ACQUISITION OF COCAINE AND HEROIN SELF-ADMINISTRATION IN RATS DEVELOPMENTALLY EXPOSED TO LEAD

A Thesis

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

MASTER OF SCIENCE

May 2005

Major Subject: Psychology

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Approved as to the style and content by:

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ABSTRACT

Acquisition of Cocaine and Heroin Self-administration in

Rats Developmentally Exposed to Lead. (May 2005)

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Dr. Paul Wellman

Rationale: The rate of acquisition of drug self-administration may serve as a predictor of later drug-taking behavior, possibly influencing vulnerability to initiate drug use. *Objectives:* The present study examined the effects of perinatal (gestation/lactation) lead exposure on adult rates of acquisition of intravenous (i.v.) heroin self-administration and cocaine self-administration using an automated procedure that included both Pavlovian and operant components. *Methods:* For Experiment 1, female rats were gavaged daily with 0 or 16 mg lead for 30 days prior to breeding with nonexposed males. Metal administration 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 and subsequently were tested daily in a preparation where sessions included an initial 3-hr autoshaping period followed by a 3-hr self-administration period. During autoshaping, heroin (.018 mg/kg) infusions were paired with the extension and retraction of a lever when a lever press was not made for 15 sec, while infusions occurred during self-administration only when a lever press was executed (FR-1). The criterion for acquisition was a 2-day period during which a mean of 10 infusions/session occurred during self-administration. Animals were given 35

days to reach criterion. *Results:* Findings from Experiment 1 showed the proportion of rats meeting the lever-press response criterion for heroin when tested as adults was lower among lead-exposed animals. In Experiment 2, cocaine (.20 mg/kg) was presented to animals that underwent the same metal-exposure regimen, surgical procedures and methods with variations only in the number of infusions that were automatically administered during the Pavlovian component. Criterion for cocaine acquisition was a mean of 50 infusions over a two-day. In Experiment 2, a greater proportion of lead-exposed animals reached the criterion for cocaine acquisition. *Conclusions:* Developmentally lead-exposed animals showed a decrease in vulnerability to initiate drug-taking behavior when presented with heroin in the adult phase, relative to controls. In contrast, developmentally lead-exposed animals showed an enhanced vulnerability to reach the criterion for cocaine self-administration. Clinical relevance of developmental exposure to lead and the attendant vulnerability to self-administer drugs of abuse is discussed.

To my family.

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INTRODUCTION

Environmental lead emissions plummeted following the phasing-out of leaded paint in 1978, and lead-based gasoline in the early 1980's (Hubbs-Tait, 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 are equal to or exceed 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 toxicity. 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 contain higher concentrations of past emissions from lead-based gasoline and particles from lead-based paint that are readily airborne (Lanphear et al., 2002).

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This thesis follows the style of Psychopharmacology.

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, despite this decline, research suggests that even present low-level, chronic lead exposure (<10 μg/dL) can produce neurophysiological and neurobehavioral deficits (Canfield et al., 2003; Needleman et al., 1990, 2002; Tong et al., 1998), even in children as young as 3-5 years of age (Canfield et al., 2003). Paradoxically, recent evidence suggests that blood lead levels in children may be negatively correlated with IQ scores and ability tests, implying that higher concentrations of lead in blood may initiate neuroprotective cellular mechanisms that are not activated in the presence of lower blood lead levels [<10 μg/dL] (Bellinger and Needleman, 2003).

Lead is stored in bone for up to three decades and can be mobilized and released into blood plasma during periods of stress and high calcium demands, such as during pregnancy (Barltrop, 1968; Gulson et al., 1997; Horiguchi et al., 1959; Roelfzema et al., 1987) and lactation (Silbergeld, 1991). Maternal blood lead levels reach a peak during the second trimester, at which point the metal is readily transferred to the fetus. Because approximately 45-70% of lead in the blood of reproductive age women originates from long-term tissue stores (Gulson et al., 1995), and lead easily crosses the placental barrier (Angell and Lavery, 1982; Barltrop, 1968; Weizsaecker, 2003), women exposed to high levels of lead prior to more stringent CDC regulations will give birth to children who have correspondingly elevated blood lead levels.

Absorption and Excretion

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 penetrates the soft bone structure (Weizaecker, 2003). Consequently, children born to lead-exposed mothers 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 ingested lead is absorbed by the body and 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).

Lead that is inhaled is more easily absorbed than ingested lead. If inhaled 20% to 40% will be 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. Smaller lead particles are more likely to be sequestered by kidney where they later can be released into the bloodstream. Larger particles are expelled by the respiratory tract or

trapped in mucus secretions and transported by ciliary action to the larynx where lead-containing particles are ultimately swallowed for more efficient excretion (Barltrop, 1979). Thus, residual lead from decades ago may continue to produce exceedingly deleterious health deficits that appear to be persistent and irreversible (Canfield et al., 2003).

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 will be a focus in this 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 of 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) antagonists may prevent cells from becoming integrated into a neural network. If this occurs, inhibitory neurotransmitter systems that are late to develop can be prematurely deleted as

unnecessary 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 (Farber and Olney, 2003).

In addition to cellular changes in utero, lead continues 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 will be expressed. ALAD-2 appears to be differentially expressed in ethnic groups, with approximately 11%-20% of Caucasians (Benkman et al., 1983) and virtually no African-Americans examined expressing this allele. This is of importance as adolescents carrying ALAD-2 performed better on a battery of neuropsychological tests compared to those homozygous for the more common variant of ALAD, 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 calcium (Silbergeld et al., 1980). Lead disruptions of mitochondria can also produce excitotoxicity in otherwise normal glutamate transmission (Lidsky and Schneider, 2003).

As with ALAD-2, glial cells also may serve to protect 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-Castigliani, 1989). Along these lines, younger astroglia, rather than older, are more efficient at clearing the blood supply of lead. 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).

Dietary Deficiency

Dietary intake mediates lead absorption and excretion. Calcium, an essential nutrient in the human diet, is a cation that is implicated in the modulation of most cellular neurotransmission occurring in the central nervous system. Lead is a non-essential cation that mimics calcium-mediated functions; readily substituting for calcium when concentrations of calcium are low, or lead is present at high concentrations (Hubbs-Tait, 2005).

On a molecular level, calcium activates a chain of essential mechanisms involved in imperative stages of cellular development, such as proliferation and differentiation. These stages of cellular development are mediated by calcium-activated protein kinase C [PKC] (Bressler and Goldstein, 1991). Intracellularly, calcium activates calmodulin which stimulates several protein kinases, cyclic-Amp, and phophodiesterases, thus affecting potassium channels (Bressler et al., 1999). Long-term potentiation (LTP), a form of neural plasticity believed to be important in learning and memory (Nihei and Guilarte, 2001), is also mediated in part by calcium ions acting as second messengers. In any of these actions, lead can take the place of calcium and enter an excitable cell that ordinarily would allow for the influx of calcium. Lead also can enter a cell via voltage-sensitive calcium channels (Kerper and Hinkle, 1997). The substitution of lead ions for calcium ions impedes the natural cascade of calcium-dependent cellular mechanisms, and therein, alters neurotransmitter function.

Calcium, iron, zinc or protein deficiencies that are more frequently encountered in economically disadvantaged individuals increase lead absorption (Hubbs-Tait, 2005;

Lidsky and Schneider, 2003). This is a particular concern for women who are pregnant or lactating and require additional nutritional supplements for the developing fetus. During pregnancy and lactation, in the absence of sufficient micronutrients, lead is more readily mobilized from maternal long-term bone stores and directly transferred to the fetus. By increasing lead levels in the fetus, maternal dietary deficiencies predispose future generations to lead-induced deficits. Multiple studies have found that adequate nutrient-intake during these periods decreases 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. Anemia that develops from severe or chronic iron deficiency is present in a higher percentage of children from low- (29%) rather than higher-income (5%) families (Mahaffey, 1995). Dietary deficiencies suffered during crucial developmental years disrupt normal synaptic neurotransmission and exert effects that are of long duration.

Chemical Neurotransmission

In addition to lead-induced neurodevelopmental toxicant effects, lead can produce neuropharmacological disruptions. Through childhood and far into adulthood, lead continues to be mobilized from bone to blood plasma where it is most active and can exert its most deleterious effects. Lead interferes with normal chemical neurotransmission in the brain and many of these effects are irreversible, even at low-

level chronic doses (Canfield et al., 2003). This section will focus on neuropharmacologically-induced lead effects.

Glutamatergic Systems

Lead is an antagonist at the glutamate receptor. It binds noncompetitively at one of the four known subunits of glutamate, N-methyl-D-aspartate (NMDA). In addition to NMDA, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate (ionotropic), and g-protein coupled (metabotropic) receptors are known to compose the glutamate ion channel. Lead allows glycine, a coagonist of glutamate, to bind to the NMDA receptor, but after depolarization when the magnesium (mg++) block is lifted, lead blocks the channel, inhibiting the influx of calcium that would normally hyperpolarize the cell (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, a noncompetitive NMDA receptor antagonist, has been used to assess the receptor status of NMDA receptors in animals developmentally exposed to lead and tested as adults. Whereas Guilarte et al. (1993) reported a 31% increase in forebrain NMDA receptors in lead-exposed rats, Ma et al. (1997) found 15-41% increases in NMDA receptors throughout the hippocampus and cortex. In contrast, 15-30% decreases in NMDA receptors in multiple brain regions have been found following postweaning lead-exposure (Cory-Slechta et al., 1997). Lead-induced changes in the sensitivity, number of receptors and brain regions affected vary depending on the length and time of lead exposure (Lasley et al., 2001).

In adults, lead exposure reduces glutamatergic receptor binding in the frontal cortex, basal ganglia and hippocampus. Dopamine receptor binding and dopamine transporter sites also have been reported in these regions (Cory-Slechta, 1995). By reducing glutamatergic activity, lead impairs LTP, perhaps accounting for lead-induced cognitive impairments, including spatial memory deficits and lower IQ scores. Opiates such as methadone and morphine exert some effects by acting at the noncompetitive site of the NMDA receptor. Further, correlations have been found between the activation of NMDA receptors and resistance to opiates and the development of tolerance (Gies et al., 1997). Disturbances of the gamma-amino-butyric-acid (GABA)ergic system in the striatum and the hippocampus also have been found (Guilarte et al., 2003).

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. Administering antagonists for these receptors alters drug self-administration in rats, typically producing a pattern of increasing behavior in a fixed ratio (FR) task, but decreasing behavior during a progressive ratio task when the same drug dose is presented (Hubner and Moreton, 1991). This pattern of behavior suggests a decrease in the rewarding potency of the drug. Whereas in an FR task animals will emit additional lever presses to obtain maximal euphoria when the cost is low (i.e., FR1), in a progressive ratio task where an

exponentially greater amount of lever-presses is required for each subsequent infusion of drug, lead-exposed animals more readily stop responding.

Disruption of dopaminergic functioning that is normally involved in not only motor control but also attention, memory and executive functioning can produce a multitude of behavioral problems, including attention deficit hyperactivity disorder and cognitive impairments (Moll et al., 2001).

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] sulpride to D2 receptors in cortical areas, but not in the caudate putamen, thalamus, or nucleus accumbens (Ma et al., 1999). Conversely, by another account, D2 receptor activity was decreased in the nucleus accumbens following postweaning lead exposure with no significant changes observed in D1, D2, or dopamine transporter (DAT) changes in the striatum (Pokora et al., 1996). One hypothesis suggests that lead depletes dopamine availability, thus, an upregulation of D2 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 receptors would be expected. The time and length 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 greatly implicated in drug-taking behavior.

Cocaine exerts its neurophysiological effects by blocking the dopamine transporter and to a lesser extent norepinephrine and serotonin transporters (Rocha et al., 1998a). Blockade of the dopamine transporter markedly increases the levels of dopamine within the synaptic cleft and the time the neurotransmitter can act on target receptors. This observation is well documented in animal experiments (Self, 2004) and supported in human studies (Schlaepfer, 1997).

Serotonergic Systems

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 at least 15 receptor subtypes for 5-HT, each with at least three different effector mechanisms, via adenylyl cyclase, phospholipase C, and ion channels (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 dihydrohytryptamine in cocaine-trained animals attenuated cocaine drug-seeking during extinction and attenuated cocaine-induced reinstatement, possibly via an increase in 5-HT2C receptors. These studies suggest serotonin may play different roles in cocaine reward depending on the phase of cocaine self-administration.

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) [see Grottick, 2000].

Serotonin 5-HT2 receptors do not exert consistently similar effects on neurotransmitter function. Fluoxetine, a selective serotonin reuptake inhibitor, has been found to act through an antagonism of 5-HT2c receptors in addition to blockade of 5-HT reuptake, suggesting contrasting actions at these two serotonin receptor subtypes may result in an increase of serotonin in the synaptic cleft (Ni and Miledi, 1997). At this juncture, the role of 5-HT as it relates to other drugs has yet to be determined, though a decrease in the functioning of the serotonin transporter has been found to mediate neuronal changes in chronic cocaine and alcohol users (Little, 1998).

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).

The dopaminergic fibers projecting from the VTA to the NAcc are the fibers that have been strongly implicated in opiate self-administration (Vezina et al., 1987). The VTA also is the site of many gamma-amino-butyric acid (GABA) neurons, which are linked to the dopamine cells in the VTA. In the absence of substantial VTA mu-opioid receptor activation, GABA interneurons modulate glutamate-stimulated dopaminergic activity, ultimately constraining the basal firing rate of dopamine projection neurons (Koob, 1992). With the application of morphine, however, there is an inhibitory effect of GABA interneurons, resulting in greater glutamate involvement in the region of the VTA and a dopamine increase in the NAcc (Kalivas and Duffy, 1995).

In addition to GABA, glutamate may be involved in the reinforcing properties of opiates. NMDA glutamate receptors and opiate receptors are co-localized on neurons throughout the CNS, suggesting interactions between the two are profound inasmuch as depolarization of a single cell may simultaneously excite both receptors (Wang et al., 1999). Several cellular models for possible interactions between NMDA and opiate receptors have been proposed based on biochemical and physiological evidence. It is generally suggested that mu-opioid receptors modulate subsequent NMDA receptor functions through phosphorylation and other second messenger systems (Trujillo, 2003).

MK-801 and other NMDA receptor antagonists have been found to block the ability of opiates to establish a conditioned place preference [CPP] (Tzsentke et al.,

1995) and may inhibit the acquisition of morphine self-administration (Semenova et al., 1999). These findings are consistent with studies examining lead/opiate interactions, inasmuch as they suggest NMDA receptor antagonists, such as lead, modify the reinforcing properties of opiates, and thus, vulnerability/susceptibility to use drugs of abuse.

Lead and Behavior

Lead exerts neurochemical disturbances that implicate glutamatergic (Lasley et al., 2001), dopaminergic (Hu and White, 1994), and GABA systems (McFarland and Kalivas, 2001) that are known to modulate drug sensitivity (Cory-Slechta, 1995) and may play a role in the neurobehavioral deficits exhibited in developmentally lead-exposed children. These deficits are consistent with studies correlating impairments in academic achievement with developmental lead exposure. Deficits in abstract thinking, attention span, conceptual reasoning, and visuospatial perception in children with moderate to high blood lead levels have been documented (Rosen and Mushak, 2001). Disturbingly, children with levels of blood lead below those considered safe exhibit increased distractibility, hyperactivity, inability to inhibit inappropriate responding, preservation of incorrect responses, poor judgment and impulse control (Brockel and Cory-Slechta, 1997; Rice, 1993), and delinquent behavior (Needleman et al., 1996).

These problems appear to involve a similar mechanism at the frontal cortex (Volkow et al., 2002). Disruptions to this area produced by developmental lead-induced impairments may explain deficits in self-monitoring behavior and inhibitory functioning of higher order thinking. With a decrease in activity of the frontal cortex, cognitive

operations that mediate appropriate judgment of behavior is replaced with automatic, non-directed, sensory-driven behaviors, such as is seen in drug addiction.

One theory is that hypodopaminergic effects impair functioning of the orbitofrontal cortex (OFC) and anterior cingulate gyrus (CG) contributing to compulsive behavior and impaired inhibition. This is particularly a problem for children as the frontal cortex is late in developing. It is of further concern in lead-exposed children who have additional developmental delays and hypodopaminergic activity at brain sites imperative for self-monitoring behavior (Cory-Slechta, 1997).

In addition to direct neurochemical changes in lead-exposed mothers, alterations in maternal care have been observed. In experimental settings, nonexposed and lead-exposed mothers 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). An additional concern with maternal lead-exposure is related to stress induced by toxic metal exposure. Studies on maternal stress have shown increased pathologic behaviors in pups, such as sensitization to drugs of abuse and delinquent behavior (Cuomo et al., 1996).

Lead/Drug Interactions

Time Course of Lead Exposure

Cocaine and opiates show contrasting effects of drug potency in animals exposed to lead at different developmental stages. Specifically, animals exposed to lead throughout gestation and lactation (perinatally) appear to have an almost uniform potentiation for the rewarding efficacy of cocaine when administered chronically. In contrast, animals exposed to lead during adulthood show an almost uniform attenuation for the rewarding efficacy of cocaine when administered chronically. By comparison, when opiates, rather than cocaine, are presented repeatedly to animals perinatally exposed to lead, or exposed to lead as adults, reinforcement potency is attenuated.

Perinatal Lead Exposure

In experiments where opiates have been presented repeatedly to animals perinatally exposed to lead, a decrease in sensitivity to the drug often is observed. For example, in an intravenous (i.v.) self-administration study rats responded fewer times for a heroin reinforcer, at least at intermediate doses (Rocha et al., 2004). Parallel results were obtained when perinatally-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 agreement with these findings, animals exposed perinatally to lead failed to show a morphine-induced conditioned place preference, suggesting a decreased rewarding sensitivity to the opiate (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 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 contrast to lead/opiate interactions, developmental lead exposure seems to potentiate the behavioral effects of cocaine. That is, perinatal lead exposure increases the stimulatory properties of cocaine when animals are tested in a locomotor chamber at either postnatal day (PND) 30 or PND 90 (Nation et al., 2000). Elsewhere, when tested in a self-administration paradigm, lead-exposed rats maintained responding at cocaine doses too low to sustain responding in untreated controls (Nation et al., 2004). Also employing an i.v. self-administration model, Nation et al., (2003) found that adult rats born to dams exposed to lead throughout gestation and lactation exhibited a greater inclination to return to drug-seeking at lower doses of a cocaine priming injection. That is, after an extinction period where saline infusions replaced cocaine infusions as the reinforcement outcome for lever responding, lead-exposed animals were more likely than nonexposed controls to return to self-administration behavior (lever responding) following intraperitoneal (i.p.) injections of very low doses of cocaine.

The studies noted above suggest the reinforcer potency of heroin is decreased by developmental lead exposure and the same metal exposure regime increases the reinforcer potency of cocaine. However, in order to obtain a more complete understanding of lead-relate vulnerability to use drugs of abuse, it is necessary to examine the drug acquisition phase.

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., 1986) 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.

Opiate patterns of drug sensitivity differ upon time of lead exposure and task examined. Rats exposed to lead as adults show a reduced behavioral response to morphine in a locomotor activity task, relative to control. However, rats developmentally exposed to lead show an enhanced response in the same task (Miller, 2001). This pattern of behavior is opposite that observed in self-administration studies where an attenuation, not enhancement, of drug responsiveness results with developmental lead exposure. Directionally opposite effects may be due to experimenter administered injections, as opposed to i.v. self-administration by the rat, and length of drug exposure.

Vulnerability to Drug Acquisition

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. Still, as with humans, not all animals that are presented with the opportunity to administer drugs of abuse 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).

Sex Differences

Sex differences have been found to result in differential sensitivity to drugs of abuse. Specifically, female rats exhibited a higher rate of cocaine (.20 mg/kg) and heroin (.015 mg/kg) acquisition, as compared to males. In the case of cocaine, a greater percentage of female rats reached the criterion set for acquisition and self-administered more drug after acquisition was met (Lynch and Carroll, 1999). Additionally, a higher percentage of females, but not males, bred for high saccharin preference reached criterion for cocaine (.20 mg/kg), but not heroin (.015 mg/kg) acquisition (Lynch and Carroll, 1999). Also, females that show preference for high levels of saccharin showed a faster rate of cocaine, but not heroin acquisition. Phenotypical differences were not seen with male rats (Carroll et al., 2002), but additional differences in groups that varied by sex and/or saccharin preference may have been uncovered if doses on the ascending, rather than descending, limb of the dose-effect curve were examined.

Appetitive Manipulations

Non-food reinforcers such as running-wheels made available concurrently with cocaine also have been shown to impede drug self-administration in females to the point where wheel-running substituted for cocaine as a reinforcer. Varying doses may have uncovered differences in male rats (Cosgrove et al., 2002). A reasonable conclusion is that hormonal factors are important in modulating drug self-administration, perhaps as a function of estrus cycle in female animals (Lynch et al., 2001, 2002).

Restricted access to food also has been shown to facilitate cocaine acquisition in an animal model of drug self-administration (Bollweg et al., 1995; Campbell and Carroll, 2001). Further studies showed that food deprivation was a factor in learning, not differences in performance such as response speed or reactivity (Bollweg et al., 1995). Moreover, food deprivation increases the association between lever pressing and a drug reward stimulus. The increase in learning may be due to food deprivation-induced increases in plasma corticosterone in rats and cortisol in humans that accumulate in the hippocampus, a structure greatly implicated in learning and memory. Conversely, blocking the effects of corticosterone with TMT (trimethyltin), a limbic forebrain neurotoxin, has been shown to interfere with acquisition of autoshaped lever responding during a progressive fixed ratio task. Further, it was shown that depriving the animal to 75% body weight reversed the decline in acquisition rates following TMT treatment. Endogenous glucocorticoids, vasopressin, and catecholamines also may be related to the learning enhancement seen following food deprivation (Bollweg et al., 1995).

Environment

The modulating influence of the environment on lead neurotoxicity was underscored in a recent study using laboratory rats (Schneider, 2001). Immediately after weaning, rat pups were put in either impoverished or enriched environments. Half of the animals in each environment were exposed to lead via drinking water. Although by the end of testing lead was no longer detectable in the blood and brain of the lead-exposed group, lead-exposed rats reared in impoverished environments showed learning deficits. Conversely, lead-exposed rats raised in enriched environments performed similarly to their unexposed counterparts. The clinical implications of this study are great, suggesting that even when lead burdens on the body are identical and diet remains the same, environmental changes are enough to ameliorate lead-induced learning deficits (Schneider et al., 2001).

Schneider (2001) suggests that environmental factors may modulate the response of the brain to a neurotoxin such as lead in the impoverished condition via a decrease in neurotrophic factor gene expression in the hippocampus. Using high-density oligonucleotide microarrays, gene expression in young adult mice raised in enriched or impoverished environments were analyzed (Rampon et al., 2000). Neural structure during growth and development, synapse formation, synaptic transmission, neuronal plasticity, cell survival and neurogenesis (particularly in hippocampal neurons) were significantly protected in the group that experienced the enriched environment (Kempermann et al., 1997).

Guilarte et al. (2003) also found lead-induced deficits could be reversed by an enriched environment. Animals that were raised in a social and novelty-enriched environment, rather than in isolation, showed neuroprotective properties against glutamate-mediated disruption of spatial learning and sparing of deficits in glutamate subunit, NMDA receptor gene expression (Hubbs-Tait, 2005).

Stress

Stress has been an environmental factor of much discussion in the vulnerability of drug abuse. Stress induction appears to be directly linked to the activation of the hypothalamic pituitary adrenal (HPA) axis (Goeders, 2002a; Goeders, 2002b; Goeders, 2003). It is hypothesized that glucocorticoid hormones function 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).

Being exposed to high levels of stress in utero may also potentiate the effects of lead and produce a susceptibility to use drugs. Cory-Slechta et al. (2004) exposed dams to lead for 2 months prior to breeding them with nonexposed males. The females were either restrained or not 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 times on each of two days. Sex differences were found implicating the interaction of sex hormones with lead-induced behavioral effects. Specifically, male offspring born to dams exposed to lead and no stress, and females born to lead-exposed dams that experienced stress

showed permanently elevated corticosterone levels in offspring. Paradoxically, males born to dams that were exposed to lead and received stress showed slightly decreased corticosterone levels.

Sex differences are known to mediate stress response. However, the mechanisms by which sex and stress interact remain unclear and outcome measures vary depending on factors such as type and duration of the stressor (Faraday, 2002). Overall, stress-induced deficits may be produced by elevated corticosterone levels mediating changes in mesocorticolimbic function, particularly in the nucleus accumbens and prefrontal cortex. Further, this study suggests lead may interact with corticosterone to indirectly enhance susceptibility to stress-induced disorders, brain dysfunctions and cognitive deficits (Cory-Slechta et al., 2004).

Genetics

In addition to the environment, genetics also modulate drug self-administration. Rats that are genetically bred to exhibit a high (vs. low) response upon presentation of a novel environment may also 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 hypothalamus-pitutiary-adrenal (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).

Genetics also may play a role in drug self-administration as seen in studies using the cocaine and dopamine reuptake inhibitor, GBR 12909. GBR 12909 reduced wheel

running in hyperactive rats, but not controls (Rhodes et al., 2001). Compulsivity (Werme, 2003), impulsivity (Poulos et al., 1995), and novelty-seeking (Bardo et al., 1996) also may have a genetic basis that leads to an increase in drug-seeking/drug-taking behavior.

Autoshaping Procedure

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 is needed whereby all animals receive the same training to make a lever-press response. The autoshaping procedure serves this purpose, allowing for the monitoring of vulnerability to self-administer drugs in control and treated animals in a context where shaping methods are uniform and systematic both between and within groups.

The autoshaping procedure consists of a combination of Pavlovian and operant components wherein animals are first trained that the pairing of an automated retractable lever and light cue (conditioned stimuli) and drug infusion (unconditioned stimulus) [Pavlovian conditioning] consistently leads to euphorigenic effects. Subsequent to Pavlovian training, rats must 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).

Design and Hypothesis

Accordingly, the purpose of the present project 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 (a concentration of lead that is considered to be low and clinically relevant) 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 .018 mg/kg heroin infusions were paired with the extension and retraction of a lever (a Pavlovian

procedure). During a subsequent 3-hr self-administration component of each daily session, .018 mg/kg heroin infusions were delivered only when a single lever press (FR-1) was executed (an operant procedure). In Experiment 2, cocaine (.20 mg/kg) was used as the reinforcement outcome. The methods, apparatus, metal exposure regimen, surgical procedures, behavioral endpoints, and all other aspects of the research conducted for Experiment 2 will be precisely as described for Experiment 1. Only the number of conditioned drug presentations during the Pavlovian session changed to account for differential usage patterns of opiates versus cocaine. That is, the number of infusions self-administered at a dose of .018 heroin (Rocha et al., 2004) are manifold lower than those self-administered at a dose of .20 cocaine (Nation et al., 2003); though, both doses are on the descending limb of the dose-effect curve for each respective drug.

Predictions

Experiment 1: Heroin

Body weights. Based on previous studies, the analysis of body weights during the period of acquisition is not expected to show significant group differences. Weekly fluctuations are expected to occur, but the pattern of change is expected to remain constant across groups. Though the litter size is not expected to be different between Group 0-mg and Group 16-mg animals, initial individual pup weights are expected to be higher for control versus lead-exposed animals. However, no differences are expected between groups by the beginning of testing.

Lead concentrations in tissue. The mean (SEM) blood lead residue values for non-exposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding,

parturition, and weaning will be assessed. Blood lead concentrations for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment will also be determined. In accordance with previous studies (Nation et al., 2000; 2003; 2004; Rocha, 2004) for both groups, blood lead levels are expected to fall below detectable limits by Day 35 of acquisition testing. In terms of the analyses that will be performed on tissue samples taken from test animals, with the exception of bone (tibia) samples for the lead group, tissue concentrations are expected to be the same for both exposure conditions. It is anticipated that lead levels in bone will remain elevated in lead-exposed animals due to the extended half-life of lead in bone (Weizaecker, 2003).

Acquisition of self-administration. Based on previous literature that suggests a pattern of attenuation to the rewarding effects of heroin in rats developmentally exposed to lead (Nation et al., 2000; 2003; 2004), a trend should be evident over the 35-day testing period for Group 16-mg animals to be less likely to reach the criterion for acquisition of heroin self-administration than their control counterparts.

A survival analysis is expected to show that Group 16-mg animals acquire at a slower rate than control rats. Proportion tests also will be performed on successive 5-session blocks and are expected to show a smaller percentage of Group 16-mg animals will meet the conditions for acquisition, overall, than Group 0-mg animals.

The strength of the differences in group self-administration responding after meeting the criterion of 10 infusions/session for 2 consecutive days is expected to be reflected in the results from a one-tailed *t*-test. If these findings are observed, they will suggest perinatal (gestation and lactation) lead exposure results in lower percentages of

animals reaching the heroin self-administration acquisition criterion, and that of the animals that do acquire, lead-exposed animals will reach criterion at a slower rate.

Experiment 2: Cocaine

Body weights. Because animals from Experiment 2 are littermates of Experiment 1, the analysis of body weights is expected to be the same across studies. During the period of acquisition, weekly fluctuations are expected to occur but the pattern of change is expected to remain uniform across groups. Also, as with Experiment 1, the litter size of animals in Experiment 2 should be comparable between Group 0-mg and Group 16-mg animals. Though initial individual pup weights are expected to be higher for control versus lead-exposed animals, no differences are expected between groups by the beginning of testing.

Lead concentrations in tissue. Because animals used Experiment 2 are littermates of animals used in Experiment 1, the predictions are the same for Experiment 2 as they were in Experiment 1.

Acquisition of cocaine self-administration. Based on the pattern of potentiation to the rewarding effects of cocaine in rats developmentally exposed to lead, a trend should be evident over the 35-day testing period for Group 16-mg animals to be more likely to meet the requirements for acquisition of cocaine self-administration than their control counterparts.

As in Experiment 1, survival analysis and a proportion test will be performed to assess differences between groups in rate of acquisition and percentage of animals to reach acquisition criterion across 5-session blocks. In contrast to Experiment 1 where

heroin will be used, results from Experiment 2 where developmentally lead-exposed animals will be presented with cocaine, Group 16-mg animals are expected to reach the criterion for cocaine acquisition at a faster rate than control rats. Proportion tests performed on successive 5-session blocks are expected to show that a greater percentage of Group 16-mg animals will meet the conditions for acquisition than Group 0-mg animals.

In Experiment 2 where cocaine will be used, Group 16-mg animals are expected to make substantially greater number of active lever responses (receive more infusions) than Group 0-mg animals, across the 35-day testing period. The strength of the differences in group self-administration responding after meeting the criterion of 50 infusions/session for 2 consecutive days is expected to be reflected in the results from a one-tailed *t*-test. If these findings are observed, they will suggest that perinatal lead exposure results in greater percentages of animals reaching the cocaine self-administration acquisition criterion, and that of the animals that do acquire, lead-exposed animals will reach criterion at a faster rate.

The results from Experiments 1 and 2 are expected to agree with previous findings that suggest developmental exposure to inorganic lead is associated with a decreased sensitivity to the reinforcing properties of opiates (Miller et al., 2000, 2001) and, conversely, with an increase in sensitivity to the reinforcing properties of cocaine (Nation et al., 2000; 2003; 2004). These effects are expected to translate into similar patterns of drug-taking behavior when assessing vulnerability to initiate high and stable levels of responding for cocaine or heroin.

MATERIALS AND METHODS

Experiment 1: Heroin

Animals

All aspects of the research reported here were approved by the Texas A&M University Laboratory Animal Care Committee. 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 16 ga gavage needle (Source, Location) 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 weights or the locomotor ability of pups (see Miller et al., 2000). Following this 30-day toxicant exposure period, females were bred with nonexposed 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 were available *ad libitum* for dams in the home cage. Litters were culled to eight pups on postnatal day (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.

The rate of pregnancy was not different between groups. On PND 21, pups used for testing were weaned and housed individually. All animals were maintained on a 12-hour light/dark cycle. Testing commenced at approximately 10:00 hrs, two hrs into the 12-hr light cycle.

Surgical Procedures

Surgery was 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., 2000; 2001; Nation et al., 2003; 2004). Using a backplate technique, implantation of chronic indwelling jugular catheters was performed using sterile techniques. Rats were anesthetized with separate injections of 50 mg/kg ketamine and 50 mg/kg sodium pentobarbital administered intraperioneally (i.p.). A .01 interior diameter (ID) Silastic tubing [Dow Corning, Midland, MI] catheter 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 exiting the back between the scapulae. A backplate consisting of two stainless steel ovals separated by propylene mesh (Ethicon, Inc; Somerville, NJ) provided an anchor for 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 channel fluid swivel [22 ga] (Instech Labs, Plymouth Meeting, PA). The swivel

design permitted an interlock with separate connecting arms located in the home cage and operant test chambers. The hinged arm allowed for a range of movement in either the home cage or test chamber. A .02 ID catheter continued from the top of the swivel to an infusion pump (Razel Scientific Instruments; Stamford, CT) that controlled the solution delivery. Animals were allowed 5 days to recover from surgery before commencing heroin self-administration testing. During this recovery period, each rat received in the home cage hourly intravenous (i.v.) infusions (200 µl) of a sterile saline solution containing heparin (1.25 U/ml), penicillin g potassium (250,000 U/ml), and streptokinase (8,000 U/ml). Following recovery, animals received automated hourly infusions (213 µl) over an 8.00 sec time frame of heparinized saline in the home cage for the duration of the study.

All animals received free access to food and water for 5 days while recovering from surgery. Subsequently, daily food allotment was restricted to 18 g of standard rat chow in order to maintain animals at approximately 85% of the mean body weight of non-food-deprived littermates (not participating in the study). Moderate food restriction has consistently been shown to accelerate cocaine acquisition and the procedure is recommended for autoshaping acquisition studies (Campbell and Carroll, 2001). Uncontaminated water was available *ad libitum* throughout the study. Animals were weighed daily prior to testing. Although initial individual pup body weights were higher for control versus lead-exposed animals, no group differences in body weight were evident at the commencement of testing operations. Food was placed in home cages following the end of each daily testing session.

Apparatus

Twelve operant conditioning chambers (Model E10-10, Coulbourn, Allentown, PA) in sound attenuating cubicles served as the test apparatus. Each chamber had two levers (left, right) 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. infusions (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. Seven control and 10 lead-exposed animals were run in two squads, and subject assignment to chambers and squad was counterbalanced by group.

Procedure

Autoshaping component. Each of the 6-hr experimental sessions consisted of two parts, an autoshaping and a self-administration component. Testing was carried out seven days per week. For the first 3 hrs of Experiment 1, during the autoshaping component, testing commenced with the retractable lever drawn outside the reach or vision of the animal. After a 480-sec time-out period, the retractable lever extended into the operant chamber at which point the animal received a .018 mg/kg heroin infusion if it pressed the lever or after 15-sec, whichever occurred first. Once again, a 480-sec time-out period was instituted. As before, the active lever was then extended into the chamber and the animal was given 15-sec to press the lever for an immediate infusion of .018 mg/kg heroin, or, if no response occurred, the animal received a noncontingent heroin infusion of .018 mg/kg heroin at the end of the 15-sec period. This cycle repeated for the first 20 min of each of the first three hours wherein 5 heroin infusions were

administered for a total of 15 heroin infusions over the three hours of autoshaping each day of testing.

With the chamber house-light off, the stimulus light above the active (right) lever was lit for the 6-s duration of the infusion and terminated immediately after. 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 .018 mg/kg heroin infusion (160 μl) was delivered to the animal following each lever retraction regardless of whether the delivery was contingent or noncontingent. After the first 20 min of each hour, following the 5 heroin 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 .018 mg/kg heroin infusions were contingent upon lever pressing under an FR-1 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 was concluded for the day.

The criterion for acquisition of heroin self-administration was a 2-day period during which a mean of 10 infusions/session occurred during the 3-hour operant phase. This criterion followed that set by previous studies in the area of acquisition using a comparable dose of heroin (Carroll et al., 2002; Lynch and Carroll, 1999). The heroin

dose (.018 mg/kg) was chosen based on data from previous studies that show this dose is highly reinforcing, and will produce high and stable rates of responding (Rocha et al., 2004).

In order to confirm patency during acquisition training, catheters were flushed twice daily with .20 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 .20 mls of heparinized saline, and these animals were checked for immediate onset of brief anesthesia. At the end of the study, each animal 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. Each of the animals included in this report tested positive for open lines.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the heroin (diacetylmorphine). Heparinized saline served as the heroin vehicle. Lead acetate and sodium acetate were obtained from Sigma Aldrich 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

The comparative rates of acquisition of cocaine self-administration were assessed using the Kaplan-Meier survival analysis, Breslow statistic (Lee, 1992). This analytical procedure is ideally suited for determining differences in rate with respect to animals reaching a set criterion (SPSS; Chicago, Il). Comparative percentages of animals reaching criterion between groups was assessed using a proportion analysis (Bruning and Kintz, 1997). In addition, an Analysis of Variance (ANOVA) test was performed on the mean number of active lever responses (infusions) and inactive lever responses for each group across the course of self-administration testing.

RESULTS

Body Weights

The analysis of body weights during the period of acquisition did not show significant group differences; $F(1,14)= .10$, $p> .05$ (mean body weight = 329.64 g $\pm$ and 326.92 g $\pm$ for Groups 0-mg and 16-mg, respectively). Weekly fluctuations did occur but the pattern of change was uniform across groups. Though the litter size was not different between Group 0-mg and Group 16-mg animals (means = 13.5 and 12.3, respectively; $p> .05$), initial individual pup weights were higher for control versus lead-exposed animals ($t(21)= 3.14$, $p< .05$). However, as indicated, no differences were seen between groups by the beginning of testing.

Acquisition of Heroin Self-administration

Figure 1 illustrates the cumulative percentage of rats in each exposure condition meeting criterion for the acquisition of heroin self-administration. It is visually apparent that over the 35-day testing period Group 16-mg animals were less likely to meet the requirements for acquisition of heroin self-administration than their control counterparts. Although during the first two weeks of testing both groups appeared to have little separation, by the third week of testing the separation was evident. All of Group 16-mg animals that would acquire did so before the second week of testing was complete. Group 0-mg animals continued to escalate in their rate of acquisition until day 28 of testing.

Though a survival analysis failed to show differences between groups in rate of acquisition ($p> .05$), proportion tests (Bruning and Kintz, 1997) performed on each of 5-

session blocks, showed that the percentage of rats that met the lever-press response criterion when tested as adults was lower among lead-exposed animals (62.5%; Group 16-mg), than in controls (87.5%; Group 0-mg), by the end of testing (35 days) [Z= 2.13, df=1, $p< .05$)]. Whereas 5 of 8 lead-exposed animals reached criterion for acquisition of heroin (10 infusions within the 3-hr instrumental session), 7 of 8 control animals reached criterion.

Figure 2 profiles the mean number of lever presses for each group across successive 5-trial blocks of acquisition training. Although the group main effect ($F(1,14)= .003$, $p> .05$) and interaction effect ($F(1,14)= .59$, $p>.05$) failed to reach an acceptable level for statistical significance, there was an unexpected trend toward higher responses from lead-exposed animals. It is apparent that both groups responded more frequently on the active (heroin) lever than the inactive lever that had no programmed consequences. Further, it is clear that both groups had reached asymptote and exhibited stable self-administration patterns over the last 10 days of acquisition.

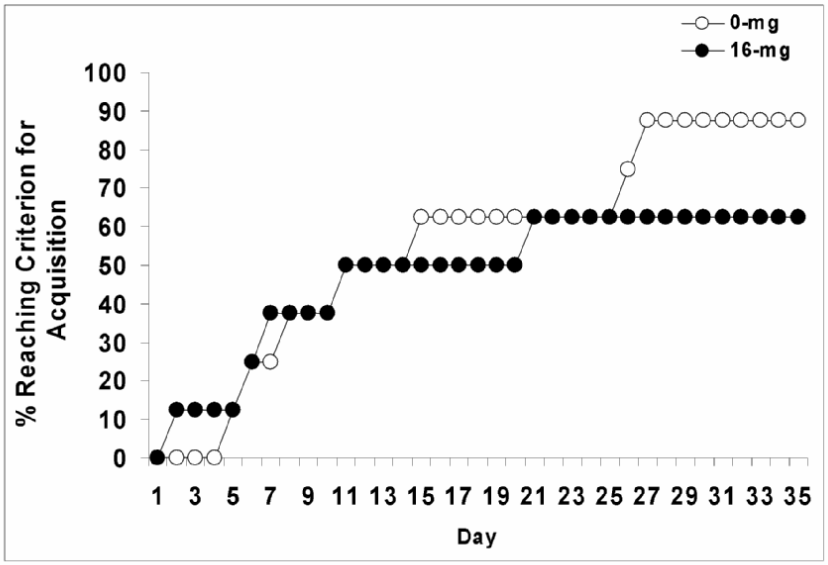

Figure 1. Cumulative percentage (%) of nonexposed (Group 0-mg/N=8) and lead-exposed (Group 16-mg/N=8) rats meeting the criterion for the acquisition of heroin (.018 mg/kg/infusion) self-administration within the 35-day limit. Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

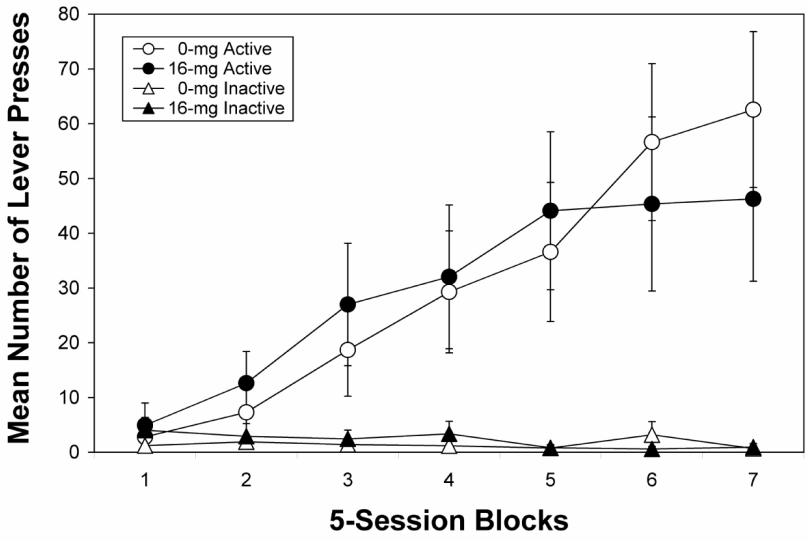

Figure 2. Mean active and inactive lever responses for heroin (.018 mg/kg/infusion) in all animals in Group 0-mg lead (N=8) and Group 16-mg lead (N=8), across successive 5-session blocks (Experiment 1). Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

Lead Concentrations in Tissue

Table 1 presents the mean (SEM) blood lead residue values for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, parturition, and weaning. Blood lead concentrations are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the experiment. As can be seen, for both groups, blood lead levels had fallen below detectable limits by Day 35 of acquisition testing (< .5 μg/dl), and in terms of the analyses performed on tissue samples taken from test animals, with the exception of tibia samples for the lead group, tissue concentrations were the same for both exposure conditions.

Table 1. *Mean (SEM) blood and tissue lead concentration values for dams, littermates and test animals in Experiment 1.*

	Group 0 mg	Group 16 mg
Blood Lead Concentration (µg/dl)		
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) *

The symbol * indicates that control and lead-exposed animals were significantly different ($p < .05$).

MATERIALS AND METHODS

Experiment 2: Cocaine

The purpose of the second experiment was to examine the relative acquisition rates, independent of major intrusions, of drug (cocaine) self-administration for offspring (rats) born to dams exposed to 0 mg or 16 mg lead prior to breeding, and throughout gestation and lactation. Because opiates and cocaine typically exert differentially opposite effects, it was hypothesized that cocaine would produced an increase in vulnerability to reach criterion for cocaine acquisition.

As in Experiment 1, adult control and lead-exposed animals were tested during daily sessions that involved an initial 3-hr autoshaping component wherein .20 mg/kg cocaine infusions were paired with the extension and retraction of a lever (a Pavlovian procedure). During a subsequent 3-hr self-administration component of each daily session, .20 cocaine infusions were delivered only when a single lever press (FR-1) was executed (an operant procedure).

Animals

All aspects of the research reported here were approved by the Texas A&M University Laboratory Animal Care Committee. Metal exposure regimen, surgical procedures, apparatus, tissue collection/analyses and statistical analyses were identical to those of Experiment 1 with only the procedure varying slightly to account for differential patterns of drug self-administration between opiates and cocaine.

Procedure

Autoshaping component. Each of the 6-hr experimental sessions consisted of two parts, an autoshaping and a self-administration component. Testing was carried out seven days per week. For the first 3 hrs of Experiment 1, during the autoshaping component, testing commenced with the retractable lever drawn 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 .20 mg/kg cocaine 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 then extended into the chamber and the animal was given 15-sec to press the lever for an immediate infusion of .20 mg/kg cocaine, or, if no response occurred the animal received a noncontingent heroin infusion of .20 mg/kg cocaine at the end of the 15-sec period. This cycle repeated for the first 20 min of each hr for 3 hrs (30 total cocaine infusions).

With the chamber house-light off, the stimulus light above the active (right) lever was lit for the 6-s duration of the infusion and terminated immediately after. 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 .20 mg/kg cocaine HCl infusion (160 µl) was delivered to the animal following each lever retraction regardless of whether the action was contingent or noncontingent. After the first 20 min of each of the three hours, following the 10 cocaine 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 .20 mg/kg cocaine HCl infusions were contingent upon lever pressing under an FR-1 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 was concluded for the day.

The criterion for acquisition of cocaine self-administration was a mean of 50 infusions per day over 2 consecutive daily self-administration sessions. This value is half of what has 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 cocaine dose (.20 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 (Campbell and Carroll, 2001).

In order to confirm patency during acquisition training, catheters were flushed twice daily with .20 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 .20 mls of heparinized saline, and these animals were checked for immediate onset of brief anesthesia. At the end of the study, each animal 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 (50.00 mg/kg sodium pentobarbital). Each of the animals included in this report tested positive for open lines.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the cocaine HCl. Heparinized saline served as the cocaine vehicle. Lead acetate and sodium acetate were obtained from Sigma Aldrich Chemical Company (St. Louis, MO).

RESULTS

Body Weights

The analysis of body weights during the period of acquisition did not show significant group differences; $F(1,15)= .06$, $p> .05$ (mean body weight = 284.09 g $\pm$ and 285.85 g $\pm$ for Groups 0-mg and 16-mg, respectively). Weekly fluctuations did occur but the pattern of change was uniform across groups. Animals used in Experiment 1 were littermates in Experiment 2. Accordingly, data on litter size is the same as in Experiment 1. Litter size was not different between Group 0-mg and Group 16-mg animals (means = 13.5 and 12.3, respectively; $p> .05$), initial individual pup weights were higher for control versus lead-exposed animals ($t(21)= 3.14$, $p< .05$). However, as indicated, no differences were seen between groups by the beginning of testing.

Acquisition of Cocaine Self-administration

Figure 3 illustrates the cumulative percentage of non-metal (0-mg lead) and metal-exposed (16-mg lead) rats meeting criterion (50 lever presses). Note, the increase in the infusion requirement to meet criterion in Experiment 2 corresponds to the greater number of lever responses made at a dose of .20 mg/kg in a cocaine dose-effect curve (Nation et al., 2003), compared to the lever responses made at a dose of .018 mg/kg in a heroin dose-effect curve (Rocha et al., 2004).

It is visually apparent that over the 35-day testing period Group 16-mg animals were more likely to meet the requirements for acquisition of cocaine self-administration than their control counterparts. Indeed, even by the fifth day of acquisition training, a greater number of lead-exposed animals (20%; Group 16-mg) reached criterion than

non-exposed animals (0%; Group 0-mg), and this pattern persisted throughout testing. This is especially evident at day 17, approximately mid-way through the 35 day testing period, where 60% of Group 16-mg animals had reached the acquisition criterion, but only 14.29% of Group 0-mg animals had reached criterion.

By the end of testing, neither Group 0-mg (42%) [3 of 7] nor Group 16-mg (80%) [8 of 10] reached 100% percentages with respect to acquisition criterion. As assessed by survival analysis, lead-exposed animals reached criterion at a faster rate across the 35-day testing period than controls [Kaplan-Meier, Breslow statistic (X^2 = 3.89, df=1, p< .05)]. In addition, there was evidence that lead-exposed animals acquired at significantly faster rates than controls.

In addition to survival analyses, proportion test were performed. Proportion tests showed a significantly greater percentage of Group 16-mg animals reached acquisition criterion than Group 0-mg animals during all but the first 5-day block (block 2, z = 3.03; block 3, z = 4.20; block 4, z = 3.78; block 5, z = 3.78; block 6, z = 3.09; block 7, z = 3.09; *ps*< .05).

Figure 4 profiles the mean number of active (infusions) and inactive lever responses per five-session blocks for all animals in both exposure conditions. A 2 Groups (0-mg, 16-mg) X 2 Levers (active, inactive) 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 this analysis revealed that lead-exposed rats self-administered cocaine at greater rates than controls. In addition to significant main effects for Levers (F(1,15) =14.95, p<.05) and Blocks of 5 Sessions

($F(6,90) = 7.54$, $p < .05$). Further, it is apparent from Figure 4 that both groups maintained stable response rates at the end of the experiment.

In addition to a greater percentage of lead-exposed animals reached criterion for cocaine acquisition and doing so at faster rates, lead-exposed animals also made substantially greater number of lever responses (received more infusions) across the remaining sessions than Group 0-mg animals. The largest separation in the number of lever responses and, consequently, drug infusions administered, was seen during the fourth 5-day block between Day 16 and Day 20 of acquisition. During the fourth 5-day block, Group 0-mg animals administered less drug (mean= 19.03 infusions) than Group 16-mg animals (mean= 62.22 infusions) per 3-hour self-administration session. Animals that reached criterion pressed for a comparable number of drug infusions during the last 5 days of testing, regardless of group ($p > .05$), with control animals maintaining a trend toward fewer responses.

The strength of the differences in group self-administration responding after meeting the criterion of a mean of 50 infusions/session for 2 consecutive days is reflected in the results from the one-tailed *t* test performed on the data shown in Figure 4, $t(9)=2.22$, $p < .05$). Thus, not only does perinatal lead exposure result in greater percentages of animals reaching the cocaine acquisition criterion and at faster rates, but after criterion is reached lead-exposed animals self-administer at greater frequencies than non-exposed animals for the duration of testing.

Lead Concentrations in Tissue

The dam and littermate blood data for animals tested in Experiment 2 are presented in Table 1 (littermates were used as test animals for Experiment 2). As is presented in Table 2, the only substantial group differences with respect to lead accumulation in tissue of test animals was in the analysis of tibia, where greater lead residues were evident in Group 16-mg relative to Group 0-mg; $p < .05$. As in Experiment 1, at termination of testing blood lead concentrations in both exposure conditions were < .5 µg/dl, i.e., below the limits of detection. Thus, all differences were observed even though lead had cleared blood in lead-exposed animals by the end of testing.

Table 2. *Mean (SEM) tissue lead concentration values for test animals in Experiment 2.*

Tissue Concentrations of Test Animals at Termination (µg/g)		
	Group Lead-0	Group Lead-16
Brain	.004 (.001)	.006 (.002)
Kidney	.008 (.001)	.031 (.002)
Liver	.004 (.001)	.006 (.001)
Tibia	.029 (.003)	1.950 (.338) *

The symbol * indicates that control and lead-exposed animals were significantly different ($p < .05$).

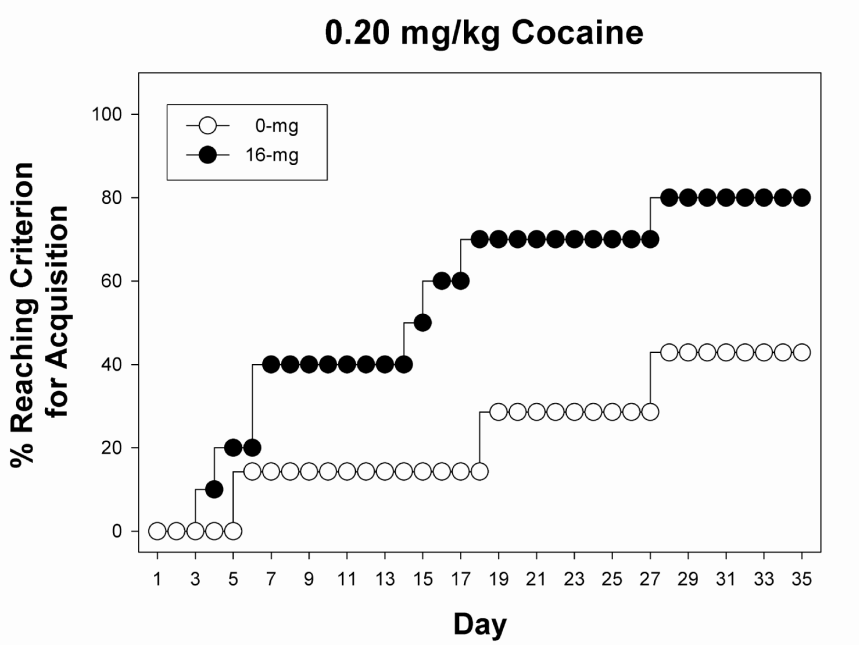

Figure 3. Cumulative percentage (%) of nonexposed (Group 0-mg/N=7) and lead-exposed (Group 16-mg/N=10) rats meeting the criterion for the acquisition of cocaine (.20 mg/kg/infusion) self-administration within the 35-day limit. Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

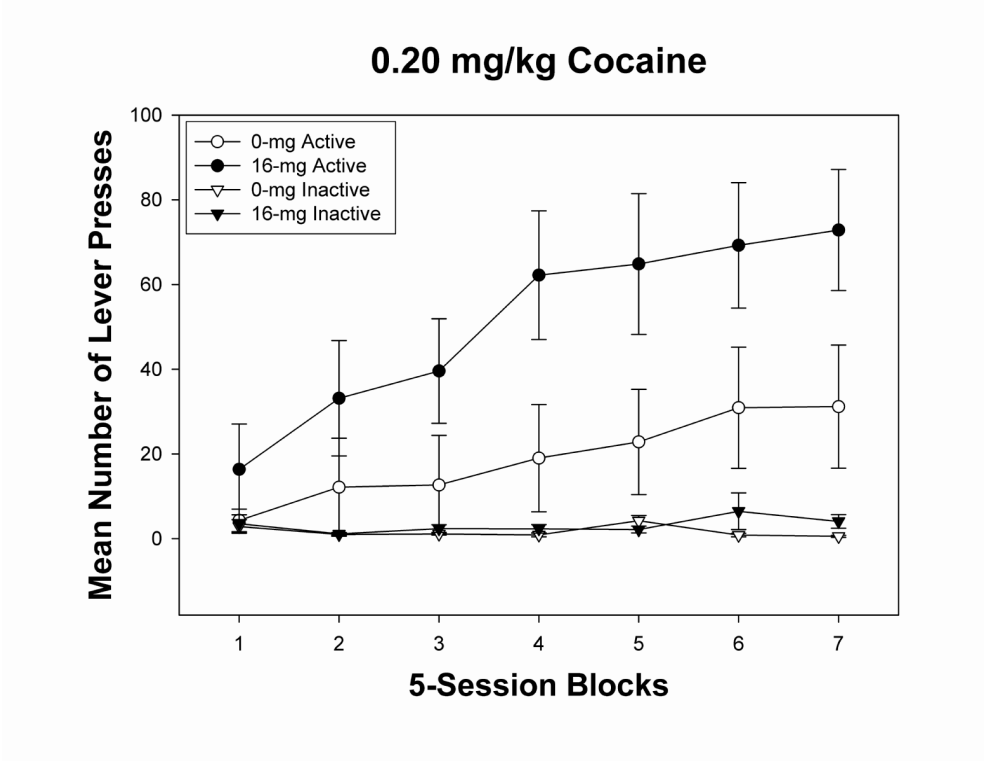

Figure 4. Mean active and inactive lever responses for cocaine (.20 mg/kg/infusion) in all animals in Group 0-mg lead (N=7) and Group 16-mg lead (N=10), across successive 5-session blocks (Experiment 1). Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

DISCUSSION, SUMMARY AND CONCLUSIONS

Employing .018 mg/kg heroin intravenous (i.v.) as the reinforcement outcome in a drug self-administration paradigm, the findings from Experiment 1 revealed that perinatal exposure to clinically relevant, low-levels of lead resulted in a smaller percentage of rats reaching the criterion for heroin acquisition (10 infusions within the 3-hour instrumental session) during a 35-day training regimen, relative to nonexposed controls. When cocaine (.20 mg/kg) was substituted as the reinforcer in the self-administration model (Experiment 2) a greater percentage of the developmentally lead-exposed animals reached criterion for acquisition as compared to control animals. Additionally, lead-exposed animals made a greater number of lever responses (self-administered more cocaine) than nonexposed animals. Whereas lead exposure appears to decrease the rewarding potency of heroin at relatively low doses, there is evidence that when animals are presented with cocaine, lead exposure increases the rewarding potency of cocaine.

It is important to note that in the studies where drugs and perinatally (gestation and lactation) lead-exposed animals were used, 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 data suggest relatively permanent neuronal alterations in cocaine/dopamine related circuitries occur during a critical period of developmental lead exposure.

Interpretive issues arise from the fact that developmental lead exposure resulted in a smaller percentage of animals acquiring heroin self-administration in Experiment 1,

but a greater percentage of animals acquiring cocaine self-administration in Experiment 2. In the case of heroin, it is possible that the pattern of group separation may derive from lead-induced decreases in the reinforcing properties of the drug, and therein, the metal functionally decreases the heroin dose. Conversely, in the case of cocaine, the metal functionally increases the cocaine dose.

This line of reasoning agrees with a recent literature on perinatal lead/heroin interactions where it has been shown that developmental lead exposure results in decreased sensitivity to the behavioral effects of heroin; whereas under the same testing conditions, lead/cocaine interactions are expressed as an increased sensitivity to the behavioral effects of cocaine. Previously, it was mentioned that developmental lead exposure produces a downward shift in the heroin dose-effect curve (Rocha et al., 2004) and decreases breaking points in a heroin-reinforced progressive ratio paradigm (Rocha et al., 2004). Combined with drug substitution studies (Miller et al., 2001), the available data suggest a decreased responsivity, or subsensitivity, to the rewarding potency of opiates. In contrast, an increase in responsivity, or supersensitivity, is evidenced in the rewarding potency of cocaine when animals are developmentally exposed to lead. Specifically, lead exposure produces a leftward shift in the cocaine dose-effect curve (Nation et al., 2004), reinstatement of drug seeking at low doses of a priming injection of cocaine occurs at levels that are too low to affect controls (Nation et al., 2003) and heightened locomotor activation by cocaine is evident (Nation et al., 2000). In each of these cases, then, animals developmentally exposed to lead expressed an attenuated

response to heroin and, conversely, an amplified response to cocaine, relative to controls.

It is reasonable to expect that the changes in the reinforcing and stimulating properties of heroin and cocaine engendered by early lead exposure may be associated with direct changes in neural pathways associated with drug reward. Areas 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 heroin and cocaine effects observed elsewhere and presently in Experiment 1 and Experiment 2. Of course, numerous other transmitter systems, e.g., cholinergic processes (Reddy et al., 2003), may concurrently interact to produce a manifold change in neural mechanisms in the immature rat brain that produces relatively permanent modifications to the sensitivity of drug reward.

The contrasting effects observed in the rewarding potency of heroin and cocaine following developmental lead exposure may be due in part to the differences in neurochemical actions of each type of drug. Lead is known to target the mesolimbic dopamine system, most conspicuously projection neurons from the ventral tegmental area to the nucleus accumbens (Cory-Slechta, 1995; Tavakoli-Nezhad et al., 2001). Because dopamine activity along this circuit is critically involved in mediating cocaine

responsiveness (Ranaldi and Wise, 2001; Wise and Bozarth, 1987), disruptions in mesolimbic dopamine functioning resulting from the presence of lead may translate into an enduring increased sensitivity to cocaine.

In the case of heroin, dopamine in the VTA also may mediate opiate reward. However, opiates also may be self-administered directly into the NAcc (Smith et al., 1987) and opiate antagonists administered into the NAcc attenuate i.v. heroin self-administration (Vaccarino et al., 1985). In addition, systemic or intra-NAcc administration of DA antagonists does not alter i.v. heroin self-administration (Ettenberg et al., 1982). Likewise, destruction of presynaptic DA terminals in the NAcc, using the neurotoxic compound 6-OHDA, selectively attenuates cocaine but not heroin self-administration (Pettit et al., 1984). These data suggest a dopamine-independent mechanism in the NAcc for opiates.

Morphological evidence demonstrates that the majority of NAcc neurons are GABAergic and comprise the final common-output neurons in the NAcc (Chang and Kitai, 1985; Kita et al., 1985). These medium, spiny GABAergic neurons receive multiple inputs including dopamine from the VTA, glutamate from the PFC, and enkephalin from local interneurons that all project primarily to the ventral pallidum (Sesack and Pickel, 1982). Opiate reinforcement is also mediated by an indirect disinhibition of dopamine neurons in the VTA and a direct inhibition of GABAergic output neurons in the NAcc (Bardo, 1998).

Glutamate (Glu) system functions also may account for the directionally opposite effects observed in heroin and cocaine sensitivity. Glutamate (Glu) seems to be more

greatly implicated in heroin self-administration than cocaine self-administration. N-methyl-D-aspartate (NMDA) is a Glu subtype and distinct genes encode NMDA receptor properties essential to calcium channel activity modulating drug reward. Moreover, lesions of the NAcc core, but not the shell, slightly reduce maintenance of heroin self-administration and impair acquisition of heroin responding (Hutcheson et al., 2001). The shell mediates the rewarding effects of cocaine, ostensibly via DA pathways. Conversely, the core of the NAcc that is essential for the acquisition of heroin self-administration, but not that of cocaine, mediates changes in NMDA function. In addition, though dopamine in the NAcc is not critical for heroin self-administration, intra-Nacc infusions of opiate receptor antagonists reduces heroin reward, eventually eliminated heroin self-administration (Vaccarino et al., 1985; 2001). In view of these and other similar findings, it must be considered that distinctive neuroadaptations in these regions resulting from perinatal lead exposure may account for the contrasting differences in lead-induced heroin or cocaine self-administration.

Other more indirect determinants that affect drug sensitivity produced by perinatal lead exposure also should be considered. Because lead-exposed pups initially exhibited lower body weights than controls, early malnutrition may have produced adverse neuroadaptations that affected later self-administration of drugs. In addition, disturbances in metabolic conversion and drug distribution/absorption may persist for lengthy periods following early lead exposure. Further, it must be considered that elevated response rates exhibited by lead-exposed animals may derive from the aforementioned increase in activity that is consistently observed with animals exposed to

the same regimen employed here (Nation et al., 2000). However, elevated locomotion in lead-exposed animals does not appear to explain the patterns of self-administration observed by Nation et al. (2004), where rats emitted fewer responses for the lowest and highest doses of cocaine, but pressed more for the intermediate doses. Pressing for the lower doses of drug was decreased suggesting drug self-administration was consistent with drug potency and not a confounding effect of locomotor stimulation.

Whatever the neurobiological array of events that ultimately results in increased acquisition of intravenous cocaine self-administration among animals perinatally exposed to lead and tested during the adult cycle, the risk implications are clear. As noted by Carroll and Lac (1997), animal models of acquisition permit an assessment of variables that may increase vulnerability to initiate drug-taking. In these studies, developmental lead exposure modulates the likelihood that an animal will self-administer a drug (heroin or cocaine) at a low dose under conditions where there is no prompting to take the drug. These data may have predictive validity regarding possible vulnerability to drug abuse in humans. Insofar as an environmental event, such as chronic low-level lead exposure, increases the choice to use a hedonic drug, abuse potential necessarily increases.

Because early lead exposure consistently has increased the reinforcement efficacy of cocaine (present data; Nation et al., 2003; 2004), there may be legitimate concerns that lead poisoning elevates the chances for transition from casual drug use to compulsive drug-seeking. In the case of heroin, antagonism of the potency of the drug by lead exposure is of concern because it represents a form of functional tolerance that

may occasion increased intake at higher doses of the drug. Previous studies (Campbell et al., 1998; Carroll and Lac, 1993) suggest that as dose increases, the likelihood to self-administer a drug also increases. Thus, even though self-administration of heroin decreased in lead-exposed animals, at higher doses, the susceptibility to administer the drug may increase for lead-exposed animals. The implications are clear; although lead-induced changes in drug potency may decrease self-administration at the dose of heroin tested, in real world situations, individuals exposed to lead during development may seek out higher, thus, more dangerous doses of heroin in order to reach an optimal effect of the drug (Corrigall and Coen, 1991; Woolverton, 1986).

Lead may be producing a burden in the organism, affecting drug sensitivity or intake via altered molecular mechanisms in a time-dependent manner (Tran-Nguyen et al., 1998). Interaction effects with lead and opiates/cocaine in various behavioral measures further suggest that the lead burdens that occur during various periods of development are crucial in mediating the resulting behavioral patterns (Miller et al., 2000; 2001; Nation et al., 2003; 2004; Rocha et al., 2004; Valles et al., 2003). Accordingly, data on developmental exposure to lead indicates a more dynamic and sometimes directionally opposite pattern of effects in animals exposed perinatally to the metal versus later on in adulthood.

A confounding variable may be that children exposed to lead are more likely to live in lower-income communities where the incidence of low maternal IQ, poor diet, delinquency and exposure to multiple environmental toxicants are high. 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).

An expanding literature shows that urban, minority children from low-income families are targeted for lead exposure and exhibit unsafe levels of metal-residue (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).

Ultimately, drug initiation such as that monitored here in Experiment 1 and Experiment 2, may or may not lead to high, stable responding for a given drug, even in the presence of environmental pollution. Genetic predisposition, experiential history, psychosocial unrest, and innumerable societal factors are major determinants of this complicated health problem. What the present acquisition findings from Experiment 1 and Experiment 2 suggest is that the scientific community and health-care providers should not ignore the growing literature that shows exposure to lead during development may alter drug sensitivity. This information not only increases our understanding of factors that enhance drug intake, but also it enhances our awareness of how environmental vectors in drug abuse may suggest preemptive strategies for decreasing vulnerability to drug addiction.

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APPENDIX

FIGURE CAPTIONS

Figure 1. Cumulative percentage (%) of nonexposed (Group 0-mg/N=8) and lead-exposed (Group 16-mg/N=8) rats meeting the criterion for the acquisition of heroin (.018 mg/kg/infusion) self-administration within the 35-day limit. Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

Figure 2. Mean active and inactive lever responses for heroin (.018 mg/kg/infusion) in all animals in Group 0-mg lead (N=8) and Group 16-mg lead (N=8), across successive 5-session blocks (Experiment 1). Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

Figure 3. Cumulative percentage (%) of nonexposed (Group 0-mg/N=7) and lead-exposed (Group 16-mg/N=10) rats meeting the criterion for the acquisition of cocaine (.20 mg/kg/infusion) self-administration within the 35-day limit. Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

Figure 4. Mean active and inactive lever responses for cocaine (.20 mg/kg/infusion) in all animals in Group 0-mg lead (N=7) and Group 16-mg lead (N=10), across successive 5-session blocks (Experiment 1). Open symbols and closed symbols represent the nonexposed and exposed conditions, respectively.

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