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Perinatal exposure to lead attenuates the conditioned reinforcing properties of cocaine in male rats

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Abstract

The purpose of this study was to examine the effects of developmental lead exposure on drug responsiveness later in the life cycle. Adult female rats were gavaged daily with 0, 8, or 16 mg lead for 30 days before breeding with non-exposed males. The respective exposure regimens were maintained throughout gestation and lactation (perinatal exposure). In Experiment 1, at postnatal day (PND) 30 or 90, pups were trained with 0, 1.25, 2.5, or 5 mg/kg cocaine HCl (IP) in a biased conditioned place preference (CPP) procedure. At both PND 30 and 90, an attenuation in CPP was present in animals exposed to 8 or 16 mg lead relative to control rats. Using an identical lead-exposure regimen, a conditioned place aversion (CPA) procedure with 0, 10, 20, or 40 mg/kg lithium chloride (IP) was employed for Experiment 2. No significant differences were present among pups from each lead-exposure group conditioned and tested at PND 30 or 90, thus suggesting that an impairment of associative mechanisms was not solely responsible for the pattern of attenuation present in Experiment 1. Subsequent analyses of blood-lead in all experiments demonstrated concentrations below 5 μg/dl for all animals at PND 30 and below detectable limits (< 1 μg/dl) at PND 90. The findings suggested attenuation in cocaine reinforcement with perinatal lead exposure even though the metal apparently had gained clearance from soft tissue.

Keywords: Cocaine; Conditioned place preference; Development; Lead

1. Introduction

Lead is distributed widely throughout the environment, and continues to be used in many industrial products. In the past 50 years, it has become known that exposure to this heavy metal is associated with a variety of neurologic and behavioral impairments [7]. Studies of human blood-lead levels consistently report that sociodemographic factors, such as low-income level, are associated with increased concentrations of the metal [27]. This is perhaps because economically disadvantaged persons are more likely to live in inner city areas that retain lead through residual paint and past automobile pollution [20].

In addition to environmental exposure to greater pollution in the inner cities, drug use is a concern for such residents [12]. Epidemiological surveys indicate children who grow up and remain in the inner city are more likely to be drug users and live in a neighborhood with heavy drug trafficking, compared to those who live in more affluent areas [11]. Although drug use is not exclusive to urban zones, clearly there is increased availability and higher rates of use in the inner city [12]. With increased rates of drug use and heightened exposure to lead, it is possible that environmental pollution and drug abuse could interact in an undetermined manner in a sizable segment of the urban population.

Animal models have indicated that adult exposure to lead, at clinically relevant concentrations, produces changes in drug-induced behaviors [4,23]. Regarding cocaine, adult exposure to lead delayed the development of locomotor sensitization, and ultimately, the magnitude of elevated responding to repeated cocaine challenges was less in metal-exposed than in control animals [24]. It also has been demonstrated that developmental exposure to lead produces an alteration in cocaine sensitization. Perinatal exposure to lead attenuated the locomotor-activating prop-

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erties of an acute presentation of cocaine relative to non-exposed animals. With repeated drug presentation, both metal-exposed and control animals developed locomotor sensitization. However, animals that received perinatal exposure to lead showed greater sensitization than control animals [25], a pattern opposite that reported with adult lead exposure [24].

These reported results of a change in sensitivity to cocaine have implications with respect to defining the abuse potential of this psychostimulant. Considerable evidence exists to demonstrate the importance of drug-related stimuli in the reinforcing properties of drugs [13]. An appropriate paradigm to study the influence of context on drug use is conditioned place preference (CPP). In this model, a drug is administered to the animal immediately before placement in an environment with unique contextual stimuli (olfactory, visual, tactile). Following several pairings of the drug and the unique context, and separate pairings of a distinctly different context and no drug (vehicle), the animal is tested for preference by being allowed free-choice access to the drug-paired and the vehicle-paired context. Place preference is then defined by some measure of partiality for one environment over another. Cocaine and other drugs of abuse have been shown to produce a place preference [2,13], and in the adult organism, this behavior can be attenuated by exposure to heavy metals at biologically relevant concentrations [21,22].

Although CPP is considered a valid model of drug reinforcement, and it is further believed that an attenuation in the development or expression of a CPP is a reflection of an antagonism of the neural mechanisms of drug reinforcement, it is also possible that any attenuation in CPP could derive from challenges to associative or cognitive processing. The CPP paradigm is based on classical (Pavlovian) conditioning principles, as contextual cues acquire secondary reinforcing properties through temporal pairing with the drug [5]. Developmental exposure to lead has been demonstrated to disrupt the acquisition of learned tasks [3,15], perhaps through modulation of neural loci associated with learning [9,10,18]. Thus, it is reasonable to suspect that any effects of the metal on cocaine CPP may be due to alterations in learning and conditioning mechanisms, rather than exclusive disturbance of cocaine pharmacodynamics or drug kinetics.

The experiments reported here present findings on the effects of perinatal lead exposure on the conditioned reinforcing effects of cocaine and lithium chloride (LiCl). Experiment 1 assessed the effects of perinatal lead exposure on cocaine CPP. To address issues related to the actions of lead on mechanisms of learning, Experiment 2 employed a conditioned place aversion (CPA) procedure in which LiCl was paired with one distinct context and vehicle-only was paired with another. To assess differential age-related effects, both experiments were conducted on separate groups of developmentally exposed animals at postnatal days (PND) 30 and 90.

2. Materials and methods

2.1. Apparatus

Place conditioning and testing were conducted in seven 20 × 60 × 20-cm wooden shuttle boxes with wooden tilt floors. At the end of each box was a microswitch interfaced to an IBM-compatible computer. A BASIC computer program was written to continuously record the number of times and duration the switch was activated through a tilt of the floor by a rat.

Half the box had white walls with a smooth white floor and the other had black walls with a black sandpaper floor. For conditioning sessions, the boxes were divided into two equal-sized compartments by removable partitions. On test sessions, the partitions were removed and a 20 × 10 × 5-cm wooden platform was installed 2 cm above the floor to divide the two compartments but allow free access by rats. In earlier investigations, subjects showed a strong preference for the black side [21,22]. To counteract this preference, a 40-W light was positioned 50 cm above the black side of each apparatus. These seven lamps provided the only illumination in the room. Following each conditioning and test session, the apparatus was cleaned with a mild soap solution. The apparatus was located in a sound-resistant room with a 40-dB white noise generator operating continuously. All conditioning and testing sessions were conducted during the light phase of the cycle.

2.2. Drugs

Lead acetate trihydrate (Sigma, St. Louis, MO) was dissolved in deionized water and presented via gavage as a 1-ml solution. Doses are expressed as the lead base. A solution of 0.4% w/v sodium acetate (Sigma) was also prepared weekly for control (0 mg lead) dosing. Cocaine HCl was provided gratis by the Research Technology Branch of NIDA. The drug was dissolved in 0.9% w/v saline vehicle, and doses are expressed as the salt. LiCl (Fisher Scientific; Fair Lawn, NJ) was also dissolved in saline, and doses are expressed as the base. Both cocaine and LiCl were administered IP at a volume of 1 ml/kg.

2.3. Experiment 1: cocaine place conditioning

2.3.1. Subjects

For both Experiment 1 and Experiment 2, a total of 123 adult female Sprague–Dawley rats (Charles River) were weighed and randomly assigned to an exposure group [0 mg lead (n=46 dams), 8 mg lead (n=40 dams), and 16 mg lead (n=37 dams)]. Because female pups were used in a separate investigation of the effects of lead exposure on neuroendocrine function, and because of the limited number of CPP test boxes, the experiments had to be completed as a series of replications based on the availability of male pups. Efforts were made to counterbalance by group, and addi-

tional animals were run in each condition at each replication as a check against reliability. Although this approach necessarily yielded different sample sizes, in all cases, a sufficient number of pups were placed in each group to ensure statistical validity. No significant differences were observed among groups in the presence of copulatory plugs, the number of dams that delivered pups, or pup mortality, and none of the available animals was discarded nor was their data excluded from analysis. However, litter size for 16 mg lead dams (mean = 10.8 pups, SEM = ± 1.3 pups) was decreased relative to controls (mean = 12.9 pups, SEM = ± 0.6 pups) and 8 mg lead dams (mean = 12.1 pups, SEM = ± 0.6 pups), further constraining the ability to maintain equivalent sample sizes across experimental conditions. For all conditions, rats were housed individually in hanging polycarbonate cages, and maintained on a 12L:12D cycle (lights on at 0500 h). Standard rat chow (Teklad) and tap water were available ad lib. Body weights and food consumption were recorded weekly.

On the day before lead exposure commenced, and again at breeding, parturition, and weaning, 100–150 μl of tail blood was drawn from the dams. The blood was mixed with heparin and frozen at − 70°C.

Following 30 days of daily intubation with 0, 8, or 16 mg lead, females were paired with a non-metal-exposed male for breeding. Males were removed once females tested positive for a copulatory plug. Females continued to receive daily administration of the appropriate lead solution through gestation and lactation, and no lead was administered to the pups by the investigators. At PND 7, litters were culled to 8–10 pups. All male pups remained in the litter, and enough females remained to maintain consistent litter size across all dams. Four male pups from each lead-exposure group were sacrificed at PND 1 for blood-lead analysis.

At PND 21, male pups were weaned and transported to a different colony room. Pups were double-housed and had ad lib access to food and water throughout the remainder of the experiment. Body weights and food consumption were recorded weekly. At PND 29, all pups were weighed and assigned randomly to Experiment 1, Experiment 2, or several other experiments conducted concurrently in the laboratory.

The animal holding and testing facility was approved by the Association for the Assessment and Accreditation of Laboratory Animals Care International (AAALAC International), and all animal maintenance and research was conducted in accordance with the guidelines provided by the University Laboratory Animal Care Committee (ULACC). The campus veterinarian monitored the health of the animals throughout the duration of the project.

2.3.2. Procedures

Separate groups of animals commenced behavioral training and testing at PND 30 and PND 90. The experimental procedures described were identical for training that began at PND 30 and PND 90.

On day 1 of behavioral training, animals were transferred from the colony to the testing room for 40 min to habituate to transportation and the sound and illumination of the room. They were not placed in the apparatus. Initial biases for the white or black chamber (pretest) were determined on day 2 as non-injected control and lead-exposed animals were given free access to either chamber for 15 min. On days 3–10, different groups of control or lead-exposed animals received one of four injections of cocaine (0, 1.25, 2.5, or 5 mg/kg) on four of the conditioning days, and four vehicle (saline) injections on the remaining four conditioning days. In all cases, the animal was confined to the side least preferred (defined as the side in which the animal spent the least amount of time on the day 2 pretest), 5 min after receiving a cocaine injection. The animal was confined to the most-preferred chamber (defined as the side in which the animal spent the most amount of time on the day 2 pretest), 5 min following a vehicle injection. The period of confinement for each conditioning trial was 30 min. Cocaine and vehicle were presented on alternating days (eight total) and the injection received first was counterbalanced for type of injection (saline, cocaine) and exposure regimen (control, lead). Animals were run in squads of seven, counterbalancing by dose and group assignment. A posttest was conducted on day 11 following the same procedure as the day 2 pretest.

2.3.3. Brain and blood-lead collection and analysis

To assess lead concentrations in the brain at PND 30, 24 h after the posttest, five animals from the 0-mg lead-exposure group and six animals from the 16-mg lead-exposure group were rendered unconscious with 60 mg/kg sodium pentobarbital (IP), and perfused with a saline/formalin mixture. Blood was collected via cardiac puncture, and the whole brain was removed and frozen for analysis. Brain samples were not collected from any pups at PND 90. All remaining pups at PND 30 and PND 90 were rendered unconscious with pentobarbital, decapitated, and approximately 1.0 ml of trunk blood was collected and later analyzed.

Lead residues in blood and brain were measured via atomic absorption spectrophotometry [26]. To assure accuracy in measurements of blood levels, certified standards (National Institutes of Health, Centers for Disease Control, and Wisconsin Blood Lead Certification Program) for blood-lead were processed before and after blood-lead samples collected from the experiments reported here. Further, spiked samples were spaced intermittently (1 per 20 experimental samples) to ensure adequate recovery. If, at any time, values from the standards were more than 5% above or below the certified mean or outside the 99% confidence interval, analysis of samples was stopped, and recommenced following diagnosis of any problem with the lead analysis procedures.

2.4. Experiment 2: lithium chloride place conditioning

2.4.1. Subjects

Pups for Experiment 2 were exposed to lead simultaneously with pups used for Experiment 1. As such, lead-exposure procedures were identical to those mentioned for Experiment 1.

2.4.2. Procedures

The conditioning and testing procedures for Experiment 2 were precisely as described for Experiment 1, with two exceptions. First, separate groups of animals received one of four LiCl doses (0, 10, 20, and 40 mg/kg) on the drug-side of the chamber during the training period (days 3–10). The injections were given 5 min before placement in the chamber and the duration of confinement was 45 min. Second, each animal was confined to the side most preferred (defined as the side in which the animal spent the most amount of time on the day 2 pretest) 5 min after receiving a LiCl injection. The animal was confined to the least-preferred chamber (defined as the side in which the animal spent the least amount of time on the day 2 pretest) 5 min following a vehicle injection. All other aspects of the study, including posttest procedures on day 11, were as described for Experiment 1.

2.5. Data analysis

Because animals for both experiments were born of the same dams, data on maternal blood-lead, body weight, and food consumption were collapsed across the experiments. Analyses of maternal blood-lead concentrations were conducted through separate one-way analysis of variance (ANOVA) tests and Newman–Keuls post hoc tests ($p < 0.05$) from each of the time points at which tail blood was collected. An exposure group (0, 8, 16 mg lead) × weeks repeated-measures ANOVA (RANOVA) was conducted for the dam food consumption and body weight analyses.

Collapsing across experiments, analysis of pup blood-lead concentrations (μg/dl) was conducted through separate one-way ANOVAs following completion of the CPP and CPA procedures at PNDs 30 and 90. Data collected from the analysis of pup brains (μg/g) at PND 30 were analyzed in a similar manner. In both experiments reported here, pups were double-housed. To analyze food intake data the total food consumption of the cage pair was halved. In an effort to assess the possibility of a sampling bias, postweaning food consumption and body weight were analyzed using a 3 exposure group (0, 8, 16 mg lead) × 2 testing period (PND 30, PND 90) × 2 experiments (CPP, CPA) ANOVA performed on the PND 30 data. Separate ANOVAs were performed on food consumption and body weight of animals tested commencing at PND 90.

Conditioning scores were defined by the change in time (measured in seconds) in the drug-paired chamber from the pretest to posttest (time on posttest minus time on pretest). A CPP or CPA occurred when the mean group conditioning score was significantly different from the vehicle-only (0 mg/kg) group. An exposure group × drug dose (0, 1.25, 2.5, or 5 mg/kg cocaine; or 0, 10, 20, or 40 mg/kg LiCl) ANOVA test was employed, and Newman–Keuls post hoc tests were used when appropriate to determine significant main effect and interaction differences ($p < 0.05$).

3. Results

3.1. Exposure regimen

No differences were noted in dam mean weekly food consumption throughout the study (0 mg lead mean = 139.7 g, SEM = ± 3.6 g; 8 mg lead mean = 136.0 g, SEM = ± 9.3 g; 16 mg lead mean = 136.7 g, SEM = ± 7.0 g) ($p>0.05$). Further, no significant differences were present among female rats in body weights at the commencement of lead exposure (0 mg lead mean = 219.0 g, SEM = ± 4.1 g; 8 mg lead mean = 220.5 g, SEM = ± 4.6 g; 16 mg lead mean = 223.3 g, SEM = ± 7.4 g) or breeding (0 mg lead mean = 264.6 g, SEM = ± 9.3 g; 8 mg lead mean = 257.7 g, SEM = ± 6.0 g; 16 mg lead mean = 264.3 g, SEM = ± 4.2 g) ($p>0.05$). With regard to blood-lead concentrations, upon arrival to the laboratory, all dams had levels below detectable limits (<1 μg/dl). However, as depicted on Table 1, a significant and dose-dependent elevation in blood-lead concentrations was present at breeding ($F(2,57) = 56.01$, $p < 0.001$), parturition ($F(2,57) = 22.65$, $p < 0.001$), and gestation ($F(2,57) = 35.71$, $p < 0.001$) in the dams following treatment with 8 or 16 mg lead, relative to animals that received daily intubation with sodium acetate (0 mg lead). There was no mortality for dams from any of the three lead-exposure conditions, and no overt signs of toxicity were noted by experimenters throughout the lead-exposure period.

The analysis of preweaning (PND 30) food consumption failed to reveal any significant differences according to exposure group, testing period, or experiment. However, the analysis of body weight at PND 30 did show a significant main effect for exposure condition, $F(2,401) = 24.82$, $p < 0.001$. Post hoc comparisons indicated that the two lead-exposure groups (8

Table 1
Mean (±SEM) blood lead concentrations for dams at breeding, parturition, and weaning (PND 21)

	Breeding (μg/dl)	Parturition (μg/dl)	Weaning (PND 21, μg/dl)
0 mg	< 1	< 1	< 1
8 mg Lead	16.6 ± 4.0*	22.0 ± 4.5*	23.0 ± 5.1*
16 mg Lead	32.5 ± 1.4*,#	31.0 ± 4.9*	33.9 ± 0.4*,#

* Denotes significant group separation ($p < 0.05$). Group blood-lead levels significantly elevated relative to the 0-mg lead group.

# Denotes significant group separation ($p < 0.05$). Group showed blood-lead levels significantly elevated from the 8-mg lead group.

and 16 mg), which did not differ, had lower body weights than controls ($p < 0.05$). None of the main effect or interaction tests involving testing period or experiment reached an acceptable level for statistical significance. Thus, there were no indications of sampling bias anywhere in the study. At PND 90, none of the main effect or interaction tests were found to be significant on either the test performed on food consumption or the test performed on body weight. Table 2 presents the relevant mean food consumption and body weight data for each exposure group at PND 30 and PND 90, collapsed across the non-significant factors of testing period or experiment.

Table 3 presents blood-lead levels (μg/dl) from the pups from each exposure group sacrificed at PND 1. Levels are also reported from the analysis of blood collected at the completion of the behavioral testing that began at PND 30 and PND 90. From the paired litter mates at PND 1, there was a lead dose-dependent elevation in blood-lead levels (16>8>0 mg lead) ($F(2,18) = 7.14$, $p < 0.05$). At PND 30, lead concentrations were below detectable levels (<1 μg/dl) in control and 8 mg lead pups, but significantly elevated levels remained in animals exposed to 16 mg lead perinatally ($F(2,87) = 6.62$, $p < 0.01$). By PND 90, concentrations in all groups were below detectable limits and no group differences were evident ($p < 0.05$). Analysis of the five whole brains from control pups and six whole brains from pups in the 16-mg lead group at PND 30 indicated lead levels below detectable limits (<1 μg/g) for all samples.

3.2. Experiment 1: cocaine place conditioning

3.2.1. Postnatal day 30

For the pups commencing place preference training at PND 30, more time was spent in the black than the white chamber on the day 2 pretest. There were no significant differences among groups (control mean = 248.8 s, SEM = ± 12.7 s; 8-mg lead-exposed mean = 286.0 s, SEM = ± 19.7 s; 16-mg lead-exposed mean = 235.9 s, SEM = ± 16.8 s) with respect to the amount of time spent in the side least preferred on the pretest ($p > 0.05$).

A two-way ANOVA (exposure group × cocaine dose) was conducted on the conditioning scores (time on the least-preferred side on the posttest minus time on that side during the pretest) yielded by day 11 testing. A significant main effect of cocaine dose was found ($F(3,106) = 11.05$, $p < 0.001$), and post hoc tests indicated that the 1.25 (mean = 129.6 s, SEM = ± 24.2 s), 2.5 mg/kg (mean = 146.7 s, SEM = ± 35.4 s), and 5 mg/kg doses (mean = 201.7 s, SEM = ± 30.7 s) yielded greater conditioning scores than the vehicle-only dose (mean = 0.65 s, SEM = ± 19.7 s). A significant main effect of exposure group also was found ($F(2,106) = 4.57$, $p < 0.05$), as the control group (mean = 191.2 s, SEM = ± 26.9 s) showed greater overall conditioning scores than the animals that received perinatal exposure to 8 mg lead (mean = 86.1 s, SEM = ± 25.2 s) or 16 mg lead (mean = 76.4 s, SEM = ± 25.5 s).

In addition to the main effects of exposure group and cocaine dose, a significant exposure group × cocaine dose interaction was found ($F(6, 106) = 7.66$, $p < 0.01$). Subsequent post hoc analyses were conducted to investigate the differential effect of perinatal lead exposure on cocaine CPP. As shown in Fig. 1A, post hoc tests showed that control animals exhibited a place preference at 1.25, 2.5, and 5 mg/kg cocaine ($p < 0.05$). Within the 8-mg lead-exposure group, a significant place preference was found at 2.5 and 5 mg/kg cocaine ($p < 0.05$). Animals that received perinatal exposure to 16 mg lead demonstrated a significant place preference at the 5-mg/kg cocaine dose only ($p < 0.05$).

Additional interaction comparisons of group separation at each dose of cocaine found that no significant differences in conditioning scores were present among groups at the 0- or 5-mg/kg cocaine dose. At the 1.25-mg/kg dose, control animals had conditioning scores significantly greater than animals in the 16-mg lead group ($p < 0.05$). At the 2.5-mg/kg dose, the relevant post hoc test indicated that control animals demonstrated conditioning scores significantly greater than those of animals in the 8- or 16-mg lead groups ($p < 0.05$).

3.2.2. Postnatal day 90

For animals commencing place preference training at PND 90, more time was spent in the black chamber than the white chamber on the day 2 pretest. There were no sig-

Table 2
Mean (± SEM) food consumption and body weight for all animals tested in CPP (Experiment 1) and CPA (Experiment 2), at postweaning (PND 30) [includes animals from both testing periods] and PND 90

		Food consumption (g)	Body weight (g)
PND 30	0 mg lead	129.6 ± 6.3	150.4 ± 15.2
	8 mg lead	126.7 ± 6.1	124.7 ± 7.0*
	16 mg lead	125.3 ± 5.8	122.6 ± 11.8*
PND 90	0 mg lead	169.2 ± 9.0	536.9 ± 15.5
	8 mg lead	162.7 ± 8.8	530.6 ± 13.6
	16 mg lead	165.4 ± 7.6	528.6 ± 7.6

* Different from the 0-mg lead group ($p < 0.05$).

Table 3
Mean (± SEM) pup blood-lead concentrations at PND 1 (parturition), PND 30, and PND 90

	PND 1 (parturition) (μg/dl)	PND 30 (μg/dl)	PND 90 (μg/dl)
0 mg Lead	< 1	< 1	< 1
8 mg Lead	12.3 ± 2.1*	< 1	< 1
16 mg Lead	21.8 ± 1.6*,#	5.0 ± 0.9*,#	< 1

* Denotes significant group separation ($p < 0.05$). Group blood-lead levels significantly elevated relative to the 0-mg lead group.

# Denotes significant group separation ($p < 0.05$). Group showed blood-lead levels significantly elevated from the 8-mg lead group.

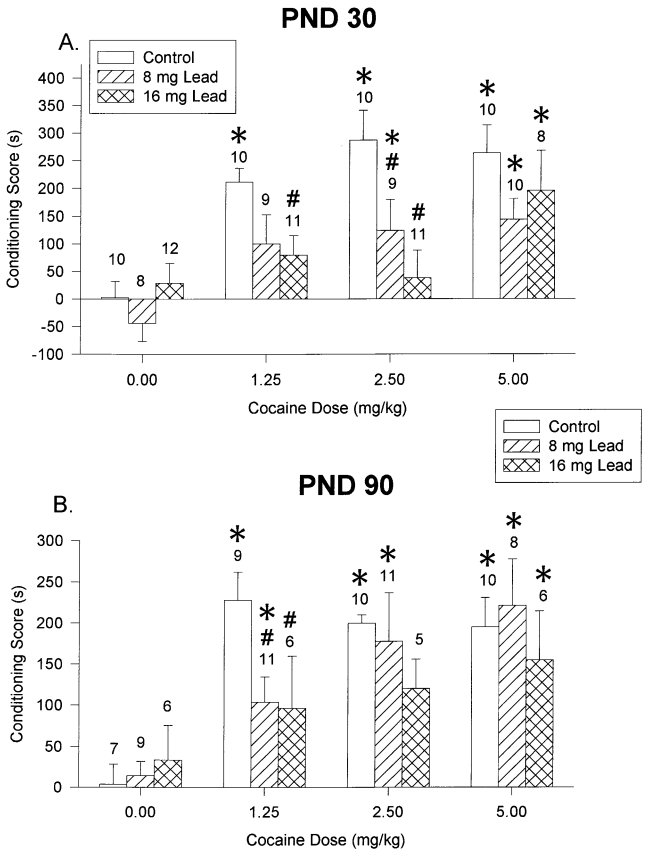

Fig. 1. (A) Mean (± SEM) conditioning scores (measured in seconds) for cocaine CPP for animals exposed perinatally to 0, 8, or 16 mg lead and tested commencing at PND 30. (B) Mean (± SEM) conditioning scores (measured in seconds) for cocaine CPP for animals commencing testing at PND 90. The numbers above the bars indicate group size. The following symbols denote significant group separation ($p < 0.05$): * The dose is significantly different from the same metal exposure condition (0, 8, or 16 mg lead) vehicle-only group; # The group showed a conditioning score significantly different from the 0-mg lead group.

nificant differences among groups (control mean = 294.2 s, SEM = ± 15.7 s; 8 mg lead-exposed mean = 291.2 s, SEM = ± 19.7 s; 16 mg lead-exposed mean = 278.7 s, SEM = ± 20.5 s) with respect to the amount of time spent in the side least preferred on the pretest ($p > 0.05$).

A two-way ANOVA (exposure group × cocaine dose) was conducted on the conditioning scores yielded by day 11 testing. A significant main effect of cocaine dose was found ($F(3,85) = 13.35$, $p < 0.001$), and post hoc tests indicated that the 1.25 (mean = 134.1 s, SEM = ± 24.8 s), 2.5 mg/kg (mean = 174.7 s, SEM = ± 26.3 s) and 5 mg/kg doses (mean = 215.0 s, SEM = ± 25.4 s) produced greater conditioning scores than the vehicle-only (mean = 7.3 s, SEM = ± 11.9 s) dose. Significant group separation was also present between the 1.25- and 5-mg/kg doses. A marginally significant main effect of exposure group also was found ($F(2,85) = 2.80$, $p = 0.066$), as the control group (mean = 174.8 s, SEM = ± 19.2 s) showed greater overall conditioning scores than the animals that received perinatal exposure to 16 mg lead (mean = 91.5 s, SEM = ± 26.3 s).

In addition to the main effects of exposure group and cocaine dose, a significant exposure group × cocaine dose interaction was found ($F(5,85) = 2.66$, $p < 0.05$). As shown in Fig. 1B, control animals demonstrated a significant place preference at 1.25, 2.5, and 5 mg/kg cocaine ($p < 0.05$). Animals in the 8-mg lead group also showed a place preference at 1.25, 2.5, and 5 mg/kg cocaine ($p < 0.05$). As was the case at PND 30, animals in the 16-mg lead-exposure group had a significant place preference at only the 5-mg/kg dose at PND 90 ($p < 0.05$).

Additional comparisons of group separation indicated no significant differences among exposure groups at 0, 2.5, and 5 mg/kg cocaine. However, at 1.25 mg/kg, control animals demonstrated conditioning scores significantly greater than those produced by animals that received perinatal exposure to 8 or 16 mg lead ($p < 0.05$).

3.3. Experiment 2: lithium chloride place conditioning

3.3.1. Postnatal day 30

For animals commencing CPA training at PND 30, more time was spent in the black chamber than the white chamber on the day 2 pretest. There were no significant differences among groups (control mean = 580.1 s, SEM = ± 12.9 s; 8 mg lead-exposed mean = 596.0 s, SEM = ± 21.8 s; 16 mg lead-exposed mean = 639.4 s, SEM = ± 27.4 s) with respect to the amount of time spent in the side most preferred on the pretest ($p > 0.05$).

A two-way ANOVA (exposure group × LiCl dose) was conducted on the conditioning scores yielded by day 11 testing. Across all exposure groups, the doses of LiCl produced a dose-dependent conditioned place avoidance, as a significant main effect of LiCl dose was found ($F(3,100) = 14.27$, $p < 0.001$). Subsequent post hoc tests indicated that the 20- (mean = − 230.6 s, SEM = ± 42.4 s) and 40-mg/kg doses (mean = − 318.1 s, SEM = ± 40.9 s) were significantly different from the vehicle-only group (mean = − 7.2 s, SEM = ± 22.1 s). The main effect of exposure group and the exposure group × LiCl dose interaction was not significant ($p > 0.05$), suggesting no differential effect of perinatal exposure to 0, 8, or 16 mg lead on LiCl conditioned place avoidance (see Fig. 2A).

3.3.2. Postnatal day 90

For the animals commencing CPA training at PND 90, more time was spent in the black chamber than the white chamber on the day 2 pretest. A one-way ANOVA on time spent in the drug-paired environment during the pretest indicated a significant main effect of exposure group ($F(2,95) = 4.93$, $p < 0.01$). Subsequent post hoc tests indicated animals that received perinatal exposure to 16 mg lead (mean = 634.4 s, SEM = ± 26.7 s) showed a greater bias for one side over the other on the pretest than animals that received perinatal exposure to 0

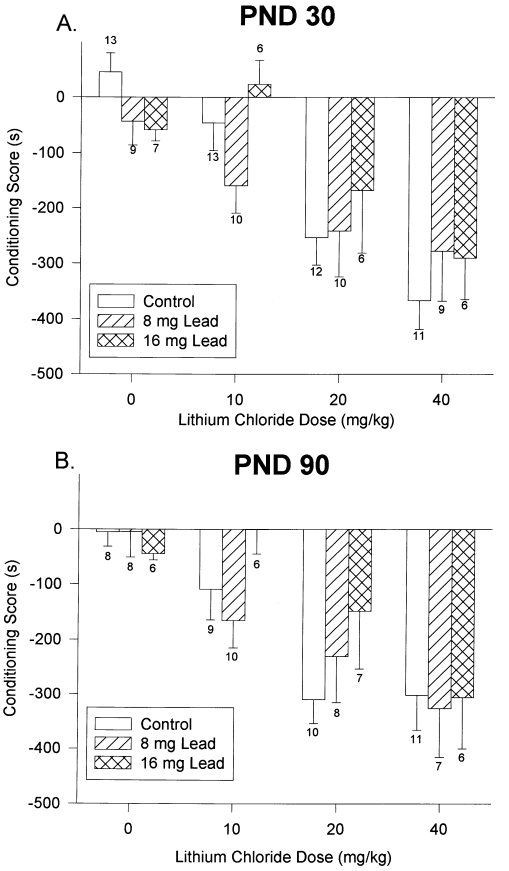

Fig. 2. (A) Mean (±SEM) conditioning scores (measured in seconds) for LiCl CPA animals exposed perinatally to 0, 8, or 16 mg lead and tested commencing at PND 30. The numbers above the bars indicate group size. (B) Mean (±SEM) conditioning scores (measured in seconds) for LiCl CPA for animals commencing testing at PND 90.

(mean = 555.2 s, SEM = ± 15.4 s) or 8 mg lead (mean = 544.6 s, SEM = ± 22.0 s).

A two-way ANOVA (exposure group × LiCl dose) was conducted on the conditioning scores yielded by day 11 testing. As noted at PND 30, a significant main effect of LiCl dose was present ($F(3,95) = 11.85$, $p < 0.001$). Post hoc tests indicated that the 20 (mean = − 239.2 s, SEM = ± 43.8 s) and 40 (mean = − 310.0 s, SEM = ± 44.0 s) mg/kg LiCl doses produced greater conditioning scores than the vehicle-only group (mean = − 15.5 s, SEM = ± 19.0 s). Also, the 40-mg/kg dose was significantly different from the 10-mg/kg group (mean = − 105.3 s, SEM = ± 32.2 s). No significant main effect of exposure group was found, nor was the exposure group × LiCl dose interaction significant ($p > 0.05$), suggesting no differential effect of perinatal exposure to lead on place avoidance (see Fig. 2B).

4. Discussion

An attenuation of the conditioned reinforcing properties of lower doses of cocaine was observed for male rats exposed perinatally to 8 or 16 mg lead in Experiment 1. These results were evident in animals tested at PND 30 and PND 90, points at which blood-lead concentrations were below 5 μg/dl (PND 30) or below the detectable limit of 1 μg/dl (PND 90). Further, the results of Experiment 2 suggest that the attenuation in cocaine conditioned reinforcement was not due solely to an impairment of cognitive or learning processes, as a dose-dependent CPA to LiCl was observed, yet no differences were noticed among lead-exposure groups.

4.1. Cocaine conditioned reinforcement

Animals from dams that received 8 or 16 mg lead daily demonstrated an attenuation in cocaine CPP relative to control pups at PND 30 and at PND 90. The absence of a complete antagonism of cocaine CPP by lead is significant regarding drug abuse. The results from Experiment 1 indicate that cocaine can have substantial reinforcing properties in lead-exposed animals. Because CPP is generally believed to measure the reinforcing properties of drugs, it can be stated that ultimately cocaine produced positively rewarding effects in both metal-exposed and control animals. The lead-exposed animals, however, required a stronger stimulus intensity (more drug) than their control counterparts to show the same amount of CPP.

Although there was some indication that the magnitude of the attenuation in cocaine CPP was related to lead concentration (greater amounts of cocaine were required for cocaine CPP in 16-mg lead animals than 8-mg lead animals at both PND 30 and PND 90), it is clear from Fig. 1 that perinatal exposure to dams gavaged daily with either 8 or 16 mg lead resulted in cocaine antagonism. Or at least this is the case for the relatively narrow range of doses used in this investigation. Perhaps a different pattern of lead-related changes in responsiveness to cocaine would be evident had other doses of the drug been employed in the CPP tests.

From the data presented, it is not possible to make definitive statements with respect to where in the central nervous system the metal might be producing the attenuation in conditioned reinforcement. A diverse body of research suggests a critical role of dopamine in cocaine reward [1,6], and low-level lead exposure is associated with a change in dopamine synthesis [14], release [16,34], and binding properties [28]. The direction of the change in dopaminergic systems by lead appears to be related to the developmental period in which the metal is presented [19,28]. Regardless, it is important to note that mesolimbic dopamine is not the exclusive modulator of drug reinforcement [1]. Alterations of opiate [31,32], and NMDA pathways [6] produce an antagonism of the conditioned reinforcing properties of cocaine, and developmental exposure to lead has been associated with impairments of these systems [8,17,30]. Whether or not the behavioral differences observed here derive from lead-induced dis-

turbances in different transmitter systems, or perhaps preferentially are the result of changes in a particular system, remains to be determined.

There are alternative explanations to action by the metal on reward beyond an antagonism of neural reward circuits. The first explanation is based on the demonstrated toxic effect of lead on kidney and liver function [33]. It is possible that the attenuation of cocaine CPP by lead is due to direct alterations in the pharmacokinetics of cocaine, such that available brain levels of the drug are not equivalent in lead and control animals. Questioning the validity of this rationale, however, are the data from adult animals that show lead exposure produces no change in the levels of cocaine or the cocaine metabolite (benzoylecgonine) in plasma or brain [26]. Still, in the absence of developmental data on the issue, peripheral accounts of the results reported here must be considered. A second alternative explanation derives from the differences in body weights among lead-exposure groups at PND 30. At the commencement of CPP training, the body weights of pups exposed to 8 or 16 mg lead perinatally were below those of control animals. Although drug administration was based on body weight, it is possible that attenuation in cocaine CPP was due to developmental deficiencies in the lead-treated pups. Certainly, body weight differences among groups might serve as an indicator of differences in normal ontogenetic development among groups. Of even greater importance is the possibility that undernutrition resulting from metabolic changes produced by lead exposure during gestation/lactation may redefine drug potency. It is reasonable to expect that toxicokinetic disturbances in transmitter function integral to the expression of cocaine effects may emerge indirectly from the effects of lead on the brain per se. Or, drug distribution and transport processes may be linked to body size and therein alter the impact of a set delivery of cocaine. Of course, no significant differences in body weight were present among groups at PND 90, yet parallel attenuation patterns in cocaine CPP were still present. So, it would seem that something other than weight differentials would have to be considered as a determinant of the lead/cocaine interaction effects observed in this study.

4.2. LiCl conditioned reinforcement

CPP is a paradigm based on the principles of classical conditioning [5]. As such, it is possible that the attenuation in cocaine CPP produced by perinatal exposure to 8 or 16 mg lead was due to compromises of associative or cognitive processes believed to mediate CPP, rather than disturbance of neural mechanisms of reward per se. However, several lines of evidence suggest that a learning impairment was not responsible solely for the effects observed here. First, a previous study demonstrated a change in the locomotor-activating properties of cocaine in animals that received perinatal exposure to lead [25]. Thus, in a behavioral preparation (locomotor activity recording) with a minimal contribution of classical conditioning [25,29], attenuation in the behavioral effects of cocaine presented acutely was produced with developmental lead exposure. Second, the results of Experiment 2 indicated that rats born to dams exposed to 8 or 16 mg lead demonstrated a CPA to LiCl in a like manner to control rats. Thus, an attenuation in cocaine CPP was present without a parallel attenuation in place avoidance, suggesting that lead-induced impairments of cognitive mechanisms alone were insufficient to account for cocaine reinforcement antagonism in this classical conditioning paradigm.

4.3. Implications

Regarding the broader implications of our findings, the results from Experiment 1 suggest a subsensitivity to cocaine potency commensurate with level of perinatal lead exposure. It follows that this change in cocaine sensitivity could result in a change in drug self-administration patterns. The present experiment and previous cited research [25] indicate that lead exposure is associated with a subsensitivity to the reinforcing and locomotor-activating properties of acute cocaine self-administration. With this subsensitivity, in a compensatory attempt to achieve a set level of arousal or reward, animals exposed to lead during development may self-administer more cocaine at larger concentrations than those not exposed to the metal. In contrast to the acute exposure case, with repeated cocaine presentation, animals exposed to lead during development have shown an enhanced locomotor sensitization to the drug [25]. As a result, it is possible that cocaine self-administration responding at higher doses may decrease, i.e., a displacement to the left in the dose–effect curve would be apparent among lead-exposed animals, consequently, fewer responses would be required to maintain a functionally greater dose of cocaine. This potential pattern of increased or decreased drug ingestion may have particular implications regarding drug lethality issues, inasmuch as the dose–effect curve for reinforcement may be shifted in a different manner than the dose–effect curve for toxicity or lethality.

Finally, measurement of blood-lead level has been the conventional index of at-risk populations [27]. In previous research from adult animals in which lead-induced deficits of drug-related behavior were demonstrated, analysis of blood-lead burdens indicated significantly elevated lead levels [4,23,24]. However, in the current investigation, blood-lead levels were reduced significantly (PND 30), or below detectable limits (PND 90), yet pronounced disturbances in drug responsiveness were observed. These results suggest that developmental exposure to lead can cause long-term changes in drug-related behaviors even when the metal is not at measurable levels in the blood. Accordingly, screening procedures that measure present tissue levels of a contaminant may not be adequate to assess the full-scaled toxic effects produced by early developmental lead exposure. The more critical issue is the level of

contaminant exposure during the period of rapid ontogenetic change, and the persistent nature of the disturbances produced during this period.

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References

[1] Bardo MT. Neuropharmacological mechanisms of drug reward — beyond dopamine in the nucleus accumbens. Crit Rev Neurobiol 1998;12:37–67.
[2] Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19:39–51.
[3] Brady K, Herrera Y, Zenick H. Influence of parental lead exposure on subsequent learning ability of offspring. Pharmacol, Biochem Behav 1975;3:561–5.
[4] Burkey RT, Nation JR, Grover CA, Bratton GR. Effects of chronic lead exposure on cocaine-induced disturbance of fixed-interval behavior. Pharmacol Biochem Behav 1997;56:117–21.
[5] Calcagnetti DJ, Schechter MD. Extinction of cocaine-induced place approach in rats: a validation of the "biased" conditioning procedure. Brain Res Bull 1993;30:695–700.
[6] Cervo L, Samanin R. Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Res 1995;673:242–50.
[7] Chisholm J, Goldstein G, Cory-Slechta DA, Weiss B, Landrigan P, Mushak P, Needleman HL, Rice D, Rosen J, Silbergeld E. Lead debate goes on. Pediatrics 1994;94:408–9.
[8] Cohn J, Cory-Slechta DA. Subsensitivity of lead-exposed rats to the accuracy-impairing and rate-altering effects of MK-801 on a multiple schedule of repeated learning and performance. Brain Res 1993;600:208–18.
[9] Cory-Slechta DA. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions. Annu Rev Pharmacol Toxicol 1995;35:391–415.
[10] Cory-Slechta DA, Garcia-Osuna M, Greenamyre JT. Lead-induced changes in NMDA receptor complex binding: correlations with learning accuracy and with sensitivity to learning impairments caused by MK-801 and NMDA administration. Behav Brain Res 1997;85:161–74.
[11] Ensminger ME, Anthony JC, McCord J. The inner city and drug use: initial findings from an epidemiological study. Drug Alcohol Depend 1997;48:175–84.
[12] Harwell TS, Spence MR, Sands A, Iguchi MY. Substance use in an inner-city family planning population. J Reprod Med 1996;41:704–10.
[13] Hoffman DC. The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res Bull 1989;23:373–87.
[14] Jadhav AL, Ramesh GT. Pb-induced alterations in tyrosine hydroxylase activity in rat brain. Mol Cell Biochem 1997;175:137–41.
[15] Jett DA, Kuhlmann AC, Guilarte TR. Intrahippocampal administration of lead (Pb) impairs performance of rats in the Morris water maze. Pharmacol Biochem Behav 1997;57:263–9.
[16] Kala SV, Jadhav AL. Low level lead exposure decreases in vivo release of dopamine in the rat nucleus accumbens: a microdialysis study. J Neurochem 1995;65:1631–5.
[17] Kitchen I. Lead toxicity and alterations in opioid systems. Neurotoxicology 1993;14:115–24.
[18] Lasley SM, Gilbert ME. Presynaptic glutamatergic function in dentate gyrus in vivo is diminished by chronic exposure to inorganic lead. Brain Res 1996;736:125–34.
[19] Luthman J, Lindqvist E, Gerhardt GA, Olson L, Hoffer B. Alterations in central monoamine systems after postnatal lead acetate treatment in rats. Environ Res 1994;65:100–18.
[20] Mielke HW. Lead in the inner cities. Am Sci 1999;87:62–73.
[21] Miller DK, Nation JR. Chronic cadmium exposure attenuates the conditioned reinforcing properties of morphine and fentanyl. Brain Res 1997;776:162–9.
[22] Miller DK, Palme KM, Najvar SA, Caudill SD, Nation JR. Chronic cadmium exposure attenuates the conditioned reinforcing properties of cocaine and other drugs in rats. Pharmacol Biochem Behav 1999;64:15–20.
[23] Nation JR, Baker DM, Taylor B, Clark DE. Dietary lead increases ethanol consumption in the rat. Behav Neurosci 1986;100:525–30.
[24] Nation JR, Livermore CL, Burkey RT. Chronic lead exposure attenuates sensitization to the locomotor-stimulating effects of cocaine. Drug Alcohol Depend 1996;41:143–9.
[25] Nation JR, Miller DK, Bratton GR. Developmental lead exposure alters the stimulatory properties of cocaine at PND 30 and PND 90. Neuropsychopharmacol in press.
[26] Nation JR, Wellman PJ, Livermore CL, Miller DK, Bratton GR. Brain and plasma levels of cocaine and benzoylecgonine in lead-exposed and cadmium-exposed rats following acute or chronic intraperitoneal administration of cocaine. Toxicol Lett 1997;92:47–57.
[27] Pirkle JL, Brody DJ, Gunter EW, Kramer RA, Paschal DC, Flegal TD, Matte TD. The decline in blood lead levels in the United States. The National Health and Nutrition Examination Surveys (NHANES). J Am Med Assoc 1994;272:284–91.
[28] Pokora MJ, Richfield EK, Cory-Slechta DA. Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: time course of effects and interactions with chronic dopamine agonist treatments. J Neurochem 1996;67:1540–50.
[29] Post RM, Lockfeld A, Squillace KM, Contel NR. Drug–environment interaction: context dependency of cocaine-induced behavioral sensitization. Life Sci 1981;28:755–60.
[30] Schulte S, Muller WE, Friedberg KD. In vitro and in vivo effects of lead on specific ^{3}H-MK-801 binding to NMDA-receptors in the brain of mice. Neurotoxicology 1995;16:309–17.
[31] Suzuki T, Mori T, Tsuji M, Misawa M, Nagase H. The role of delta-opioid receptor subtypes in cocaine- and methamphetamine-induced place preferences. Life Sci 1994;55:PL339–44.
[32] Suzuki T, Shiozaki Y, Masukawa Y, Misawa M, Nagase H. The role of mu- and kappa-opioid receptors in cocaine-induced conditioned place preference. Jpn J Pharmacol 1992;58:435–42.
[33] Waalkes MP, Klaassen CD. Concentration of metallothionein in major organs of rats after administration of various metals. Fundam Appl Toxicol 1985;5:473–7.
[34] Zuch CL, O'Mara DJ, Cory-Slechta DA. Low-level lead exposure selectively enhances dopamine overflow in nucleus accumbens: an in vivo electrochemistry time course assessment. Toxicol Appl Pharmacol 1998;150:174–85.