PERINATAL LEAD EXPOSURE SENSITIZES RATS TO THE REWARDING EFFECTS OF COCAINE, BUT NOT COCAINE/3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA) COMBINATIONS

A Senior Honors Thesis

by

AARON LYNN CARDON

Submitted to the Office of Honors Programs & Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE RESEARCH FELLOWS

April 2002

Group: Life Sciences 1
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Jack R. Nation (Fellows Advisor)

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April 2002

Group: Life Sciences 1
Perinatal Lead Exposure Sensitizes Rats to the Rewarding Effects of Cocaine, but not Cocaine/3,4-Methylenedioxymethamphetamine (MDMA) Combinations.

(April 2002)

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Early-age lead exposure has been shown to have various behavioral effects later in life, including learning deficits and mental retardation. Recent evidence indicates that developmental lead exposure may serve as a risk factor for drug abuse later in life by increasing the reward potency of cocaine and other drugs of abuse. In an attempt to extend these findings, the current study looked at perinatal lead exposure as a risk factor for later self-administration of cocaine and cocaine/3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”). Female Sprague-Dawley rats were exposed daily to either 0 mg (n=6) or 16 mg (n=7) lead acetate via gavage. After 30 days of initial exposure, dams were bred with unexposed males. The exposure regimen continued throughout breeding, gestation, and lactation up to weaning at post-natal day (PND) 21. On PND 60, male pups from control and lead-exposed dams were implanted with a jugular catheter under surgical anesthesia. Subjects were trained to lever-press for .500 mg/kg/inf. cocaine. Following shaping, operant responding rates were examined for four doses of cocaine (.030, .060, .125, and .250 mg/kg/inf.) and cocaine/MDMA combinations (the four cocaine doses combined with .1 mg/kg/inf. MDMA). Analysis of results revealed that animals exposed to lead responded at higher rates for all doses of cocaine, with significant differences at low doses (.030 and .060 mg/kg/inf.). MDMA universally and dose-dependently suppressed responding for cocaine (significantly at .060 and .125 mg/kg/inf.). There were, however, no group differences in rate of responding for cocaine/MDMA combinations. The results imply that early lead exposure serves as a risk factor for later drug abuse by increasing the reward potency of cocaine and
increasing sensitivity to the suppressive effect of MDMA combinations. Further research to more fully characterize the relationship between environmental lead exposure and drug abuse and to determine the underlying mechanisms responsible for the observed behavioral effects may prove vital to our understanding of risk factors involved the selection and intake of commonly abused drugs.
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INTRODUCTION

Environmental pollution continues to present health risks in the United States. Of the many hazardous environmental chemicals that are potential sources of risk, heavy metals such as lead are possibly of greatest concern. Those living in the inner city are much more likely to have elevated blood lead levels (BLLs) that put them at risk for health problems (Pirkle, LeBoutillier, & Brooks, 1992; Vanderschmidt, Lang, Knight, & Vanderschmidt, 1993). A recent report by the Centers for Disease Control and Prevention (CDC) indicates that although the percentage of children nationwide with elevated BLLs above the current guidelines of 10 ug/dl, has been decreasing, up to 7.6% of children nationwide may still be at risk for the effects of lead poisoning (CDC, 2000).

Relative to the adult, the developing brain is more vulnerable to metal-toxicity (Cory-Slechta, 1997; Needleman, 1992; Tesman & Hills, 1994; Winneke, Altmann, Turfeld, Behler, Gutsmuths, & Mangold 1994). Various toxic effects of childhood lead exposure such as learning deficits and retardation (Needleman, 1992; Winter, 1982) persist long after blood metal residues return to normal (Banks, Ferretti, & Shucard, 1997; CDC, 1991). In animal studies of developmental lead exposure, the literature describes how both appetitively (e.g., Newland, Ng, Baggs, Gentry, Weiss, & Miller, 1986; Rice, 1993) and aversively (Winneke, Liienthal, & Werner, 1982) motivated behavior is impaired.

Heavy metal exposure has long-term effects on various neurotransmitter systems. A body of evidence indicates neonatal lead exposure, relative to the adult case, is appreciably

This thesis follows the style and format of Neurotoxicology.
more likely to alter glutamate receptor binding and NMDA stimulated changes in behavior (Guilarte, 1997; Petit, LeBoutillier & Brooks, 1992; Rajanna, Rajanna, Hall, & Yallapragada, 1997). Other lead/neurotransmitter studies indicate developmental lead exposure interferes with the synthesis and release of dopamine (Lasley, 1992).

The rewarding effects of commonly abused drugs are believed to be regulated by their effects on neurotransmitter release. Cocaine and 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) both act on the post-synaptic reuptake mechanism to increase extracellular levels of dopamine, serotonin, and norepinephrine (McKim, 1997). Long-lasting disturbances in dopaminergic and glutamate function such as those caused by early-age lead exposure may affect selection and intake patterns of many commonly abused drugs (Wise & Bozarth, 1987).

Thus, it is not surprising that lead exposure effects on various animal models of drug abuse have been reported. In adult rats, lead contamination has been observed to increase alcohol consumption (Nation, Baker, Fantasia, Ruscher, & Clark, 1987; Nation, Baker, Taylor, & Clark, 1988), disturb behavioral effects of cocaine exposure (Grover, Nation, & Bratton, 1993; Nation, Miller, & Bratton, 2000), and antagonize stimulatory properties of morphine (Miller, Nation, Jost, Schell, & Bratton, 2000). Further, it has been recently shown that perinatal lead exposure alters responsiveness to cocaine when the drug is presented during the adult cycle, long after the toxicants have been cleared from the soft tissues (blood, brain) (Nation, Smith, & Bratton, 2002).

These demonstrated relationships between lead exposure and drug effects, in combination with the continued widespread national issues associated with drug abuse, make a compelling argument for research to more fully characterize the relationships between heavy metal exposure and drug abuse. The recent and growing trend in the drug subculture of using polydrug combinations indicates that research examining polydrug interactions is long overdue. The current study examined the effects of perinatal lead exposure on later self-administration of cocaine and cocaine/MDMA combinations. Cocaine is a powerful reinforcing stimulus, and is readily self-administered by various mammalian species, including rats (McKim, 1997). MDMA
self-administration, however, has been reported in only a few studies involving rhesus monkeys (Beardsley, Balster, & Harris, 1986) and baboons (Sannerud, Brady, & Griffiths, 1989). Reliable self-administration of MDMA has not been reported in rats.

To determine the relationships between early-age lead exposure, cocaine, and MDMA, we employed an animal model that tested adult male rats for self-administration of cocaine and cocaine/MDMA combinations long after lead-exposure had ceased. Animals born to dams exposed to 0 mg or 16 mg lead daily prior to and throughout breeding, gestation, and lactation were tested in free-operant responding for various doses of cocaine (.030-.25 mg/kg/intravenous infusion) and cocaine/MDMA combinations (cocaine doses combined with .1 mg/kg/inf. MDMA) beginning on postnatal day 70. Based on the neurotransmitter evidence and on previous behavioral research (Nation et al., 2002), we hypothesized that perinatal lead exposure would sensitize animals to the rewarding effects of both cocaine and cocaine/MDMA combinations. In a free-operant responding paradigm of self-administration, this sensitization is represented by a leftward shift (higher responding for lower doses) in the dose-effect curve for the drugs. We further expected MDMA to augment the primary rewarding effects of cocaine, resulting in a similar leftward shift in the dose-effect curve (relative to cocaine administered alone).
METHODS

All aspects of the research reported here were approved by the Texas A&M University Laboratory Animal Care Committee.

Animals

For 30 days female Sprague-Dawley (Harlan; Houston, TX) rats were exposed daily to 0 (sodium acetate, n=6) or 16 mg lead (as lead acetate, n=7) using a 16 ga oral gavage needle. The respective solutions were dissolved in a volume of 1.0 mL-deionized water. After 30 days of initial exposure, females were bred with nonexposed males. Cages were checked daily for copulatory plugs, and males were removed as soon as plugs were found. Females received their daily doses of control or lead solution throughout gestation and lactation. Use of the gavage procedure permitted a perinatal lead exposure regimen wherein pups were unable to gain postnatal access to lead via routes other than maternal milk. Rat chow and tap water were available ad libitum in the home cage.

Body weights of offspring were recorded on postnatal day (PND) 1 and litters were culled to 8 pups. Body weights were recorded weekly for each pup. Pups remained with dams until PND 21, at which point all male pups were separated into cages containing 2-3 pups each and given ad libitum access to standard rat chow and tap water with no added lead. Food intake was measured weekly for male offspring after PND 21. On PND 50, animals were separated into individual housing, where they remained for the remainder of the study. To avoid confounds of litter effects, only one pup from each litter was used in the experiment. Other pups were used for related studies on lead toxicology and behavioral pharmacology. All animals were maintained on a 12-hr light/dark cycle and individually housed from PND 50 until the study was completed.
**Surgery**

On PND 60, under aseptic conditions, chronic indwelling jugular catheters were implanted in controls (n=6) and lead-exposed (n=7) animals. Anesthesia was produced with separate intraperitoneal (i.p.) injections of 50-mg/kg ketamine and 50-mg/kg sodium pentobarbital. A catheter consisting of .02 interior diameter silastic tubing (Dow Corning) and a silicon mount was inserted into the right jugular vein and held in place with silk thread. The catheter was passed subcutaneously through the body of the animal and exited the top of the head between the ears. The catheter was attached to a 22 ga metal tube approximately 5 cm in length which was mounted to the skull using jewelers' screws and dental acrylic. To maintain catheter patency throughout the study, rats received daily infusions (.1 mL) of a sterile saline solution containing heparin (1.25 U/mL), ampicillin sodium (170 mg/mL), and streptokinase (8,000 U/mL). Rats were allowed a 7-day recovery period before the onset of self-administration training.

**Self-Administration Training/Testing**

Sixteen free-operant self-administration chambers (Med Associates) in sound-attenuating cubicles served as the test apparatus. Each chamber contained two levers and a stimulus light above each lever. Infusion pumps (Med Associates) delivered drug solution to each of the boxes. A 20-mL syringe delivered i.v. infusions (.1 mL) over a 12.0 sec interval. IBM computers controlled the system using OPN software to control drug delivery and record data from 8 chambers each. Subjects were randomly assigned to chambers, and testing occurred at the same time each day during the light phase of the cycle.

All test animals were water deprived for 24-hr prior to commencing shaping to lever press for a .500 mg/kg infusion of cocaine HCl (administered as the salt). Shaping occurred in 2-hr daily sessions on a fixed-ratio (FR-1) schedule wherein each depression of the right ("active") lever activated the 20-mL syringe infusion pump, delivering an infusion of drug. The right stimulus light was illuminated for the duration of each drug infusion. The houselights inside the test chambers were turned off during self-administration training and testing. Lever responses
during the drug-delivery phase had no programmed consequence and are not reported here. Left ("inactive") lever responses were recorded but had no programmed consequence.

Once animals acquired the lever press response and responding stabilized at FR-1, water was again made available ad libitum in the home cage and animals were shifted to an FR-2 baseline schedule of i.v. cocaine reinforcement wherein two active lever responses resulted in a delivery of .500 mg/kg cocaine.

Dose-effect testing began after responding stabilized (<20% variation across 2 sessions) at FR-2 for .500 mg/kg infusions. Two stable baseline sessions of responding at each dose of cocaine (.125, .25, .06, and .03, in order) were followed by two test sessions consisting of the cocaine dose from the previous session in combination with .1 mg/kg/inf. MDMA [administered as the salt]. Number of active lever responses was recorded for each daily session.

**Blood Sampling**

To confirm exposure levels, blood samples were collected from dams and pups at various points during the experiment and analyzed for lead levels using atomic absorption spectrophotometry. For dams, .1-.15 mL of tail-blood was drawn one day prior to breeding, at day 10 of gestation, and PND 1. Blood samples were collected from littermates sacrificed at PND 1 and PND 21. Under lethal anesthesia produced by sodium pentobarbital (150 mg/kg i.p.), blood was collected via cardiac puncture from dams at PND 21. Test animals and littermates were sacrificed and blood samples collected in the same manner at the end of dose-effect testing.

**Statistics**

All data were analyzed using SysStat 7.0. Separate one-way analyses of variance (ANOVAs) were used to analyze litter size and blood and tissue lead levels by Group (0 or 16-mg lead). Body weights and food intake for pups were analyzed by Group across Weeks using separate repeated measures two-way ANOVAs (Groups X Weeks). To analyze the effects of lead on responding for cocaine and cocaine/MDMA combinations, active lever responses from the last baseline session and from the last test session were analyzed by Group across Dose
using separate repeated measures two-way ANOVAs. Another repeated measures two-way ANOVA was used to analyze the effects of MDMA on responding for cocaine across dose, using MDMA (presence or absence) and Dose as the independent variables.
RESULTS

Consistent with the stated hypothesis and previous findings (Nation et al., 2002), the results of our experiment indicate that lead exposure during gestation and lactation sensitizes animals to the rewarding effects of cocaine when the drug is offered as a reinforcer much later in life (i.e. produces a leftward shift in the dose-effect curve). Contrary to our expectations, however, we found that MDMA had a generally suppressive effect on cocaine responding.

Body Weights and Food Intake

Analysis revealed no significant difference (p>0.05) between Group 0-mg Lead and Group 16-mg Lead in terms of either body weights or food intake. Figures 1 and 2 display representative data (seven weeks beginning around PND 21) by group for body weights and food intake, respectively.

Fig. 1 Average Weekly Food Intake by Group. Analysis showed no significant difference across Groups (p>0.05).

Fig. 2 Average Weekly Body Weights by Group. Analysis showed no significant difference across Groups (p>0.05).
Cocaine Self-Administration

Examination of responding for cocaine delivered alone revealed that at the lower doses of .030 and .060 mg/kg cocaine there was evidence that lead-exposed animals (Group 16-mg Lead) responded at higher rates than controls (Group 0-mg Lead). Figure 3 shows the dose-effect curve for each group. Group 16-mg Lead animals responded significantly more frequently for cocaine, with a main effect of Groups ($F(1,11)=9.04, p<.05$). Responding by Group 16-mg Lead was significantly greater at both .030 mg/kg ($F(1,11)=7.82, p<.05$) and .060 mg/kg ($F(1,11)=9.33, p<.05$). Responding for the higher doses of .125 mg/kg (mean responses/session $= 132.2 \pm 34.6$ SEM and $178.5 \pm 17.08$ SEM for Groups 0-mg Lead and 16-mg Lead, respectively; $p>.05$) and .250 mg/kg (mean responses/session $= 90.4 \pm 14.33$ SEM and $100.2 \pm 13.0$ SEM for Groups 0-mg Lead and 16-mg Lead, respectively; $p>.05$), while higher in Group 16-mg Lead, was not significantly different across Groups.

![Responding for Cocaine by Group](image)

Fig. 3 Dose-Effect Responding for Cocaine by Group. Early exposure to lead was found to produce the expected left shift in the dose-effect curve, providing evidence that lead exposure increased cocaine's reward potency. Responding in Group 16-mg Lead was higher at all doses, with significant differences (*) at .030 and .060 mg/kg/inf.
Cocaine/MDMA Self-Administration

Comparison of responding for cocaine alone versus cocaine/MDMA combinations revealed that MDMA suppressed responding with a significant MDMA X Dose interaction effect ($F(1,24)=11.40$, $p<.01$). MDMA significantly suppressed responding at doses of .060 ($F(1,24)=10.72$, $p<.01$) and .125 mg/kg ($F(1,24)=8.22$, $p<.01$) cocaine. Figure 4 shows responses for cocaine baseline doses and for combination doses (averages collapsed across Groups 16-mg Lead and 0-mg Lead, which did not differ, $p>0.05$).

**Fig. 4 Effect of MDMA on Responding for Cocaine (collapsed across Groups).** Contrary to expectations, MDMA was found to dose-dependently suppress responding for cocaine, with significant (*) effects at .060 and .125 mg/kg/inf.

Finally, Table 1 presents lead concentration data for dams, littersmates, and test animals. It is evident from these data that the behavioral effects observed among test animals occurred after lead had cleared soft tissues such as blood and brain.
Table 1 Blood Lead Levels for Dams and Pups at Various Experimental Points. Reported as average (SEM); significant differences (p<.05) marked by *.

<table>
<thead>
<tr>
<th>Experimental Point</th>
<th>Group 0-mg Lead</th>
<th>Group 16-mg Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dams</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 30 Exposure</td>
<td>.01 (.002)</td>
<td>.31 (.029)*</td>
</tr>
<tr>
<td>Day 10 Gestation</td>
<td>.03 (.011)</td>
<td>.29 (.034)*</td>
</tr>
<tr>
<td>PND 2</td>
<td>.01 (.003)</td>
<td>.40 (.056)*</td>
</tr>
<tr>
<td>PND 21</td>
<td>.01 (.001)</td>
<td>.34 (.080)*</td>
</tr>
<tr>
<td><strong>Littermates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PND 1</td>
<td>.02 (.004)</td>
<td>.46 (.060)*</td>
</tr>
<tr>
<td>PND 21</td>
<td>.01 (.004)</td>
<td>.12 (.023)*</td>
</tr>
<tr>
<td><strong>Subjects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End Dose Effect</td>
<td>.03 (.001)</td>
<td>.02 (.005)</td>
</tr>
</tbody>
</table>
DISCUSSION

Consistent with predictions and previous findings (Nation, 2002), early-age lead exposure was found to produce a left shift in the dose-effect curve. This provides further evidence that developmental heavy-metal toxicity sensitizes rats to the rewarding effects of cocaine (i.e., increases the reward potency of cocaine) when the drug is presented much later in life. Interestingly, however, our results failed to show any effect of early lead exposure on rates of self-administration of cocaine/MDMA combinations. While these data seem to imply that early lead exposure has no effect on the reward potency of the drug combination, the significant difference of responding for cocaine implies a different interpretation.

Our results indicate that MDMA suppresses responding for cocaine at peak doses with a "floor" effect, wherein presence of the drug ... causes responding to decrease to very low levels relative to responding for cocaine alone. The drug suppresses responding in a dose-dependent fashion (with the strongest effect at lower doses). Despite increased responding for cocaine alone by animals exposed to lead, however, responding for the drug combination did not vary between the two groups. The drug therefore had a greater suppressive effect in lead-exposed animals than in controls. This indicates either a differential sensitivity to the effect of the drug based on early lead exposure or a limit to the reward potency of cocaine determined by the presence of MDMA. To determine which, if either, of these explanations is correct, further research should focus on testing the effects of various doses of MDMA on responding for the drug combination.

The observed suppressive effect may be due to either a general sedative effect of the drug or receptor competition between the two drugs. Anecdotal reports indicate that, unlike most amphetamine derivatives, MDMA often has a sedative effect in humans. The floor effect on cocaine responding indicates that once a certain level of MDMA is attained in the blood, the sedative properties may overcome the rewarding properties of cocaine, thereby compromising
the reinforcing properties of the stimulant (and producing the observed suppression in responding).

However, since MDMA and cocaine both work on the same post-synaptic reuptake mechanism, it is also possible that receptor competition accounts for the observed interaction. MDMA acts most potently to increase serotonin release, whereas cocaine’s most potent effects are on dopamine. If the presence of MDMA effectively blocks dopamine reuptake receptors from interaction with cocaine, the observed lower responding (implying lower reward potency) would be expected. However, receptor competition at the serotonin or norepinephrine reuptake sites could be equally important in explaining the observed effects. Therefore, future research should focus on the interaction between cocaine and more selective pharmacological agents to more fully characterize the underlying mechanism creating the observed interaction.

As a final note, the observed interaction between cocaine and MDMA in vivo underlines the importance of future polydrug combination research. Our results indicate that polydrug abuse changes the risks involved in drug abuse. Given the growing trend in polydrug abuse and our poor understanding of the subject, future research involving various polydrug combinations could potentially benefit our ability to understand and treat drug abuse.
REFERENCES


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  - Animal Handling and Care (injections, surgery, etc.)
  - Lab Procedures (Tail flick and hot plate analgesic response tests)

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  Assisted in research examining the role of various experimental compounds on
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  - Lab Procedures as described above

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  Rewarding Effects of Cocaine, but not Cocaine/3,4-Methylenedioxymethamphetamine
  (MDMA) Combinations. (In preparation)

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