels of adenyly cyclase, cAMP and cAMP-dependent protein kinase (PKA). Upregulation of the cAMP system has been attributed with the stimulation of gene transcription and protein synthesis through cAMP response element-binding (CREB) that may be responsible in the functional tolerance, craving and relapse that follow chronic drug use (Glatt and Snyder, 1993; Self and Nestler, 1998). In the case of short-term adaptive responses, adenylyl cyclase, through cAMP and cAMP-dependent protein kinase (PKA), phosphorylates K+ channels and produces prolonged action potentials that lead to a larger influx of calcium and other neurotransmitters through calcium-gated channels.

The D1-like agonists SKF 82958 and SKF 77434 produce a downward shift in cocaine self-administration in the rat (Caine *et al*, 2000). No such downward shift in

self-administration is evident with pretreatment with D2-like agonists. Elsewhere it has been shown that D1-like antagonists such as SCH 23390 or SCH 39166 selectively reduce cocaine self-administration through attenuating the reinforcing properties of the drug (Maldonado *et al*, 1993; Richardson and Roberts, 1996).

Studies on alterations to dopaminergic systems following lead-exposure continue to yield inconsistent findings. Chronic, post-weaning exposure to lead has been shown to significantly decrease binding of [^{125}I] sulpride, a well-characterized ligand for the D2-like family of receptors in cortical areas, but not in the caudate putamen, thalamus, or nucleus accumbens (Ma *et al*, 1999). Conversely, by another account, D2-like receptor activity was decreased in the nucleus accumbens following postweaning lead exposure with no significant changes observed in D1-like, D2-like, or dopamine transporter (DAT) changes in the striatum (Pokora *et al*, 1996). One hypothesis is that lead exposure in the adult phase depletes dopamine availability, thus, an upregulation of D2-like receptors would be expected. However, another hypothesis is that lead stimulates an overflow of dopamine into the nucleus accumbens, thus, a down-regulation or subsensitivity of D2-like receptors would be expected. The time and duration of lead exposure, dose of the dopamine agonist used, and dosing-measurement intervals may all contribute to differing findings in this area. What remains clear is that chronic lead exposure appears to interact with dopamine neuromechanisms implicated in drug-taking behavior.

Serotonergic Systems. As noted, dopaminergic systems have been of primary focus in the study of drug-induced reward potency and dependence for many years.

However, other transmitter systems such as serotonin (5-HT) also are believed to be of importance in drug-seeking/drug-taking behavior. There are several receptor subtypes for each of the four main classes of serotonin receptors (i.e., 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$). Each of the serotonin receptor subtypes has various mechanisms via adenylyl cyclase, phospholipase C, and ion channels that regulate neuroplastic changes important in the development of drug addiction (Saxena, 1995).

In general, serotonin has been found to be sufficient in initiating self-administration behavior in genetically altered, dopamine-transporter-deficient mice (Rocha *et al*, 1998a). In a study using 5-HT1b receptor knock-out mice, the absence of serotonin 5-HT1b receptors increased the reinforcing effects of cocaine during maintenance (Rocha *et al*, 1998b). However, Tran-Nguyen *et al*, (2001) reported that 5-HT lesions by 5,7 dihydroxytryptamine in cocaine-trained animals attenuated cocaine drug-seeking during extinction and attenuated cocaine-induced reinstatement, possibly via an increase in 5-HT2c receptors.

5HT2c receptors also have been found to inhibit VTA dopaminergic cell body firing, likely through an enhancement of GABA function (Di Matteo *et al*, 1999). In accordance, the 5-HT2c agonist, Ro 60-0175 produced a reduction of extracellular dopamine levels in the NAcc and frontal cortex (Millan *et al*, 1998). Conversely, the selective 5-HT2 antagonist SB 242, 084 increases VTA cell firing and accumbens/frontal cortical DA release (Kennett *et al*, 1997). These studies suggest that different serotonin receptor subtypes play different roles in cocaine reward depending on the phase of cocaine self-administration.

Endorphin Systems. Opiate receptors in the VTA (Van Ree *et al*, 2000) and possibly the NAcc play a role in the reinforcing effects of opiates (Xi and Stein, 2002). The opiate system modulates electrical brain-stimulation reward, sexual motivation, and potentiates the reinforcing effects of other drugs such as cocaine (Van Ree *et al*, 2000). Various subtypes of opiate receptors are known to exist. Mu-opioid receptors are attributed with the reinforcing effects of opiates, whereas kappa-opioid receptors may modulate drug-taking behavior. A kappa-opioid receptor agonist, U50,488H produced a leftward shift in both morphine and cocaine dose-effect curves, suggesting kappa-opioid agonists may increase sensitivity to the rewarding properties of various drugs of abuse (Kuzmin *et al*, 1997).

GABA. The dopaminergic fibers projecting from the VTA to the NAcc have been strongly implicated in opiate self-administration (Stewart and Vezina, 1988). The VTA also is the site of many GABAergic neurons, which are linked to the dopamine cells in the VTA. In the absence of µ-opioid receptor activation in the VTA, GABA interneurons inhibit glutamate-stimulated dopaminergic activity. Inhibition of glutamate by GABA, ultimately results in a reduction in the basal firing rate of dopamine projection neurons (Koob, 1992). With the application of a µ-opioid receptor agonist (e.g., morphine), however, GABA interneurons are unable to exert inhibitory effects on glutamate, resulting in greater glutamate activation in the region of the VTA and a dopamine increase in the NAcc (Kalivas and Duffy, 1995).

Drug Abuse

Substance abuse or addiction is a primary, chronic, neurobiologic disease, with genetic, psychosocial, and environmental factors influencing its development and manifestations. Addiction is characterized by compulsive use that persists despite the presence of adverse stimuli and harm; e.g., electric shock in concurrence with the presence of a drug in rats (Vanderschuren and Everitt, 2004) and loss of custody of a child or incarceration in the human population. An individual who suffers from substance abuse is unable to cut back on use despite knowledge that a physical or psychological problem is being caused or exacerbated. In addition, impairment or distress results from obtaining the drug or from recovering from drug effects. Failure to fulfill social, occupational, or recreational roles also can ensue (DSM-IV, 1994).

Tolerance can develop following chronic substance use and is characterized by the need for greater intake of a drug in order to produce the same effect. Substance users may take higher doses of the drug, take the drug more frequently, or may change their method of drug intake. Sensitization also develops after chronic substance use and is characterized by an increase in drug efficacy that requires a smaller amount of drug be administered to reach the optimal effect. Withdrawal occurs in the absence of the drug. In the case of many psychostimulants, fatigue, long, disturbed periods of sleep, irritability, intense hunger, depression, psychotic reactions and anxiety may be experienced. Withdrawal from most drugs is accompanied by anhedonia which is characterized by a loss of the capacity to derive pleasure from activities that an individual once enjoyed (Koob and Le Moal, 1997).

Severity of Drug Problem

The cost of addictive disorders spans the price of health, wellbeing, and quality of life to the individual and the individual's family and loved ones. The national direct and indirect costs of drug abuse have been estimated at $66.9 billion. Alcohol abuse raises the total another $98.6 billion, and tobacco adds an additional $72 billion (U.S. Department of Commerce, 1992).

By some accounts, public health problems that can be attributed to drug and alcohol use include violence against women, motor vehicle crashes, the transmission of acquired immunodeficiency syndrome (AIDS) and other sexually transmitted diseases, school failure, unintended pregnancy, low work productivity, homelessness, and suicide (Center for Substance Abuse Prevention, 1994).

Human vs. Animal Models

In preclinical behavioral research, animal models are used to inform the scientific community about human patterns of behavior. Manipulating the transition phases of drug addiction easily is accomplished in animal subjects and offers the benefit of appropriate controls to more accurately assess the role of biological, genetic, and environmental factors in drug abuse.

Concordance exists between pre-clinical and clinical studies of drug use and abuse. For example, the mesolimbic dopamine pathway system that greatly is implicated in the rewarding efficacy of a drug appears to be conserved across species, and research in animal models has been useful in understanding reward-motivated behavior in humans. Similarly to humans, not all animals will self–administer drugs of

abuse in the presence of aversive stimuli. One study suggests that, in the case of drug addiction—as with humans— about 70% of rats will ultimately continue to self-administer cocaine when the infusion is accompanied by an aversive unconditioned stimulus [i.e., an electric shock in the rat model] (Vanderschuren and Everitt, 2004).

Phases of Addiction

Addiction is a chronically relapsing disorder that is characterized by three major elements: (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented (Koob, 1997). Drug abuse progresses from presentation of the drug to high and stable drug-seeking (appetitive) and drug-taking (consummatory) behavior. This initial shift is defined as "acquisition" of drug use. Following is the "maintenance" phase where drug-seeking and drug-taking behavior can be examined in order to measure the rewarding efficacy of a drug at various doses, etc. Maintenance patterns of drug use are intermittently interrupted by periods of the cessation of drug-taking. These periods of abstinence (whether forced or voluntary) may be characterized by negative withdrawal symptoms that typically carry opposite effects of those that are present during drug taking. For example, chronic heroin use often produces lethargy, constipation, and euphoria. However, when chronic heroin use is ceased, insomnia, diarrhea, and anhedonia can occur (Koob *et al*, 1997). These negative symptoms may be a key factor for the next phase of drug addiction: relapse [referred to as reinstatement in animal models] (See, 2005). Relapse occurs when drug self-administration ensues following abstinence. Extinction of drug-taking behavior is the

desired, but most difficult, endpoint of drug treatment programs. Extinction of drug-taking behavior may be realized in the first attempt. However, following chronic drug use, multiple attempts of extinguishing drug self-administration may be necessary.

Commonly Abused Drugs

Cocaine. Cocaine is derived from the leaf of the coca plant. It is a stimulant of the central nervous system and an appetite suppressant with abuse liability. Cocaine leaves were chewed by ancient civilizations in Peru and other South American countries, perhaps as early as 3,000 B.C., according to archaeological records. In 1877, one of the first documented cases of the medical use of cocaine was published in the U.S. in the Boston Medical and Surgical Journal. During the next decade cocaine was used as a cure-all in the treatment of everything from dyspepsia to opiate addiction. Cocaine also was used as an anesthetic, as a physical and mental stimulant, and as a diuretic. Other treatments included fever, colds, and sinus conditions. Cocaine also was prescribed to pregnant women for "vomiting of pregnancy" and postpartum cervical lacerations (Kandall, 1996). Eventually, around the year 1885, cocaine found its way to commercial folk medicines easily accessed by the public in the form of over-the-counter remedies and tonics.

Neurochemistry. Dopamine (DA) is the neurotransmitter that is perhaps most strongly implicated in the reinforcing potency of drugs of abuse, particularly in the mesocorticolimbic pathway system that originates in the VTA and projects to the NAcc, consequently decreasing activity in the prefrontal cortex. Cocaine exerts its neurophysiological effects by blocking the dopamine transporter and to a lesser extent

norepinephrine and serotonin transporters (Rothman and Baumann, 2003). Blockade of presynaptic VTA-dopaminergic transporter activity markedly increases the levels of dopamine within the synaptic cleft and in turn increases the action of the neurotransmitter on target receptors. This observation is well documented in animal experiments (Self, 2004) and supported in human studies (Schlaepfer, 1997). Central dopaminergic systems are involved in not only motor control but also attention, memory and executive processes and disruption of these systems can result in a multitude of behavioral problems, including attention deficit hyperactivity disorder and cognitive impairments (Moll *et al*, 2001).

Glutamate is another neurotransmitter that augments the reinforcing property of cocaine. Glutamate stimulation in NAcc increases the rewarding efficacy of cocaine, but is not necessary for maintaining cocaine self-administration (Cornish *et al*, 1999; Pulvirenti *et al*, 1992). Sensitization occurs via chronic administration of cocaine into NAcc. Sensitization is prevented using MK-801 (an NMDA receptor antagonist) in VTA, but not induced by exciting NMDA receptors in the VTA, suggesting a vital though not necessary role for VTA dopaminergic projections to the NAcc (Vanderschuren and Kalivas, 2000). GABA and dopamine also have been shown to modulate cocaine-induced reinstatement, possibly via a dorsal prefrontal cortex-NAcc core-ventral pallidum circuit (McFarland and Kalivas, 2001).

Opiates. Opiates are derived from the opium poppy and have been used for the last 2300 years to produce euphoria, analgesia, and suppression of diarrhea and cough. During the temperance movement (e.g., early 20th century) some women used opiates as

a more acceptable alternative to alcohol. For example, in 1782 it was common practice for upper-class, Victorian women to take a dose of opium every morning (Kandall, 1996). This practice was accepted by society in general and physicians in particular who viewed women as more in need of medicinal intervention than their male counterparts (Kandall, 1996).

Neurochemistry. The dopaminergic fibers projecting from the VTA to the NAcc have been strongly implicated in opiate self-administration (Koob, 1992). The VTA also is the site of many GABAergic neurons that are linked to the VTA-dopaminergic cells. GABA interneurons exert an inhibitory function on glutamate-stimulated dopaminergic activity, ultimately constraining the basal firing rate of dopamine projection neurons (Koob, 1992). Opiates, however, bind to μ-opioid receptors to block the inhibitory effect of GABA interneurons, thus resulting in a glutamate-mediated increase in NAcc dopamine levels (Kalivas and Duffy, 1995). VTA GABAergic and glutamatergic inputs originating in forebrain regions respond to D1-like dopamine receptor blockade by decreasing drug reward, lending to the possibility of dendritically modulated dopamine cell activity by indirect stimulation (Ranaldi and Wise, 2001).

The opiate system modulates electrical brain-stimulation reward, sexual motivation, and potentiates the reinforcing effects of other drugs such as cocaine (Van Ree *et al*, 2000). Various subtypes of opiate receptors, including μ-opioid receptors are linked to the reinforcing effects of opiates, whereas kappa-opioid receptors may modulate drug-taking behavior.

Methamphetamine. Methamphetamine is a Schedule II drug, meaning that the drug has a high potential for abuse that can lead to severe psychological or physical dependence, but offers medicinal treatments approved in the United States (e.g., treatment of obesity, narcolepsy and attention-deficit-disorder).

In recent years, the use of synthetic drugs has increased in popularity with methamphetamine and MDMA (ecstasy) at the forefront. Methamphetamine use has spread across the country from the West Coast (e.g., Hawaii and California) to the East Coast, severely impacting the Midwest, Northwest and some areas of the South. Between 1992 and 2003, treatment admission rates nationwide for methamphetamine and amphetamine increased 470 percent in the population aged 12 or older. According to the 2004 National Survey on Drug Use and Health, approximately 11.7 million Americans ages 12 and older (i.e., 4.9 percent of this population) reported trying methamphetamine at least once during their lifetime, and the average age of new users was 22.1 years. However, there is a trend toward decreases in youth (8th, 10th, and 12th graders) methamphetamine use nationwide.

Children who reside in or near clandestine methamphetamine labs are at a great risk of being physically harmed in the toxic environment they inhabit due to explosions from the use of volatile solvents in the synthesis of methamphetamine, or less noticeably, to the noxious fumes that can cause damage to the maturing brain and body. Children of families who produce or consume methamphetamine often suffer from malnutrition, neglect and/or abuse.

History.   Methamphetamine was first synthesized from ephedrine by the Japanese in 1893. It became widely used during World War II (1940s) when the Japanese, American, and German soldiers used it to combat fatigue and increase performance. The popularity of methamphetamine increased in the United States in the early 1960s when motorcycle gangs began to distribute methamphetamine throughout the West Coast (Meredith *et al*, 2005). Following anti-drug legislation in 1988 the traditional method of producing methamphetamine was replaced by the ephedrine/pseudoephedrine reduction method. This new method was cheaper, simpler, and more potent than its predecessor, resulting in a more addictive and a more easily obtainable version of the drug (Meredith *et al*, 2005).

Physiology.   As a powerful stimulant, methamphetamine, even in small doses, can result in a number of central nervous system (CNS) actions including increases in wakefulness, physical activity, respiration and euphoria, along with decreases in appetite. Other CNS effects include irritability, insomnia, confusion, tremors, convulsions, anxiety, paranoia, formication (e.g., the sensation of insects creeping over/under the skin), and violent/aggressive behavior. Cardiovascular problems include rapid heart rate, irregular heartbeat, increased blood pressure, and irreversible, stroke-producing damage to small blood vessels in the brain. High doses of the drug can produce lethal levels of hyperthermia, as well as cause convulsions. Methamphetamine is often injected i.v., contributing to increased transmission of hepatitis and HIV/AIDS. Increased sexual appetite and decreased sexual inhibitions also are characteristic of MA-use, further contributing to the spread of sexually transmitted diseases (STDs), not the

least of which are HIV and AIDS. It is unclear whether MA-induced impairments and loss of brain function can be fully recovered.

Although there are no physical manifestations of a withdrawal syndrome when methamphetamine use is ceased, there are several symptoms that occur when a chronic user stops taking the drug. These include depression, anxiety, fatigue, paranoia, aggression, and an intense craving for the drug. To date, there are no safe and tested medications for treating methamphetamine addiction, although currently available behavioral treatments have shown some success. The development of an immunization strategy based on monoclonal antibodies for the treatment of methamphetamine overdose is currently underway.

Neurochemistry. Methamphetamine, like cocaine and heroin, exerts powerful addictive properties. However, methamphetamine and cocaine exert different neuropharmacologic functions. Unlike cocaine, methamphetamine is a substrate for dopamine, norepinephrine, and serotonin transporters. That is, methamphetamine binds to the substrate portion of the transporter and is carried into the cell. Once in the cell, methamphetamine inhibits vesicle storage of the neurotransmitter and degradation by monoamine oxidase (MAO), causing a buildup of the neurotransmitter in the cytoplasm. The neurotransmitter is then bound to the inward-facing portion of the transporter and is carried into the synaptic cleft. In this way, methamphetamine not only blocks the reuptake of the neurotransmitter by the transporter, but also causes the transporter to run in reverse (Ranaldi and Poeggel, 2002). Perhaps due to overexcitation of serotonergic and dopaminergic neurotransmission, methamphetamine (but not cocaine) produces

profound neurotoxicity of these two neurotransmitters in particular, within the caudate nucleus. Amphetamine is structurally similar to methamphetamine and shares many of the same mechanisms of action. Amphetamine, like methamphetamine increases synaptic availability of dopamine, norepinephrine, and serotonin via reversal of the transporter function; both also deplete dopamine in the caudate nucleus in response to chronic administration. However, methamphetamine exerts differential affinity of reuptake blockade for multiple neurotransmitters when compared to other psychostimulants such as amphetamine and cocaine (Rothman and Baumann, 2003).

Lead and Methamphetamine. The effects of perinatal lead exposure on attendant methamphetamine use and abuse have not been studied, to date. However, lead acetate and the production of methamphetamine are greatly correlated. Previously, phenyl-2-propanone (P-2-P), a precursor in the production of methamphetamine was easily available from chemical supply companies, but in attempts to decrease the illicit production of methamphetamine, P-2-P and other precursors have been classified as controlled substances (schedule II). As a result, lead acetate is used as an easily obtainable and inexpensive reagent in the manufacture of methamphetamine. Both chronic, low-level lead exposure and acute, high-level lead exposure through methamphetamine production or use may present long term health risks from persistently elevated blood lead levels (Norton *et al*, 1996).

Factors in Addiction

Sex Differences. Sex differences have been found to result in differing sensitivity to drugs of abuse. Specifically, female rats reached acquisition criterion for

cocaine (.20 mg/kg), heroin (.015 mg/kg), and methamphetamine [MA] (.02 mg/kg) self-administration at a faster rate than males (Lynch and Carroll, 1999; Roth and Carroll, 2004). In the case of both cocaine and methamphetamine, a greater percentage of female rats reached the criterion set for acquisition and self-administered more of the drug after acquisition was met (Lynch and Carroll, 1999; Roth and Carroll, 2004). Elsewhere, a higher percentage of females than males, bred for high saccharin preference, reached criterion for cocaine (.20 mg/kg), but not heroin (.015 mg/kg) acquisition (Lynch and Carroll, 1999). In addition, in a progressive-ratio task that is a measure of drug potency, female rats reached higher breaking points than males, suggesting that the motivation to continue to self-administer methamphetamine is increased in females, even after the initial phase of acquisition of methamphetamine self-administration. After an extinction period, cocaine-priming (1.0 and 3.0 mg/kg) injections produced an increase in responding on the active lever for saline infusions, and the levels of reinstatement responding were greater in female, rather than in male rats (Lynch and Carroll, 2000).

Appetitive Manipulations. Restricted access to food also has been shown to facilitate drug self-administration of psychostimulants in an animal model (Bollweg *et al*, 1995; Campbell and Carroll, 2001). Enhanced associative learning, increases in locomotor activity, motivation to obtain the drug, or the rewarding efficacy of the drug may be responsible for the facilitation of drug acquisition. Food deprivation is associated with increases in plasma corticosterone in rats and cortisol in humans. These stress-related hormones accumulate in the hippocampus, a structure greatly implicated in

learning and memory. Corticosterone also can act directly on striatum, including the accumbens. TMT (trimethyltin), a limbic forebrain neurotoxin that blocks the effects of corticosterone, was used to examine the importance of corticosterone in the drug acquisition of food-deprived animals. Rats that were food-deprived to 75% of their normal body weight were administered TMT during the drug acquisition phase and, unlike controls with normal levels of corticosterone, corticosterone-deficient rats showed a decline in acquisition rates. In addition, endogenous glucocorticoids, vasopressin, and catecholamines have been shown to modulate the strengthened association between lever pressing and drug reward observed following food deprivation (Bollweg *et al*, 1995).

Environment. Environmental factors may also modulate the effects of lead neurotoxicity (Guilarte *et al,* 2003). Guilarte found that lead-induced deficits could be reversed by an enriched environment. Immediately after weaning (postnatal day 21), offspring born to dams that were exposed to dietary lead were randomly assigned to either an enriched or impoverished environment. Rat pups in the impoverished environment were housed in isolation with no additional stimuli. The enriched environment provided interaction with seven littermates, toys, hanging hammocks, platforms, tunnels and a running wheel. Although by the end of testing in the adult phase (postnatal day 60) lead was no longer detectable in the blood or brain of the lead-exposed group, lead-exposed rats reared in an impoverished environment exhibited impairments in a water maze task. The water maze task is a measure of spatial ability and is believed to require glutamate-dependent, long-term potentiation [LTP] (i.e., a substrate of learning and memory) in the rat hippocampus. Lead-exposed animals raised

in the enriched environment performed comparable to control animals. In fact, lead-exposed rats in the enriched environment showed a reversal in the decline of brain-derived neurotrophic factor (BDNF) mRNA and recovery of deficits in NMDA receptor subunit 1 (NR1) gene expression that were observed in lead-exposed animals. Deficits in the NR1 subunit impair the proper functioning of gene and protein expression necessary for rat hippocampal LTP (Hubbs-Tait *et al*, 2005). The clinical implications of this study are substantial, suggesting that even when lead burdens on the body are high and diet is controlled, environmental changes are enough to ameliorate lead-induced learning deficits.

<u>Stress</u>.   Stress is an environmental factor that has been the topic of much discussion in the area of substance abuse. Stress induction appears to be directly linked to the activation of the hypothalamic pituitary adrenal (HPA) axis (Goeders, 2002a; Goeders, 2002b; Goeders, 2003). Glucocorticoid hormones have been shown to play a role in the long-term maintenance of the sensitized state, whereas suppression of stress-induced corticosterone secretion abolishes the enhanced behavioral responsiveness to amphetamine and morphine produced by different stressors (Koob and Le Moal, 1997).

Manipulation of the GABA receptor complex is known to alter anxiety and stress related behaviors (Nutt and Malizia, 2001), potentially altering cocaine reinforcement (Goeders, 2002a). $GABA_A$ antagonists, such as bicuculline, possess anxiogenic properties and therein amplify cocaine reward values (Thielen and Shekahar, 2002). Similarly, lead contamination disrupts GABA availability by decreasing amounts of evoked release. In a study where bicuculline was given to perinatally-exposed animals,

a decrease in cocaine (.06 mg/kg) responding was observed perhaps due to an increase in the functional reward value of cocaine (.06 mg/kg), when compared to non-metal-exposed controls (Valles *et al*, 2005).

Exposure to high levels of stress *in utero* may also potentiate the effects of lead and produce a susceptibility to drug abuse. Cory-Slechta *et al* (2004) exposed dams to lead for 2 months prior to breeding them with nonexposed males. Female rats were either restrained (stressed) or unrestrained (non-stressed) on gestation days 16 and 17, days crucial to the development of brain structures, such as hypothalamic nuclei, hippocampus, striatum, and frontal cortex (Weinstock *et al*, 1998). Restraint was conducted for 45 minutes three different times on each of two days. The findings implicated an interaction between lead exposure, sex hormones, and maternal stress that would be present in offspring into adulthood. Specifically, male offspring born to dams exposed to lead had the highest amount of elevated corticosterone that appeared to be permanently elevated. The same elevated levels of corticosterone were only observed in females born to dams that experienced both lead-exposure and stress. This study suggests that lead may interact with stress hormones to indirectly enhance susceptibility to stress-induced disorders, brain dysfunction and cognitive deficits (Cory-Slechta *et al*, 2004).

Genetics. In addition to the environment, genetics also may play a role in drug self-administration. Rats that are genetically bred to exhibit a high (vs. low) locomotor response upon presentation of a novel environment also may exhibit an increased sensitivity to the rewarding effects of hedonic drugs (Hooks *et al*, 1994; Piazza *et al*,

1991). The greater sensitivity in high-responders may be correlated with a prolonged

secretion of corticosterone in the HPA axis in response to stress. This effect also may be

mediated by higher sensitivity to the behavioral and dopamine-activating effects of

glucocorticoids (Koob and Le Moal, 2000).

Lead/Drug Interactions

Animals exposed to lead at different time periods exhibit variable rewarding

efficacy for cocaine and opiates. The most pronounced differences occur depending on

whether animals are exposed to lead in the adult phase, or are offspring to lead-exposed

dams (i.e., perinatal lead exposure).

Perinatal Lead Exposure

Cocaine. Sensitization can be characterized by enhanced locomotor activating

effects following chronic exposure to stimulants and opiates, and is attributed with the

development of neuroadaptations associated with addiction (Robinson and Berridge,

1993). An increased sensitivity to the behavioral effects of cocaine often is observed in

perinatally lead-exposed animals. That is, perinatal lead exposure increased the

stimulatory properties of cocaine when animals were tested in a locomotor chamber at

either postnatal day (PND) 30 or PND 90 (Nation *et al,* 2000). In an acquisition study

characterized by minimal experimenter intrusions, a higher percentage of perinatally

lead-exposed animals, than controls, reached the criterion for acquisition of i.v. cocaine

self-administration, and did so at a faster rate (Rocha *et al*, 2005). Also, Nation *et al*

(2004) found that rats perinatally exposed to lead maintained responding at cocaine

doses too low to sustain responding in untreated controls. In addition, perinatal lead

exposure resulted in a greater inclination to return to drug-seeking (relapse) at lower

doses of a cocaine priming injection, when compared to rats not exposed to lead (Nation

et al, 2003). That is, after an extinction period in which saline infusions replaced

cocaine infusions as the reinforcement outcome for lever responding, lead-exposed

animals were more likely than non-exposed controls to return to self-administration

behavior (lever responding) following intraperitoneal (i.p.) injections of very low doses

of cocaine.

In contrast to an increased propensity to initiate and self-administer cocaine when

presented chronically to animals perinatally exposed to lead, a decreased sensitivity is

revealed when cocaine is presented in other paradigms. That is, cocaine i.p. injections at

the lower doses tested (1.25 and 5 mg/kg) produced an attenuation of drug reinforcement

in a CPP study, when administered over 10 days (Miller *et al,* 2000a). Similarly, a

decrease in sensitivity to cocaine was observed in a drug discrimination preparation

(Miller *et al,* 2001) in animals exposed to lead early in development, in comparison to

control animals.

Opiates. A pattern of attenuation is evident in animals perinatally exposed to

lead when an opiate is used as a reinforcer. In a CPP study, animals perinatally-exposed

to lead failed to show a morphine-induced conditioned preference to the side of the

chamber previously associated with the administration of morphine (Valles *et al,* 2003).

Additionally, in a drug-discrimination study using the kappa-opioid agonist U69,539 that

attenuates cocaine reinforcement, cocaine discrimination was shown to be impaired in

control, but not lead exposed animals (Miller *et al*, 2001). Findings further suggest that

developmental lead exposure disrupts the opiate system to a point where the reinforcing efficacy of the drug is significantly reduced when animals are tested as adults. In an intravenous (i.v.) self-administration study, lead-exposed rats responded fewer times for a heroin reinforcer, at least at intermediate doses (Rocha *et al*, 2004). Parallel results were obtained when perinatally lead-exposed animals were tested on a progressive-ratio task, i.e., lead-exposed animals ceased lever pressing for heroin reinforcements at lower ratios than their control counterparts (Rocha *et al*, 2004). In other tasks the same pattern of attenuation is observed.

Adult Lead Exposure

In the case of cocaine, patterns of drug sensitivity differ depending on whether animals are exposed to lead perinatally or during the adult phase. When animals are exposed to low-levels of lead in adulthood, the locomotor-stimulating properties of cocaine are attenuated (Nation *et al*, 1996b) and the impact of cocaine on schedule-controlled operant responding (Burkey *et al*, 1997) is reduced. These patterns are directionally opposite from those observed in animals exposed to low-levels of lead perinatally.

Animals that were exposed to dietary lead as adults received a cocaine injection for 14 days prior to daily 1 hr locomotor test sessions. On day 15, animals in all groups received a saline injection, and on day 16 of testing all animals received an i.p. cocaine challenge of one of four doses (3, 10, 20 and 40 mg/kg). Though both groups showed an increase in cocaine responding, the effects of cocaine on locomotor sensitization were

less pronounced in lead-exposed animals than controls at the 20 mg/kg dose (Nation *et al*, 1996b).

Opiate patterns of drug sensitivity differ upon time of lead exposure and task examined. Locomotor tasks yield opposite results depending on the time of lead exposure. Rats developmentally exposed to lead show an enhanced behavioral response to morphine in a locomotor activity task, relative to control animals. However, rats exposed to lead during the adult phase show a reduced locomotor response (Miller *et al*, 2001). The effects of adult lead exposure on drug self-administration have not been examined, to date.

OBJECTIVES

As indicated, methamphetamine use has spread across the country severely impacting major cities and rural counties in the West, Midwest, Northwest and certain areas of the South with no regard for socio-economic status or ethnic group (Meredith *et al*, 2005). The effects of perinatal lead exposure on attendant methamphetamine use and abuse have not been studied, to date. However, lead acetate and the production of methamphetamine are clearly related. Although the incidence of lead-exposure has decreased among the general U.S. population, there is evidence to suggest that lead acetate is being used in the manufacture of methamphetamine, perhaps elevating blood lead levels of individuals using lead-contaminated methamphetamine (Norton *et al*, 1996). Blood lead stores from the mother will be mobilized to her fetus during pregnancy. Furthermore, because lead is stored in bone for a half-life of 20-30 years, the fetus will show lead-induced deficits into adulthood (Gulson *et al*, 1997). Children of families who produce or consume MA, or live in an area where it is produced, are at great risk for malnutrition, neglect and/or abuse. Perhaps producing an additive deleterious effect, calcium, iron, zinc or protein deficiencies due to malnutrition may increase lead absorption (Hubbs-Tait *et al*, 2005; Lidsky and Schneider, 2003).

According to a Substance Abuse and Mental Health Services Administration national survey, approximately 25.2 percent of the people who used cocaine in 2002 were reported to have become dependent on or had become abusers of the drug, whereas 53 percent of those who used heroin were reported to have become dependent on or

abusers of heroin. Yet, as with humans, not all animals that are presented with the opportunity to administer drugs of abuse will do so.

The list of factors that inhibit/expedite acquisition include the following: sex differences, appetitive manipulations, impoverished/enriched environment, stress, and genetics. In addition, individual differences such as high levels of locomotor activity in a novel environment and greater impulsivity have been shown to be correlated with an enhanced vulnerability to initiate drug self-administration (Jentsch *et al*, 2000).

For the present project, four studies were completed to examine the effects of perinatal lead exposure on the transition from methamphetamine selection, use, and abuse. The initial phase of drug self-administration is acquisition (Experiment 1). The acquisition phase is a predictor of later drug-taking behavior, possibly influencing the transition from drug use to abuse. Acquisition patterns were examined using an automated procedure that included a Pavlovian-training component immediately followed by an operant component during which the criterion for acquisition of self-administration was assessed (Carroll and Lac, 1997).

Maintenance studies can be conducted using various schedules of reinforcement. An FR-2 schedule of reinforcement was used to characterize the methamphetamine dose-effect curve (Experiment 2) for control and lead-exposed animals, using several doses of methamphetamine in a within group design (saline, .01, .02, .04, .08 mg/kg). In addition, a progressive ratio schedule of reinforcement (Experiment 3) was conducted in order to more directly examine the rewarding potency of each methamphetamine dose (Arnold and Roberts, 1997). In addition, the progressive ratio paradigm was used to

clarify interpretation of FR-2 results. With an FR-2 schedule of reinforcement, a decrease in lever-pressing behavior can be due to both an agonistic (less drug is needed to achieve an optimal high) or antagonistic effect (the drug is not self-administered because it fails to be reinforcing) [Arnold and Roberts, 1997]. Finally, reinstatement (a clinically relevant model for human relapse) [Experiment 4] was examined in perinatally lead-exposed rats. After stable responding for methamphetamine was reached, a five-hr procedure was conducted. In the first hr, methamphetamine was available, but was then replaced with saline for the remainder of the 4 hrs of the 5 hr testing session. At the beginning of hr 5, animals received an i.p. injection of one of four (saline, 0.5, 1.0, 1.5 mg/kg) doses of methamphetamine and once again were placed in the operant chamber where only saline was available.

EXPERIMENT 1

Most investigations on drug self-administration in animals focus on phases of drug use following the initial transition from presentation of the drug to subsequent high and stable responding. Drug maintenance, extinction, and relapse (i.e., varying drug doses, organismic and pharmacological manipulations) are all phases of drug-taking that are more commonly studied in animal models of drug use/abuse. In these studies, the environment is manipulated in order to accelerate acquisition of the lever-pressing response in order to permit detailed assessments of parameters related to drug-selection and use. Techniques such as shaping, a day or more of total food and/or water deprivation, priming, etc., are commonly used to accelerate acquisition, therein permitting lengthier periods for evaluation of other, relevant issues.

In order to examine group differences in vulnerability to initiate drug use, a systematic procedure was employed whereby all animals received the same training to make a lever-press response. The autoshaping procedure as described by Carroll and Lac (1993; 1997; 1998) serves this purpose, allowing for the monitoring of vulnerability to self-administer drugs in control and lead-treated animals in a context where shaping methods are automatic and systematic both between and within groups.

Methods

Accordingly, the purpose of Experiment 1 was to examine relative acquisition rates of psychoactive drug self-administration for offspring (rats) born to dams exposed to 0-mg or 16-mg lead prior to breeding, and throughout gestation and lactation. In Experiment 1, adult control and lead-exposed animals were tested during daily sessions

that involved an initial 3-hr autoshaping component wherein .02 mg/kg

methamphetamine infusions were paired with the extension and retraction of a lever (a

Pavlovian procedure).

Animals

All aspects of the research reported here were approved by the Texas A&M

University Laboratory Animal Care Committee (AUP #2002-287). For 30 days, adult

female Sprague-Dawley rats (Harlan; Houston, TX) were exposed to 0 (sodium acetate)

or 16-mg lead (as lead acetate) daily using a 18 ga gavage needle to administer the

respective solutions in a volume of 1.0 ml deionized water. This procedure has been

used in previous developmental lead studies to ensure stable blood/tissue levels (cf.

Nation *et al,* 2000; 2003; 2004; Rocha *et al,* 2004). The present lead concentration was

selected based on previous studies that found it produces differential behavioral effects

while not altering dam body weights or the locomotor ability of pups (see Miller *et al,*

2000b). Following this 30-day toxicant exposure period, females were bred with non-

exposed males. Once females tested positive for copulatory plugs, the males were

removed from the home cage. Females continued to receive their daily doses of the

control solution or lead acetate solution throughout the gestation and lactation periods.

Standard rat chow (Teklad, Madison, WI) and tap water was available *ad libitum* for

dams in the home cage. Litters were culled to eight pups on PND 1, and only one pup

from each litter was used in the experiment in order to avoid confounds that are

sometimes evident in studies involving toxic exposure (Holson and Pearce, 1992).

For control and lead-exposed dams, 100-150 µl of tail-blood was drawn at breeding, parturition (PND 1), and weaning (PND 21). In addition, at the point of termination of the experiment, brain, kidney, liver, and bone (tibia) were harvested from test animals for lead concentration analyses. Littermates of test animals were sacrificed on PND 1 and PND 21, and blood samples were collected for subsequent analyses.

 Rate of pregnancy did not differ between groups ($p>0.05$). On PND 21, pups used for testing were weaned and housed individually. All animals were maintained on a 12-hr light/dark cycle. Testing commenced at approximately 10:00 hrs, two hrs into the 12-hr light cycle.

Surgical Procedure

Surgeries were performed at PND 60, which is a point demonstrated to be well within the adult timeframe of behavioral change produced by developmental lead exposure (Miller *et al*, 2000a; Miller *et al*, 2001; Nation *et al*, 2003; Nation *et al*, 2004). Using a backplate technique, implantation of chronic indwelling jugular catheters was performed using sterile techniques (Nation *et al,* 2003; 2004). Rats were anesthetized using a combination of 50 mg/kg ketamine and 20 mg/kg xylazine. A catheter consisting of 0.25-mm ID Silastic tubing (Dow Corning, Midland, MI) was inserted into the right jugular vein and sutured to muscle tissue in the area of the vein. Using an 11-ga stainless steel tube as a guide, the catheter was passed subcutaneously through the body of the animal and exited the back between the scapulae. A backplate consisting of two stainless steel ovals separated by polypropylene mesh (Ethicon, Somerville, NJ) was sutured to muscle tissue below the skin. The backplate accommodated a spring leash,

through which the catheter was threaded. Connecting to the backplate at one end, the

other end of the leash was connected to a single fluid channel swivel. The swivel design

permitted an interlock with separate connecting arms located in the home cage and

operant test chambers. The movable arm allowed for free movement and delivery of

appropriate solutions in either the home cage or test chamber. A 0.51-mm ID catheter

continued from the top of the swivel to an infusion pump that controlled solution

delivery. The rats were allowed 7 days to recover from surgery before commencing

methamphetamine self-administration testing. During this recovery period, each rat

received in the home cage automated hourly intravenous infusions (200 µl) of a sterile

saline solution containing heparin (1.25 U/ml). Once methamphetamine self-

administration testing commenced, the cannulae were flushed with .2 mls of the

heparinized saline solution prior to and following daily test sessions. Each animal

continued to receive hourly infusions in the home cage of heparinized saline throughout

testing. Catheter patency was checked an equal number of times for each animal during

testing, and at the completion of the study, by administering an intravenous infusion of

7.5 mg/kg sodium pentobarbital.

Apparatus

Twelve operant conditioning chambers (Model E-10-10, Coulbourn, Allentown,

PA) in sound attenuating cubicles served as the test apparatus. Each chamber had two

levers and a stimulus light located above each lever. Infusion pumps (Razel Scientific

Instruments; Stamford, CT) controlled drug delivery to each of the boxes. A 20-ml

syringe delivered i.v. inf (160 µl) over a 6.00 sec time frame. The system was interfaced

with 2 IBM computers, each controlling drug delivery and recording data from 6

chambers.

Procedure

Only in the acquisition study (Experiment 1) were animals restricted to 18 g of

standard rat chow in order to maintain animals at approximately 85% of the mean free-

feeding weight. This food restriction regimen is similar to that used in other laboratories

(e.g., Campbell and Carroll, 2001) and has consistently been shown to accelerate drug

acquisition and self-administration (Roth and Carroll, 2004), and the procedure is

recommended for autoshaping studies. Food restriction was not conducted in

subsequent experiments where active priming injections, etc. were performed to shape

behavior. Animals were weighed daily prior to testing. Food was placed in home cages

following the end of each daily testing session. Uncontaminated water was available *ad

libitum* throughout the study.

The autoshaping procedure consisted of a combination of Pavlovian and operant

components wherein animals were first trained to associate the pairing of an automated

retractable lever and light cue (conditioned stimuli) and drug infusion (unconditioned

stimulus) [Pavlovian conditioning] with the experience of euphorigenic effects (Carroll

and Lac, 1993; 1997; 1998). Subsequent to repeated daily Pavlovian training, rats were

required to learn to press the correct lever (response) in order to receive the drug

reinforcement (stimulus) [operant conditioning]. This procedure was originally

developed to train animals to acquire food-reinforced behavior (Brown and Jenkins,

1968). However, more recently, this procedure has been used to study the acquisition of

drug self-administration in a completely automated procedure, devoid of experimenter manipulations that would otherwise vary unsystematically between and within groups (Campbell and Carroll, 2000; Carroll and Lac, 1993; Carroll *et al*, 2002; Kakade and Dayan, 2002; Roth and Carroll, 2004). With autoshaping, the presentation of drug and the stimuli associated with the drug infusions during the Pavlovian conditioning session is consistent and invariable for all animals (Carroll and Lac, 1993).

Autoshaping Component. Control (Group 0-mg; N=7) and lead-exposed (Group 16-mg; N=8) animals were run in two squads, and subject assignment to Coulborn operant chambers and squad was counterbalanced. Each of the 6-hr experimental sessions consisted of two parts, an autoshaping and a self-administration component (3-hrs each). Testing was carried out seven days per week. For the first 3 hrs, during the autoshaping component, testing commenced with the retractable lever withdrawn from the cage (i.e., outside the reach or vision of the animal). After a 90-sec time-out period, the retractable lever extended into the operant chamber at which point the animal received a methamphetamine infusion if it pressed the lever or after 15-sec, whichever occurred first. Once again, a 90-sec time-out period was instituted. As before, the active lever was extended into the chamber and the animal was given 15-sec to press the lever for an immediate infusion of .02 mg/kg methamphetamine, or, if no response occurred, the animal received a non-contingent infusion of .02 mg/kg methamphetamine infusion at the end of the 15-sec period. This cycle repeated for the first 20 min of each hr for 3 hrs (30 total methamphetamine infusions).

With the chamber house-light off, the stimulus light above the active (right) lever was lit for the 6-sec duration and terminated immediately after in the case of both contingent and noncontingent conditions. The inactive (left) lever remained extended inside the chamber throughout the study. Responses on the inactive lever, as well as responses during an infusion, were recorded but had no programmed consequences. As indicated, a .02 mg/kg methamphetamine infusion (.160 ml) was delivered to the animal following each lever retraction regardless of contingency. After the first 20 min of each hr, following the 10 methamphetamine infusions, all stimulus lights were extinguished and the active lever remained retracted for a 40 min time-out session, until testing recommenced at the beginning of the next hr.

Self-administration Component. For the second 3-hr component of the experiment, the retractable lever remained extended and .02 mg/kg methamphetamine infusions were contingent upon lever pressing under an FR-2 schedule. As before, responses on the left lever and responses during an infusion delivery were recorded, but had no programmed consequences. At the end of the 3-hr self-administration period, testing concluded for the day.

The criterion for acquisition of methamphetamine self-administration was a mean of 30 infusions per day over 2 consecutive daily self-administration sessions. This value is half of what had been set previously in studies that used twice the duration of testing time (i.e., 6-hr autoshaping and 6-hr self-administration) [Carroll and Lac, 1997; Carroll and Lac, 1998]. The methamphetamine dose (.02 mg/kg) was chosen based on data

from previous studies that show this dose is marginally reinforcing, and does not produce satiation or motoric impairments (Roth and Carroll, 2004).

In order to confirm patency during acquisition training, catheters were flushed twice daily with 0.2 mls of a heparinized saline solution; once prior to and once following each daily testing session. Catheters of questionable patency were flushed with .05 mls of pentobarbital (7.50 mg/ml) followed by 0.2 mls of heparinized saline and these animals were checked for immediate onset of brief anesthesia. At the end of the study, all animals in both exposure conditions received an i.v. infusion of 7.50 mg/kg sodium pentobarbital. Again, catheter patency was verified by rapid onset of brief anesthesia.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the (+,-)methamphetamine HCl administered as the salt. Heparinized saline served as the (+,-)methamphetamine vehicle. Lead acetate was obtained from Sigma Chemical Company (St. Louis, MO).

Tissue Collection and Analyses

After animals recovered from patency verification, control (Group 0-mg) and lead-exposed (Group 16-mg) test animals were anesthetized with sodium pentobarbital (50.00 mg/kg i.p.). Following blood collection via cardiac puncture, brain was rapidly harvested along with kidney, liver, and bone (tibia). Following collection of blood and tissue samples, lead residues were measured via atomic absorption spectrophotometry as described in a detailed report from our laboratory (Dearth *et al,* 2003).

Statistical Procedures

Animal body weights during the period of acquisition testing were analyzed using a Groups x Weeks repeated measures analysis of variance (ANOVA) test, with Weeks serving as the within factor. In all cases, Neuman-Keuls post hoc procedure for determining mean differences was used for individual comparisons.

The comparative number of rats meeting the methamphetamine self-administration acquisition criterion was assessed using a survival analyses test, which is ideally suited for evaluation of performance patterns where animals reach criterion at different rates (Lee, 1992).

Results

Body Weights

The analysis of body weights during the period of acquisition testing (mean body weights= 337g±5.30 and 309±7.11 for Groups 0-mg and 16-mg, respectively; $p > .05$) did not show significant group differences. Weekly fluctuations did occur but the pattern of change was uniform across groups.

Acquisition of Methamphetamine Self-administration

Figure 1 illustrates the cumulative percentage of non-exposed (Group 0-mg) and lead-exposed (Group 16-mg) rats meeting criterion (30 lever presses). Five of the eight (62.5%) animals in Group 0-mg reached acquisition by day 17 of testing- approximately half-way through the 35-day testing period; whereas none of the Group 16-mg animals had reached criterion for acquisition at that point in testing. Ultimately, fewer lead-exposed animals achieved the requirements for acquisition of methamphetamine
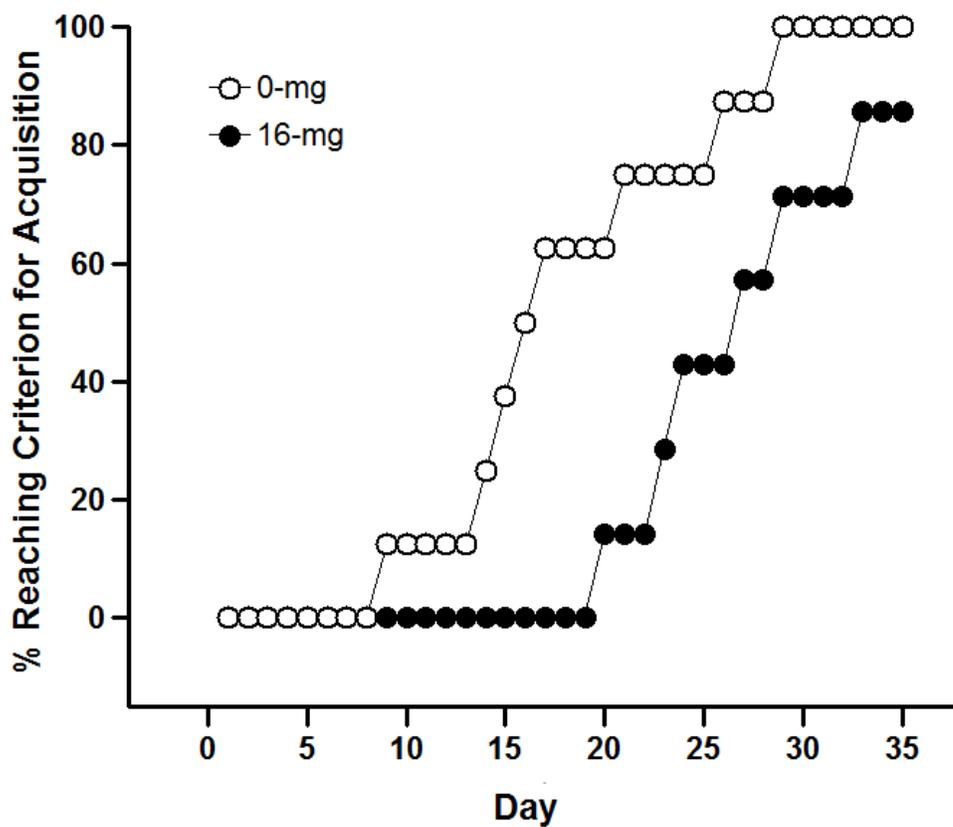
Fig. 1 Cumulative percentage (%) of Group 0-mg (*n*= 8) and Group 16-mg (*n=7*) rats meeting the criterion for the acquisition of methamphetamine (.02 mg/kg) self-administration within the 35-day limit. Open symbols and closed symbols represent nonexposed and lead-exposed conditions, respectively.

self-administration with a cumulative percentage of 85.71% (6 out of 7), in comparison to 100% acquisition rate in the control group (8 out of 8) by the end of testing. As is visually apparent in Figure 1, rates of acquisition of methamphetamine self-administration were more rapid for control animals, in comparison to lead-exposed animals (survival analysis; Kaplan-Meier, Breslow statistic) [X^2= 5.30=, p< 0.05]). Once animals reached acquisition, stable responding was maintained.

Figure 2 profiles the mean number of active (.02 mg/kg) and inactive lever responses per 5 session blocks for all animals in both exposure conditions. Control animals averaged 1.2 responses on the inactive lever and lead-exposed animals averaged 1.59 lever responses across the 7 week testing period [data not shown]. A 2 Groups x 2 Levers x 7 Blocks of 5 Sessions (1-7) repeated measures ANOVA was performed on these data, with Levers and Blocks of 5 Sessions serving as within factors. Overall, the findings from these analyses revealed that lead-exposed rats administered methamphetamine at lower rates than control animals.

Tissue Analyses

Table 1 presents the mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning for animals that underwent the identical lead exposure regimen as described in Experiments 1-4 of this work. The tissue analyses corresponding to the animals in Experiments 1-4 were not available by the completion of this dissertation. However, over the years, the perinatal (gestation/lactation) lead exposure regimen
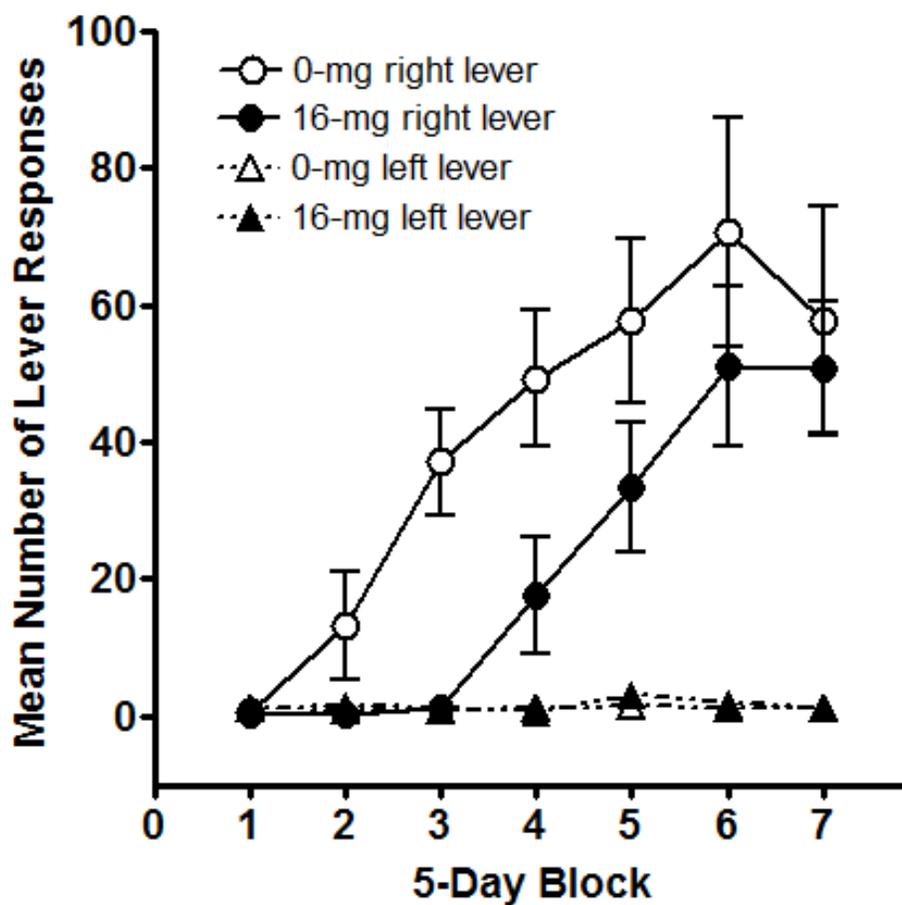
Fig. 2 Mean active (.02 mg/kg methamphetamine) and inactive lever presses for all animals in Groups 0-mg and 16-mg across successive 5-session blocks. Open symbols and closed symbols represent the nonexposed and lead-exposed conditions, respectively.

outlined in this work, as conducted by Nation *et al* (2003; 2004) and by researchers in his lab (Rocha *et al*, 2004; 2005; Valles *et al,* 2005), has yielded almost identical blood and tissue data across various time points (i.e., gestation day 10, parturition, weaning and completion of study). Thus, there is reason to believe that tissue analyses representative of that which has been found in previous studies of perinatal lead exposure by Nation *et al* will be expected once again in Experiments 1-4. Preliminary tissue data [data not shown] collected from animals in the present work, parallels that found in earlier studies and serves to further justify the presentation of previously collected and analyzed tissue data (c.f., Rocha *et* al, 2005).

By the completion of acquisition testing in Experiment 1, blood lead levels of lead-exposed animals consistently have been shown to return to control levels, i.e., in both groups lead levels had fallen below detectable limits (<1 µg/dl).

Blood lead concentrations, a conventional marker of lead toxicity, are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. Also, lead concentrations in tissues are indicated for dams sacrificed at weaning.

Table 1 *Mean (SEM) blood and tissue lead concentration values for dams, littermates and test animals. The symbol* * *indicates that control and lead-exposed animals were significantly different (p< .05).*

Blood Lead Concentration (µg/dl)		
	Group 0-mg	Group 16-mg
Dams		
Breeding	1.7 (.2)	37.1 (.6) *
Parturition (PND 1)	1.1 (.1)	58.8 (.3) *
Weaning (PND 21)	2.3 (.003)	38.9 (.3) *
Littermates		
PND 1	1.8 (.03)	83.2 (.2) *
PND 21	1.2 (.01)	13.9 (.03) *
Test Animals		
Termination	< .5	< .5
Tissue Concentrations of Test Animals at Termination (µg/g)		
	Group Lead-0	Group Lead-16
Brain	.003 (.001)	.006 (.002)
Kidney	.010 (.002)	.034 (.003)
Liver	.004 (.001)	.006 (.001)
Tibia	.035 (.002)	2.023 (.317) *

EXPERIMENT 2

In Experiment 2, littermates of lead-exposed animals and control animals were tested on a fixed-ratio 2 (FR-2) using a full range of methamphetamine doses (.01, .02, .04, .08 mg/kg) to characterize the dose-effect curve (Roth and Carroll, 2004). Self-administration studies with rats often employ a simple fixed ratio (FR) schedule of reinforcement. The FR schedule is useful for exploring patterns of drug intake and can be used in the preliminary examination of drugs with abuse potential (Arnold and Roberts, 1997).

Methods

Animals

As in Experiment 1, animal maintenance and research were conducted in accordance with the guidelines provided by the Texas A&M University Laboratory Animal Care Committee, and the Public Health Service Policy outlined in the publication of the Guide for the Care and Use of Laboratory Animals (1996). The surgical procedure, apparatus and gavage protocol were as outlined for Experiment 1.

Surgical Procedure

Cather implantation and other surgical procedures were as described in Experiment 1.

Apparatus

The apparatus was the same as described for Experiment 1. The retractable lever remained extended and an i.v. inf .04 mg/kg of methamphetamine was contingent upon lever pressing.

Procedure

All control (*n*=7) and lead-exposed (*n*=8) test animals were shaped to lever press for an infusion of .04 mg/kg methamphetamine on a FR-1 schedule where each depression of the right (active) lever activated the 20-ml syringe infusion pump and resulted in an infusion of methamphetamine and simultaneous illumination of the stimulus light above the lever. Shaping under conditions of methamphetamine reinforcement continued for 5 days. Lever responses on the left (inactive) lever were recorded but had no programmed consequences.

For 7 days after the shaping phase, all animals were trained on a FR-2 reinforcement schedule under which delivery of .04 mg/kg of methamphetamine served as the baseline-training dose. Dose–effect testing began the day immediately following the final day of baseline testing.

During the period of dose–effect testing, drug was available for two successive days at each dose in descending order (.08, .04, .02, .01, saline). Within this self-administration testing schedule, a stability criterion was imposed between dose–effect test sessions. As a result, during consecutive 2-hr daily baseline sessions FR-2 schedule responding for .04 mg/kg methamphetamine varied less than 20% for all animals in both groups in over 90% of the sessions.

Tissue Collection and Analyses

In a manner parallel to the tissue analyses previously collected and analyzed by Nation *et al* employing an identical perinatal lead exposure regimen, by the completion of testing in Experiment 2, blood lead levels are expected to return to control levels in

both control and lead-exposed groups (i.e., lead levels are expected to have fallen below

detectable limits) [see Table 1].

Statistical Procedures

As in Experiment 1, animal body weights during the period of acquisition testing

were analyzed using a Groups x Weeks repeated measures analysis of variance

(ANOVA) test, with Weeks serving as the within factor. In all cases, Neuman-Keuls

post hoc procedure for determining mean differences was used for individual

comparisons. The mean total number of lever presses for each 2-day testing session at

each dose (saline, .01, .02, .04, .08) of methamphetamine, were analyzed using analysis

of variance (ANOVA) tests.

<center>**Results**</center>

Body Weights

Body weights for animals during reinstatement testing did not differ between

groups (mean body weights= 357g±5.58 and 364g±7.49 for Groups 0-mg and 16-mg,

respectively; $p< .05$). Weekly fluctuations did occur but the pattern of change remained

uniform across Groups.

Methamphetamine Self-Administration

Baseline Stability. Table 2 presents the mean active (methamphetamine) lever

responses and SEM values (in parenthesis) over the respective 2-day baseline training

sessions that preceded each testing dose for control (Group 0-mg) and lead-exposed

(Group 16-mg) animals. It is apparent that significant shifts in baseline responding did

not occur over the course of dose–effect testing for either group, and that self-

administration responding at the baseline dose of .04 mg/kg was essentially the same for

Group 0-mg and Group 16-mg (average range). The finding of a nonsignificant Group

main effect provided statistical confirmation to this effect.

Table 2 *Mean active (methamphetamine) lever responses and (SEM) values over the respective 2-day baseline training sessions that preceded each testing dose for control (Group 0-mg) and lead-exposed (Group 16-mg) animals.*

	Saline	**.01**	**.02**	**.04**	**.08**
Group 0-mg	61.80 (6.62)	76.02 (11.12)	66.75 (4.89)	71.30 (6.46)	66.67 (5.98)
Group 16-mg	52.01 (6.10)	74.83 (6.98)	65.98 (4.63)	63.92 (4.36)	65.83 (4.93)

Dose-Effect Data

Active methamphetamine self-administration and inactive lever responding are

depicted graphically in Figure 3. The statistical unit was the average number of lever

responses for each animal across the two test sessions at each dose of methamphetamine.

At all methamphetamine doses over the course of dose-effect testing, Group 16-mg

animals responded less frequently than Group 0-mg animals. Group differences were

significant at saline (F (1, 14)=38.91, $p<0.05$), .01 mg/kg (F (1, 14)=4.88, $p<.05$), and

.02 mg/kg (F (1,14)=3.67, $p<.05$) doses. The results of the Group x Dose analysis

performed on the number of active lever presses showed a significant effect of Group (F

(1, 13)=13.03, $p<0.05$). The only other effect that reached an acceptable level of

statistical significance was the main effect of Dose (F (4, 52)=97.90, $p<0.05$).
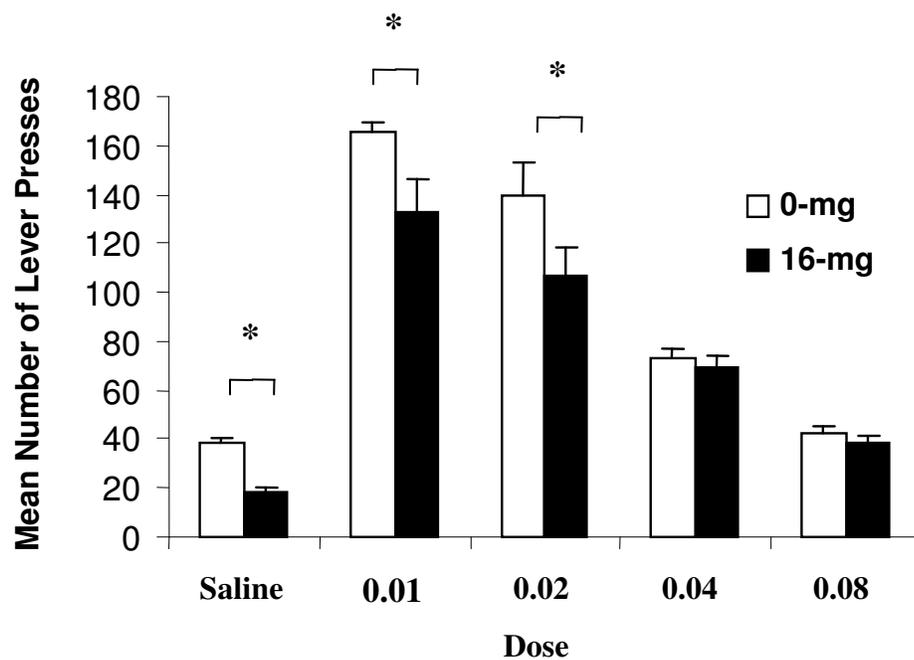
Fig. 3 Mean (SEM) active lever presses for Group 0-mg (*n*=7) and 16-mg (*n*=8)
at each dose of methamphetamine during dose-effect testing. The *asterisk*
indicates that control and lead-exposed animals were significantly different
(*p*< 0.05).

In terms of the analysis of inactive lever responding, neither the main effect for

Group nor the Groups x Dose interaction was found to be significant. A significant

effect of Dose was found (F (4, 52)= 97.89, $p<0.05$), and subsequent comparisons

indicated inactive lever responding was more frequent at higher test doses of methamphetamine. Active lever responding was greater than inactive lever responding at every test dose ($p<0.05$).

At the end of Experiment 2, as in Experiment 1, each animal in both exposure conditions received an i.v. infusion of 7.50 mg/kg sodium pentobarbital. Catheter patency was verified by rapid onset of brief anesthesia. Each of the animals included in this report had open catheters throughout the study.

Tissue Analyses

The mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning in a comparable set of animals as was used in Experiment 2 are presented in Table 1. Likewise, blood lead concentrations, a conventional marker of lead toxicity, are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. Lead concentrations in tissues are indicated for dams sacrificed at weaning (see, Table 1).

EXPERIMENT 3

The findings from Experiment 2 reflect a general decrease in the self-administration of methamphetamine among animals developmentally exposed to lead. In other animal studies of hedonic drug use, such a behavioral shift is characteristic of receptor antagonism and may derive from the diminished reinforcement potency of the drug (Caine *et al*, 2000). It is unclear from Experiment 2 what produced the equilibration in responding at the higher doses of methamphetamine. Higher doses of methamphetamine may have overridden lead effects that were responsible for the decreased responding in lead-exposed animals at the lower doses of the drug tested. Also, higher doses of methamphetamine may have produced satiation in both control and lead-exposed animals at a similar rate. Low rate FR schedules of reinforcement pose some weaknesses in that they may not offer more than a general index of rate of drug intake. Thus, simple FR schedules may be inappropriate in studies attempting to assess changes in the reinforcing effects of drugs of abuse. A more challenging schedule of reinforcement, such as progressive ratio (PR), has been cited as offering a more direct measure of the rewarding efficacy of a given dose of a drug (Arnold and Roberts, 1997).

In an effort to clarify interactive effects between perinatal lead exposure and methamphetamine self-administration testing during the adult cycle, Experiment 3 tested non-exposed animals (Group 0-mg) and animals exposed to lead (Group 16-mg) during gestation and lactation on a PR task in which i.v. methamphetamine deliveries served as the reinforcer. In PR procedures, rats complete increasing FR requirements to obtain successive infusions of the reinforcer.

Methods

Animals

As before, female rats were gavaged daily with 0-mg or 16-mg lead for 30 days prior to breeding with non-exposed males. Metal administration continued through pregnancy and lactation and was discontinued at weaning (PND 21). Animals born to control or lead-exposed dams received indwelling jugular catheters as adults and were randomly assigned to one of the four studies. In Experiment 3, animals were tested on a PR schedule in order to more explicitly determine the nature of the change in sensitivity to the drug.

Surgical Procedure

Surgeries were performed at PND 60 using a different surgical procedure than in Experiments 1, 2, and 4. Using a headplate technique, implantation of chronic indwelling jugular catheters was performed under aseptic conditions. Rats were anesthetized with a combination of intraperitoneal (i.p.) injections of ketamine (60 mg/kg) and xylazine (20 mg/kg). Immediately after surgery rats were administered an intramuscular (i.m.) injection of penicillin g potassium (250,000 U/ml). A stereotaxic instrument was used for preparation of the skull of rat prior to implantation of the catheter. Following implantation into the jugular vein, the tubing was passed subcutaneously to the skull of the rat. A pedestal was constructed of dental acrylic fixing the assembly to the skull. The rat was allowed 7 days recovery from surgery before testing began. During this recovery period, each rat received daily i.v. infusions (0.1 ml) of sterile saline solution containing heparin (1.25 U/ml). Cannulae were flushed

daily with heparin saline both prior to and following each testing session.

Apparatus

Experiment 3 employed different apparatus than Experiments 1, 2, and 4. Sixteen operant conditioning chambers (Med Associates, ENV-001; St. Albans, VT) in sound-attenuating cubicles served as the test apparatus. Each chamber had two levers and a stimulus light located above each lever. Infusion pumps (model A with 1-rpm motors, Razel Scientific Instruments; Stamford, CT) controlled drug delivery to each of the boxes. A 20-ml syringe delivered i.v. infusions (100 µl) over a 12.0-s time frame.

All animals received free access to food and water for 7 days while recovering from surgery. Uncontaminated water was available *ad libitum* throughout the study. Animals were weighed daily prior to testing. Food was placed in home cages following the end of each daily testing session.

Procedure

Shaping procedures and baseline training followed the format of Experiments 2 and 4, i.e., methamphetamine was used to shape animals for 5 days, at which point all animals were switched to the baseline-training dose of methamphetamine (.04 mg/kg) for 8 days. The PR schedule followed closely the procedures outlined by Duvauchelle *et al* (1998), as well as those summarized in a previous report by Hubner and Koob (1990). The PR schedule used in this investigation involved an exponential equation

in which the reinforcement number is a natural logarithmic function of the ratio value:

ratio =5 x exp (reinforcer number x 0.2) –5. For example, for 16 reinforcements, the

ratio progresses as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118.

The PR session ended according to which occurred first; 3 hrs or when the

animal failed to complete the ratio for a particular reinforcer within 1 hr from the

delivery of the previous reinforcer (breaking point). For both control and lead-exposed

animals, a within-subjects assessment procedure was administered with animals

receiving doses of vehicle (heparinized saline), .01, .02, .04, .08 mg/kg per infusion

methamphetamine in a random order. As in Experiments 2 and 4, a stability criterion

was imposed between PR tests sessions. Animals were required to respond for .04

mg/kg methamphetamine across two consecutive 2-hr daily baseline sessions. After

stability was obtained on the baseline dose (responding varied less than 20% across

sessions), animals were shifted to the PR schedule for a two-day testing session.

Contingencies were in place until the breaking point/3-hr session length criteria was

satisfied. Another method of analysis that considers the amount of time the animal

continues to pursue a drug within a given PR schedule is often included. This measure

of Session Duration adds to the understanding of active drug-seeking and reflects the

motivation to obtain the drug as well as to index reinforcer efficacy. Latency to initiate

the first active lever press response also was considered as a behavioral endpoint.

Catheter patency was assessed daily by flushing the catheter with 0.1 ml of a sterile

saline solution containing heparin (1.25 U/ml).

Tissue Collection and Analyses

The mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning for animals that underwent a comparable perinatal lead-exposure regimen are presented as a point of reference (see, Table 1). Likewise, blood lead concentrations, a conventional marker of lead toxicity, are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. Also, lead concentrations in tissues are indicated for dams sacrificed at weaning (see, Table 1).

Statistical Procedures

The mean total number of lever presses for each 2-day testing session at each dose (saline, .01, .02, .04, .08) of methamphetamine, were analyzed using analysis of variance (ANOVA) tests.

<div align="center">Results</div>

Body Weights

Body weights for animals during progressive ratio testing did not differ between groups (mean body weights= 364g±7.12 and 373g±8.42 for Groups 0-mg and 16-mg, respectively; $p > .05$). Weekly fluctuation did occur but the pattern of change remained stable across groups.

Progressive Ratio Data

Baseline Stability. Table 3 presents the mean and SEM baseline values

(.04mg/kg methamphetamine (active) lever responses) during the 2-day baseline session

that preceded each respective PR testing session at each dose. As expected, baseline

performance remained stable for each exposure condition throughout the period of

testing.

Table 3. *Mean and (SEM) values for average number of active (methamphetamine)*
lever responses during the 2-day baseline session that preceded each respective PR
testing session at each dose.

	Saline	**.01**	**.02**	**.04**	**.08**
Group 0-mg	46.18 (9.09)	46.67 (5.19)	43.86 (4.48)	47.19 (7.38)	46.88 (5.02)
Group 16-mg	49.50 (8.63)	34.02 (6.20)	44.33 (5.76)	41.30 (3.20)	49.16 (7.31)

Total Number of Lever Presses. Figure 4 shows the respective total mean number of lever presses on the active (methamphetamine) lever for Groups 0-mg (±) and 16-mg (±) at the different doses of methamphetamine reinforcement. The results of the Group x Dose repeated-measures ANOVA on total number of lever presses revealed a main effect for Dose ($F_{(4, 48)} = 21.49$, $p < 0.05$), as well as a main effect for Group ($F_{(1,12)} = 34.01$, $p < 0.05$). Individual comparisons indicated that these differences derived from decreasing total number of lever responses per session in lead-exposed animals associated with lower methamphetamine reward outcomes, and that the total number of lever presses per session for Group 16-mg was uniformly lower than the responses of Group 0-mg animals, across the various doses employed in PR testing.

Tissue Analyses

The mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning in a comparable set of animals as was used in Experiment 3 are presented in Table 1. Likewise, blood lead concentrations, a conventional marker of lead toxicity, are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. Lead concentrations in tissues are indicated for dams sacrificed at weaning (see, Table 1).
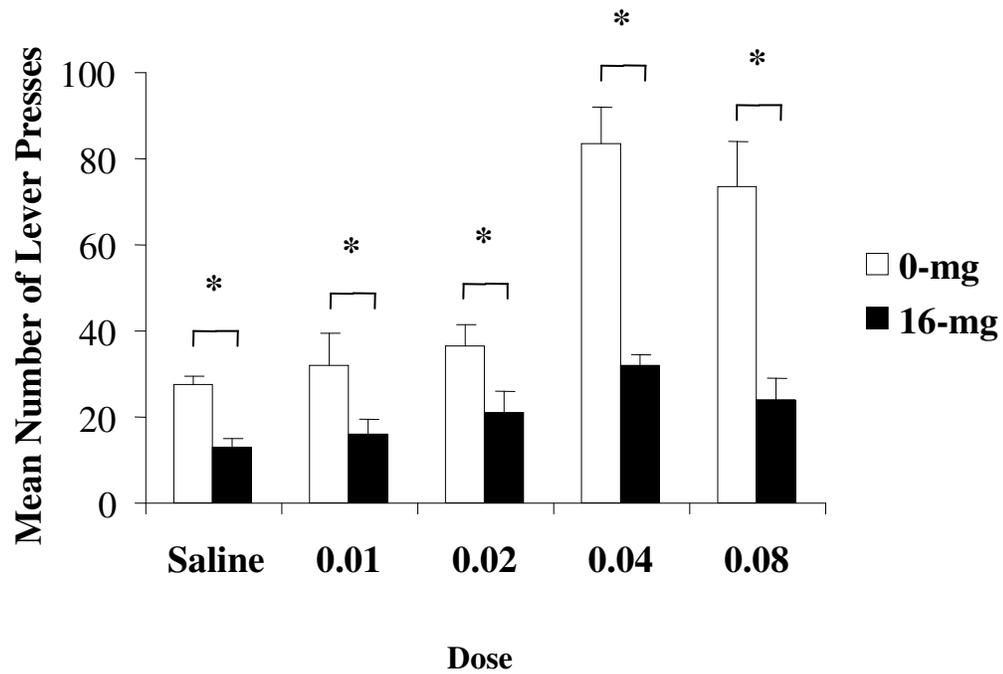
Fig. 4 Mean (SEM) total number of lever presses in a Session, for Groups 0-mg and 16-mg at each methamphetamine dose tested during the progressive-ratio procedure. The *asterisk* indicates that control and lead-exposed animals were significantly different ($p<0.05$).

EXPERIMENT 4

Drug abuse is characterized by a high incidence of relapse to drug taking that can become manifest after years of abstinence from drug administration (Cornish *et al*, 1999). Relapse to drug abuse arises from intense craving that can be triggered by a single drug prime or from a conditioned environmental stimulus such as a stressor or a drug-associated cue. Elevated dopamine transmission in the nucleus accumbens is thought to be a primary mediator of addiction to most drugs of abuse, and methamphetamine abuse is no exception. However, glutamate transmission, and not dopamine transmission, is more highly implicated after repeated exposure to stimulants. For example, craving-induced relapse has been attributed to glutamate, and not dopamine transmission in the nucleus accumbens. Craving is recognized to be a primary mediator of drug-induced reinstatement of drug-seeking behavior (Cornish *et al*, 1999).

Some effects that persist for up to 1 month following the cessation of drug self-administration can be observed in the nucleus accumbens. Supersensitivity to D1-like-mediated responses (Henry and White, 1991; Wolf *et al*, 1994), decreased levels of inhibitory G proteins that inhibit cAMP formation (Self *et al*, 1995; Striplin and Kalivas, 1993; Terwilliger *et al*, 1991), and increased levels of adenyl cyclase that aid in the conversion of cAMP and cAMP-dependent PKA, may be responsible for the functional relapse that follows chronic drug use.

Methods

Animals

The lead exposure regimen followed that of previous experiments.

Surgical Procedure

As in Experiments 1 and 2, a backplate surgical technique was used in Experiment 4 to anchor the indwelling jugular catheters to the animals. Surgeries were performed on test offspring at PND 90. As before, animals were allowed 7 days to recover post-surgery.

Apparatus

As in Experiments 1 and 2, Experiment 4 employed twelve operant conditioning chambers (Model E-10-10, Coulbourn, Allentown, PA) in sound attenuating cubicles.

Procedure

At PND 90, animals were tested in daily 2-hr sessions for steady baseline self-administration of methamphetamine (.04 mg/kg per inf) on a fixed-ratio schedule where two lever presses resulted in drug delivery (FR-2 schedule). After steady-state responding was established, methamphetamine reinstatement responding was assessed for each group within an extinction paradigm. During the initial 1 hr of reinstatement testing, the previous baseline contingencies were in place, i.e., animals operated under an FR-2 schedule for an infusion of .04 mg/kg methamphetamine. During the 2 hr, 3 hr, and 4 hr of testing saline infusions were substituted for methamphetamine infusions. After responding was extinguished during hr 4, reinstatement of responding was tested by administering an intraperitoneal (i.p.) priming injection of either saline, 0.5, or 1.5

mg/kg methamphetamine. Following these injections, lever responding for saline infusions was monitored during hr 5. Between the respective reinstatement test sessions animals were given two days of .04 mg/kg/inf methamphetamine baseline testing on a FR-2 schedule in order to reestablish baseline responding and to measure possible shifts in tolerance that may have occurred following chronic exposure to the psychostimulant.

Tissue Collection and Analyses

The mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning in a comparable set of animals as was used in Experiment 4 are presented in Table 1. Likewise, blood lead concentrations, a conventional marker of lead toxicity, are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. Lead concentrations in tissues are indicated for dams sacrificed at weaning (see, Table 1).

Statistical Procedures

Statistical analyses of behavioral profiles included a 2 Groups (0-mg, 16-mg) x 4 Dose (saline, 0.5, 1.5) repeated measures ANOVA performed on number of responses on baseline days prior to reinstatement testing. Separate 2 Groups x 2 Levers x 4 Doses repeated measures ANOVAs were performed on the hourly data, with Group serving as the between factor and Dose serving as the within factors.

Results

Body Weights

Experiment 4 animals started testing at a later PND. Thus, body weights and lead concentrations for animals in Experiment 4 were higher than for animals in Experiments 1, 2, and 3. Body weights for animals during reinstatement testing did not differ between groups (mean body weights= 364g±5.31 and 373g±7.12 for Groups 0-mg and 16-mg, respectively; $p>$ .05). By the completion of testing, blood lead levels of lead-exposed animals had returned to control levels, i.e., in both groups lead levels had fallen below detectable limits (<1 µg/dl) [data not shown].

Baseline Data

Separate analysis of variance (ANOVA) tests were performed on the number of lever presses made during baseline (hr 1) and during each successive three hr sessions of extinction testing at each reinstatement dose. For reinstatement testing (hr 5), the behavioral endpoint was the percent change from baseline (hr 1), i.e., hr 5 performance was compared to hr 1 performance at each reinstatement dose for each animal.

Reinstatement Data

Statistical analyses of behavioral profiles included a 2 Groups (0-mg, 16-mg) x 2 Levers (inactive, active) x 4 Dose (saline, 0.5, 1.0, 1.5) repeated measures ANOVA performed on number of responses on baseline days prior to reinstatement testing. Although more active than inactive lever responses were exhibited, the results of the analysis failed to indicate significant differences by Group or Dose (all $F<1$). Consequently, it was established that the baseline dose of .04 mg/kg methamphetamine

remained stable across reinstatement testing Group 0-mg (52.03±10.60) and Group 16-mg (52.18±7.16) were not differ statistically.

Figure 5 (left panel) presents the inactive and active (methamphetamine) lever responses for Group 0-mg and Group 16-mg during the initial 1-hr session at each dose of reinstatement. During this initial period baseline conditions were in place, i.e., FR-2 responding resulted in an infusion of .04 mg/kg methamphetamine. Shown in the additional panels of Fig. 5 are the response records of the two exposure groups across the 3 hrs of extinction (hrs 2-4) where completion of the FR-2 resulted in illumination of the stimulus light, but only a heparinized saline solution was delivered. Separate 2 Groups x 2 Levers x 4 Doses repeated measures ANOVAs were performed on the hourly data, with Group serving as the between factor and Dose serving as the within factors. The results of the analysis of hr 1 failed to show a significant main effect of Groups, and the Groups X Reinstatement Dose interaction was found to be nonsignificant (all $Fs < 1$). Both groups responded significantly more on the active lever that resulted in methamphetamine deliveries, relative to inactive lever responding [$F (1,14)=272.59$, $p<0.01$]. These findings basically replicate the aforementioned baseline results. Although there was some indication of group separation during hr 2, the analysis of the data from hr 2 revealed only a significant main effect of Levers [$F (1,14)=67.53$, $p<0.05$]. It is visually apparent from Figure 5 that during hrs 3 and 4 inactive and active lever responding had virtually ceased for both exposure conditions.

The methamphetamine percent of baseline reinstatement data for hr 5 (reinstatement testing) for Groups (0-mg, 16-mg) X 4 Reinstatement Dose (saline, 0.5,

1.0, 1.5 mg/kg) repeated measures ANOVA was performed on the percent of baseline

measure (see Figure 6). Clearly, there was a dose-dependent increase in reinstatement

on the active (methamphetamine) lever for both control and lead-exposed conditions as

supported by a main effect for Reinstatement Dose [$F_{(3,42)}=10.90$, $p<.05$]. More

importantly regarding the rationale that formed the basis for conducting Experiment 4, a

main effect of Groups was found ($F_{(3, 42)}=4.59$, $p<.01$). Subsequent comparisons

revealed that this effect was due to lower percent of baseline responding on the part of

Group 16-mg animals, and this effect was prominently due to lower percent of baseline

responding by lead-exposed animals at the reinstatement doses of .50 mg/kg and 1.50

mg/kg methamphetamine ($F_{(3,42)}=4.59$, $p<.01$).

Tissue Analyses

The mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and

metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and

weaning in a comparable set of animals as was used in Experiment 3 are presented in

Table 1. Likewise, blood lead concentrations, a conventional marker of lead toxicity, are

shown for littermates at PND 1 and PND 21, as well as for test animals at the

termination of the experiment. Lead concentrations in tissues are indicated for dams

sacrificed at weaning (see, Table 1).
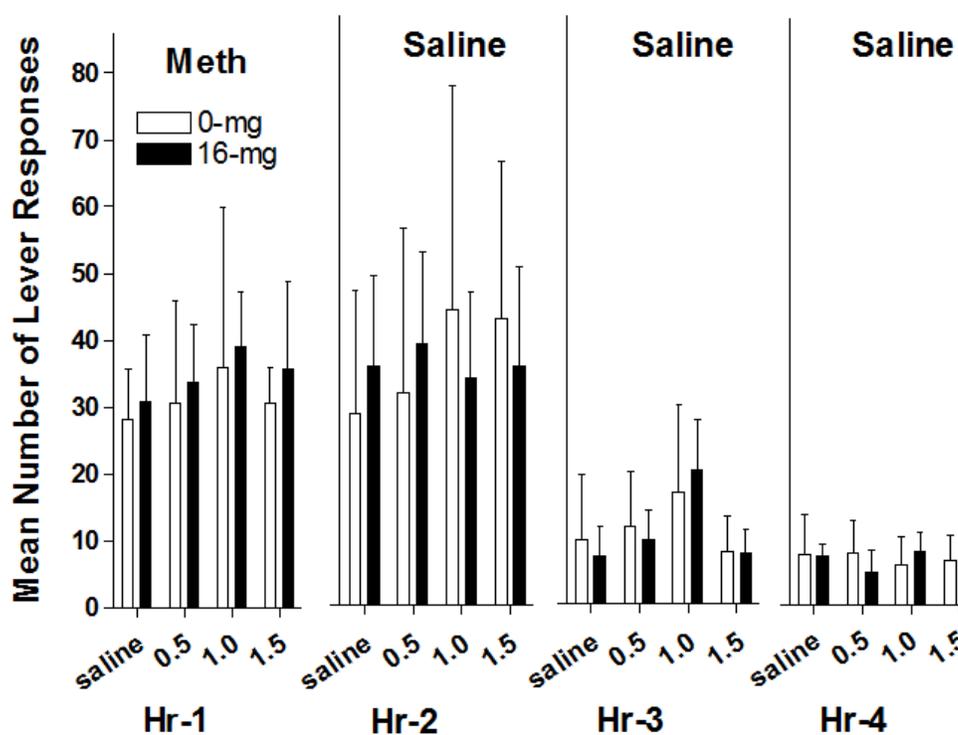
Fig. 5 Mean (SEM) number of active lever responses for Groups 0-mg (*n=9*) and 16-mg (*n=7*) during hrs 1-4. Methamphetamine (.04 mg/kg) was available during hr 1 and saline infusions were delivered during hrs 2-4.
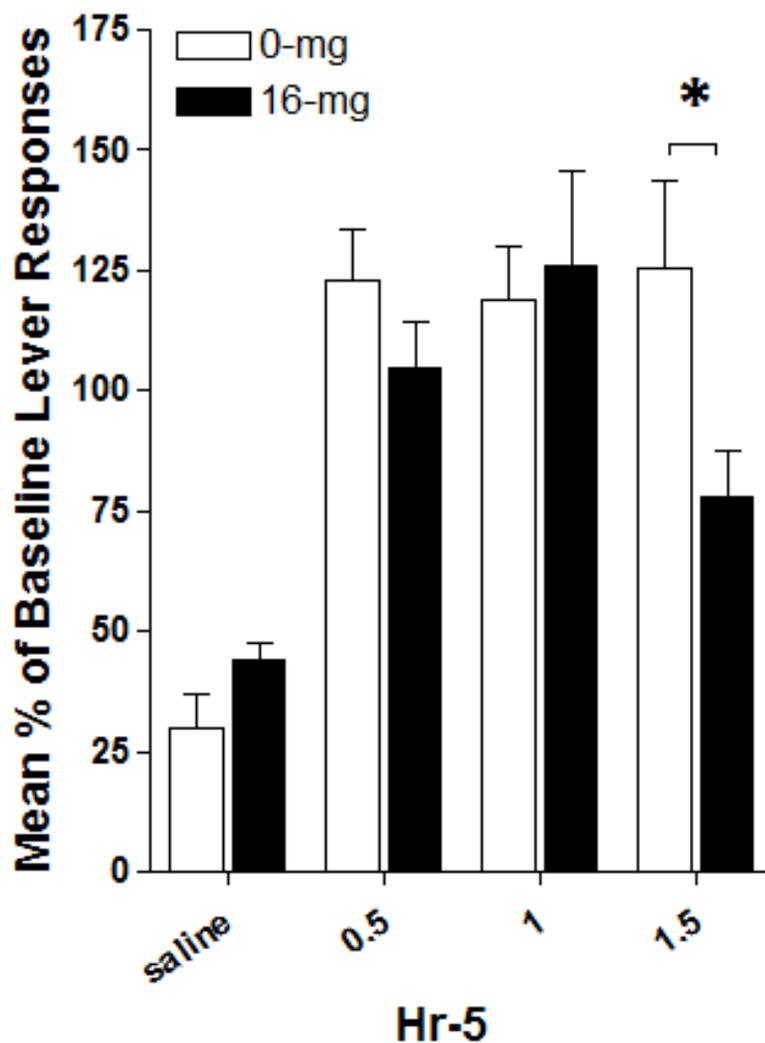
Fig. 6 The mean (SEM) % of baseline responding for Groups 0-mg and 16-mg during hr 5 (reinstatement testing) after saline was substituted for .04 mg/kg methamphetamine (i.v.). Drug priming injections (i.p.) preceded reinstatement testing. The *symbol * above the bar* indicates that Group 16-mg administered more saline infusions following the 1.5 mg/kg prime than the control counterparts (Group 0-mg); $p < 0.05$.

DISCUSSION AND CONCLUSIONS

Findings from Experiment 1 revealed that perinatal (gestation/lactation) exposure to environmentally relevant levels of lead resulted in a smaller percentage of rats reaching the criterion for intravenous (i.v.) methamphetamine (.02 mg/kg) acquisition (30 infusions within the 3-hr instrumental session) during a 35-day training regimen, relative to non-exposed controls. In Experiment 2, animals were tested in an FR-2 schedule of reinforcement employing various doses of methamphetamine. The dose-effect curve yielded a biphasic pattern of attenuation of the self-administration of methamphetamine in lead-exposed animals, with group differences significant at saline and the lower doses tested (i.e., .01 mg/kg and .02 mg/kg). In Experiment 3, a progressive ratio task showed significant group differences in the rewarding efficacy of methamphetamine. Overall, lead-exposed animals showed an attenuated response to the doses of methamphetamine tested, in comparison to control animals. Finally, Experiment 4 tested the propensity for animals to relapse to methamphetamine self-administration after a period of forced abstinence. As expected, animals perinatally exposed to lead were less likely to reinstate methamphetamine drug-seeking following a drug-cue (i.e., methamphetamine injection [i.p.]) when compared to controls. Group differences were most significant at the highest priming dose of methamphetamine tested (1.5 mg/kg). Generally, lead exposure appeared to decrease the rewarding potency of methamphetamine across various doses when compared to animals not exposed to the heavy metal.

The interactive patterns evident for perinatal lead exposure and methamphetamine agree with earlier findings associated with heroin self-administration, but are at odds with the results from previous investigations of the effects of developmental lead exposure on cocaine self-administration. That is, early lead exposure has been shown to attenuate the i.v. self-administration of heroin across a broad range of doses (Rocha *et al*, 2004). In contrast the reinforcing efficacy of cocaine is apparently amplified by perinatal lead exposure in an acquisition preparation identical to that used in Experiment 1 (Rocha *et al,* 2005), and the reinstatement procedure used in Experiment 4 was associated with enhanced cocaine relapse responding in lead-exposed animals following post-extinction administration of various priming injections of cocaine (Nation et al 2003). Moreover, a leftward displacement has been observed in the cocaine dose-effect curve among animals developmentally exposed to lead (Nation *et al,* 2004).

The differential effects of perinatal lead exposure on the phenomenology of self-administration of various psychoactive drugs is not altogether surprising given the complexity of the patterns of neuroadapation associated with chronic drug taking (Self 2004; Wolf *et al*, 2004). Though methamphetamine and cocaine are categorized as psychostimulants, both drugs are not chemically identical. Pharmacologically, methamphetamine, but not cocaine, reverses the uptake site for dopamine, norepinephrine, and serotonin not only increasing the length of time the neurotransmitters remain in the synaptic cleft, but also further releasing neurotransmitters into the synapse. In this way, methamphetamine produces a rapid,

though arguably reversible, decrease in serotonin and dopamine receptor function in striatal synaptosomes. Perhaps due to overexcitation of serotonergic and dopaminergic neurotransmission, methamphetamine (but not cocaine) produces profound neurotoxicity at dopamine and serotonin nerve endings, especially in the caudate nucleus (Rothman *et al*, 2003).

In the case of methamphetamine, GABAergic, not glutamatergic, transmission appears to be more highly implicated in behavioral sensitization. Valproate, a mood stabilizing anticonvulsant that increases GABAergic transmission by inhibiting GABA transminase (GABA-T), inhibits the development of methamphetamine- and cocaine-induced behavioral locomotor sensitization. Repeated administrations of valproate also decrease the expression of methamphetamine-induced behavioral sensitization in animals, but are unable to suppress the same expression of sensitization after a cocaine challenge (Li *et al*, 2005). Gabapentin, another drug that increases GABAergic inhibition, was found to attenuate the expression of sensitization to methamphetamine, but not to that of cocaine (Itzhak and Martin, 2000). These studies suggest that GABA plays a larger role in the expression of methamphetamine sensitization, than that of cocaine. Perhaps of importance, lead contamination is known to disrupt GABA availability by decreasing amounts of evoked release (Lasley *et al*, 1999).

Different neurochemical functions can be observed between methamphetamine and cocaine using microdialysis techniques. For example, rats responded differently to a challenge injection during a withdrawal period from either cocaine or methamphetamine (Zhang *et al*, 2001). Methamphetamine was shown to produce a larger accumulation of

dopamine in the extracellular space than cocaine. Specifically, a challenge injection of

methamphetamine on day 11 following 10 daily injections resulted in a greater elevation

of extracellular dopamine in the caudate putamen when compared to acute exposure of

methamphetamine. By contrast, a challenge injection of cocaine resulted in lower

dopamine levels in the caudate putamen than those observed for acute exposure of

cocaine. In addition, a cocaine challenge increased glutamate overflow in the caudate

putamen and the nucleus accumbens; whereas, a methamphetamine challenge increased

glutamate overflow in the caudate putamen, but it decreased glutamate in the nucleus

accumbens (Zhang *et al*, 2001). These findings suggest that some brain regions are

highly implicated in specific functions of drug addiction, more so than others (e.g., VTA

is mainly involved in the development of sensitization to psychostimulants, whereas the

nucleus accumbens is involved in the expression of sensitization). Thus, some

neurotransmitters may be more highly implicated in a particular phase of drug addiction

(i.e., acquisition, maintenance, extinction) and may exert more pronounced effects on

specific drugs of abuse when a particular brain region is activated more so than another.

In addition, neurochemical changes that occur during initial drug administration differ

both quantitatively and qualitatively from those observed after chronic drug exposure.

Given the differences between various drugs of abuse, what is at issue is

identifying the precise effects of lead exposure on mechanisms underlying functional

changes in sensitivity to drugs possessing abuse liability. Although there is a sizeable

literature on the effects of postweaning lead exposure on relevant drug-related neural

systems (Cory-Slechta, 1995), our understanding of the effects of preweaning lead

exposure on neural mechanisms central to defining drug reactivity is limited (Devoto *et al*, 2001). Even more importantly, virtually no information exists regarding the potential enduring mechanistic changes caused by early lead exposure in instances where the exposure regimen has been discontinued, as was the case in the present investigations. Surely, the present behavioral findings argue that perinatal lead exposure produces long-lasting perturbations in neural mechanisms that regulate methamphetamine intake and the propensity for relapse.

The observed differences between control animals and those exposed to lead, perhaps are due to lead-effects early in development that rewire the brain circuitry and create differing levels of involvement by the major neurotransmitters implicated in drug-seeking and –taking. The same brain regions that are integral in modulating the rewarding effects of drugs with abuse liability also are sites for the accumulation and possible negative effects of lead toxicity (Cory-Slechta *et al*, 1997). Gene and protein expression of specific glutamate subunits in the morphologically immature brain is known to be impaired by developmental lead exposure (Guilarte, 1998; Guilarte and McGlothan, 2003; Guilarte and Miceli, 1992; Guilarte *et al*, 2003). Perhaps alterations in glutamatergic function contribute to long-lasting changes of methamphetamine, (Ohmori *et al*, 1994; Mark *et al*, 2004; Sonsalla *et al*, 1989) heroin (Xi and Stein, 2002) and cocaine (Kalivas, 2004) effects observed elsewhere and presently in Experiments 1-4.

An expanding literature shows that urban, minority children from low-income families are more frequently targets for lead exposure and exhibit unsafe levels of metal

traces in blood and residual teeth (Brody *et al*, 1994). Inasmuch as the urban sub-population also is presented with increased challenges associated with drug use and abuse, the health industry should be especially vigilant to potential links between environmental pollution and drug-abuse liability (Ensminger *et al*, 1997).

Both direct and indirect determinants of drug sensitivity that may be altered by perinatal lead exposure should be considered. Because lead-exposed pups initially exhibited lower body weights than controls, early malnutrition may have produced adverse neuroadaptations that affected self-administration of drugs into the adult phase. In addition, disturbances in metabolic conversion and drug distribution/absorption may persist for lengthy periods following early lead exposure. Another confounding variable may be that children exposed to lead are more likely to live in communities where the incidence of low maternal IQ, poor diet, delinquency and exposure to multiple environmental toxicants are high. Particularly salient to children of families who produce or consume methamphetamine are the risks of malnutrition. Calcium, iron, zinc or protein deficiencies that are characteristic of malnourished individuals have consistently been shown to increase lead absorption (Hubbs-Tait *et al*, 2005; Lidsky and Schneider, 2003), perhaps further contributing to lead-induced cognitive and behavioral disturbances. Socioeconomic factors that are highly correlated with populations in enriched/impoverished areas are concerns that must be taken into account in the interpretation of epidemiologic findings (Hubbs-Tait *et al*, 2005).

It is important to note that in comparable studies using the identical perinatal (gestation and lactation) lead exposure regimen that were used presently in Experiments

1-4, lead had gained clearance from blood, brain, liver, kidney and bone (tibia) by the

end of testing, yet the altered behavioral effects persisted (Nation *et al*, 2003; 2004;

Rocha *et al*, 2004). These tissue analyses suggest that relatively permanent neuronal

alterations in methamphetamine related circuitries occur during a critical period of

developmental lead exposure. Disturbingly, it is possible that lead-induced alterations to

the organism are taking place *in utero* and have long-lasting and far-reaching effects into

adulthood. This fact is of particular salience among the methamphetamine- using and -

producing population. Although the incidence of lead-exposure has decreased among

the general U.S. population, there is evidence to suggest that lead acetate is being used in

the manufacture of methamphetamine, thus elevating blood lead levels of individuals

using lead-contaminated methamphetamine (Gulson *et al*, 1997).

The findings reported here of lead-based antagonism of methamphetamine drug-

taking and –seeking must be interpreted cautiously. Attenuation of the psychoactive

properties of drugs with abuse potential may functionally translate into a form of

tolerance or counteradaptation (Koob and Le Moal, 1997; Nation *et al*, 1996a). Under

such conditions drug self-administration has been shown to increase (Corrigall and

Coen, 1991; Koob *et al,* 1987), perhaps in a compensatory effort to regulate the level of

subjective affect (Koob and Le Moal, 1997). Accordingly, it must be considered that the

apparent antagonism of methamphetamine by developmental lead exposure may actually

dispose a drug user to take in greater amounts of the drug once drug-taking behaviors are

reliably repeated. In this regard, the present findings could be instructive with respect to

identifying possible external (environmental) risk factors associated with the movement

from methamphetamine use to methamphetamine abuse.

In summary, drug abuse is a multifaceted problem causing society billions of dollars in loss annually and inestimable costs to the individual, his/her family, and loved ones. Various genetic and psychosocial factors no doubt present a propensity to seek out and administer drugs of abuse. Experiments 1, 2, 3 and 4 of this work help to further elucidate the role of environmental toxicants as possible activating sources in drug selection, drug-seeking, use, and abuse. The data collected from these four studies, along with previous data on the subject, further strengthen the possibility that pollutants in the environment may play a modulatory role in the devastating and destructive health issue that is substance abuse.

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