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Developmental Lead Exposure Alters Methamphetamine Self-administration in the Male Rat: Acquisition and Reinstatement

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Abstract

The rate of acquisition of drug self-administration and the return to drug seeking are important elements of the overall drug profile, and are essential factors in understanding risks associated with drug abuse. Experiment 1 examined the effects of perinatal (gestation/lactation) lead exposure on adult rates of acquisition of intravenous (i.v.) methamphetamine self-administration. Experiment 2 investigated the effects of perinatal lead exposure on drug-maintained responding in a reinstatement (relapse) paradigm. In Experiment 1, female rats were gavaged daily with 0 or 16-mg lead for 30 days prior to breeding with nonexposed males. Lead exposure continued through gestation and lactation and was discontinued at weaning (postnatal day [PND] 21). Male rats born to control or lead-exposed dams were tested daily as adults in an acquisition paradigm that incorporated both Pavlovian and operant components. An initial 3-hr autoshaping period preceded a 3-hr self-administration period. For 35 daily training sessions i.v. methamphetamine infusions [inf] (0.02 mg/kg) were paired with the extension and retraction of a lever (autoshaping), while inf occurred during self-administration only when a lever press was executed (FR-1). In Experiment 2 animals developmentally exposed to lead were trained on a FR-2 to self-administer methamphetamine (0.04 mg/kg/inf) and then placed on an extinction schedule prior to receiving intraperitoneal (i.p.) priming injections of saline, 0.50, 1.00, or 1.50 mg/kg methamphetamine. The findings from Experiment 1 showed that acquisition was delayed in rats born to lead-exposed dams gavaged daily with 16-mg lead throughout gestation and lactation when a 0.02 mg/kg/inf of methamphetamine served as the reinforcement outcome. Additional data from Experiment 2 indicated priming cues (injections of methamphetamine [i.p.]) administered after extinction were less likely to occasion a return to drug seeking (relapse) in the 16-mg group relative to the 0-mg control group. These results suggest perinatal lead exposure alters patterns of methamphetamine self-administration during the adult cycle.

Keywords

Autoshaping; Developmental lead; Methamphetamine; Self-administration

1. Introduction

With the forced removal of lead additives in the 1970s, North America has witnessed substantial declines in environmental lead concentrations over the last two decades. And although some in the public sector, and even within the scientific community, have assumed

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the public health threats associated with lead toxicity have been largely removed it is clear this is not the case (Hubbs-Tait et al., 2005). Especially in the inner cities and among minorities an alarmingly high percentage of children register blood lead levels that exceed the allowable limits set forth by the Centers for Disease Control and Prevention (Kemp et al., 2007; Mielke, 1999; Pirkle et al., 1998). Coupled with a compelling literature on developmental low-level lead-induced disturbance in cognitive function (Bellinger, 2006; Canfield et al., 2003), it is becoming increasingly apparent that a range of behaviors impacted by such early lead exposure may extend to include drug selection and use, and potentially may affect matters relating to addiction. For instance, developmental lead exposure has been shown to increase the acquisition and maintenance of cocaine self-administration at low doses of the drug when animals are tested as adults (Rocha et al., 2005; Valles et al., 2005), and it increases the likelihood of relapse in a cocaine self-administration setting (Nation et al., 2003). Elsewhere, the modulatory role of developmental lead exposure in the redefinition of the reinforcing efficacy of heroin has been established (Rocha et al., 2004).

To date, no attempts have been made to explore potential interactive relations between early lead exposure and methamphetamine, a psychostimulant that currently is gaining distribution worldwide (Anglin et al., 2000; Yan et al., 2006). The psychoactive effects of methamphetamine are associated with disturbances in a variety of neurochemical pathways, but perhaps most conspicuously in dopaminergic and GABA-ergic systems. Both increased dopamine (DA) levels and decreased DA transporter (DAT) activity have been reported in rodents administered methamphetamine (Broom and Yamamoto, 2005; Brown and Yamamoto, 2003; Cadet et al., 2003; but see Shepard et al., 2006). With respect to gamma-aminobutyric acid (GABA) and attendant anxiety-related involvement in the effects of methamphetamine administration, the anxiogenic compound yohimbine increases drug sensitivity (Shepard et al., 2004), and the GABA agonist baclofen attenuates the reinforcing effects of methamphetamine (e.g., Ranaldi and Poeggel, 2002). These data are of particular interest here inasmuch as lead exposure is known to decrease dopamine activity (Cory-Slechta, 1995; Devoto et al., 2001; Lasley and Lane, 1988) as well as GABA function (Lasley and Gilbert, 2002; Lasley et al., 1999).

Given the apparent links between lead exposure and neural mechanisms ostensibly involved in determining methamphetamine sensitivity, it seems reasonable to examine both the possible relationship between developmental lead exposure and the acquisition of methamphetamine self-administration responding, as well as the return to drug seeking following extinction (reinstatement [relapse]). It has been argued that the rate of acquisition of drug self-administration may serve as a predictor of later drug-taking behavior, possibly influencing the transition from drug use to addiction (refer to Rocha et al., 2005). Accordingly, in Experiment 1 an acquisition paradigm developed by Carroll and associates (Campbell and Carroll, 2001; Carroll and Lac, 1997) was employed to train lead-exposed and control animals to press a lever for a methamphetamine-reinforcement in a consistent manner, with minimal intrusions. In this preparation, Pavlovian conditioning is first used to shape behavior, then operant conditioning is tested in order to measure rate of methamphetamine acquisition in the animals. Experiment 2 focused on the effects of lead exposure on reinstatement responding which is considered to be a valid animal model of relapse (McFarland et al., 2003; Shalev et al., 2002; Yan et al., 2006). It is within this context that findings from Experiments 1 and 2 may have clinical implications for drug addiction. In both experiments the exposure regimen consisted of perinatal (gestation/lactation) lead exposure.

2. Experiment 1

2.1 Methods

2.1.1 Animals—The research design and conduct of the experiment were approved by the Texas A&M University Laboratory Animal Care Committee, and all aspects of the research followed the guidelines outlined in the Public Health Service Policy for the Care and Use of Laboratory Animals (PHS Policy, 1996). Adult female and male Sprague-Dawley rats (Harlan; Houston, TX) were used for breeding, and only male offspring were tested in this investigation.

2.1.2 Lead Exposure Regimen—For 30 days, adult female rats were exposed to 0 (sodium acetate) or 16-mg lead (as lead acetate) daily using a 16 ga gavage needle to administer the respective solutions in a volume of 1.0 ml deionized water. This procedure has been used in our previous developmental lead studies to ensure stable blood/tissue levels (cf. Miller et al., 2000; Nation et al., 2000, 2003; Rocha et al., 2004; Valles et al., 2005). The present lead concentration was selected based on previous investigations that found it produces differential behavioral effects while not altering dam weights or the locomotor ability of pups (Miller et al., 2000). Following this 30-day toxicant exposure period, females were bred with nonexposed males. Once females tested positive for copulatory plugs, males were removed from the home cage. Females continued to receive their daily dose 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 toxicant 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-hr light/dark cycle. Testing commenced at approximately 10:00 hrs, two hrs into the 12-hr light cycle.

2.1.3 Surgical Procedures—Surgery was performed on test offspring at PND 60, which is a point demonstrated to be within the adult timeframe of behavioral change produced by developmental lead exposure (Miller et al., 2000, 2001; Nation et al., 2000, 2003). Using a backplate surgical procedure, implantation of chronic indwelling jugular catheters was performed using sterile techniques as described in detail elsewhere (Nation et al., 2003). The rats were allowed 5 days to recover from surgery before commencing methamphetamine self-administration testing. During this recovery period, each rat received in the home cage hourly intravenous (i.v.) infusions [inf] (200 µl) of a sterile saline solution containing heparin (1.25 U/ml) and penicillin g potassium (250,000 U/ml). Following recovery, over an 8.00 sec time frame, animals received automated hourly inf (213 µl) 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 was restricted to 18 g of standard rat chow in order to maintain animals at approximately 85% of their mean free-feeding body weight. This food-restriction regimen is similar to that used in other laboratories (e.g., Campbell and Carroll, 2001). Moderate food restriction consistently has been shown to accelerate methamphetamine acquisition (Roth and Carroll, 2004), and the procedure is recommended for autoshaping acquisition studies. Uncontaminated water was available *ad*

libitum throughout the study. Animals were weighed daily prior to testing. Food was placed in home cages following the end of each daily testing session.

2.1.4 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 and a stimulus light located above each lever. Infusion pumps (Razel Scientific Instruments; Stamford, CT) controlled drug delivery to each of the boxes. A 20-ml syringe delivered i.v. inf (160 μ l) 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.

2.1.5 Procedure

Autoshaping component: Control (Group 0-mg; $n=7$) and lead-exposed (Group 16-mg; $n=8$) animals were run in two squads, and subject assignment to chambers and squad was counterbalanced by group. 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 each daily session of Experiment 1, during the autoshaping component, testing commenced with a 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 0.02 mg/kg (+,-)methamphetamine HCl inf administered as the salt if it pressed the lever or after 15-sec, whichever occurred first. Once again, a 90-sec time-out period was instituted. As was the case following the first 90-sec time-out period, the active lever was then extended into the chamber and the animal was given 15-sec to press the lever for an immediate inf of 0.02 mg/kg methamphetamine, or, if no response occurred the animal received a noncontingent inf of 0.02 mg/kg methamphetamine at the end of the 15-sec period. This cycle repeated for the first 20 min of each hr for the initial 3 hrs (30 total methamphetamine inf).

With the chamber house-light off throughout training, the stimulus light above the active (right) lever was lit for the 6-sec duration of the inf 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 inf, were recorded but had no programmed consequences. As indicated, a 0.02 mg/kg methamphetamine inf (160 μ l) 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 hr, following the 10 methamphetamine inf, 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 each session, the retractable lever remained extended and 0.02 mg/kg methamphetamine inf were contingent upon lever pressing under an FR-1 schedule of reinforcement. As before, responses on the left lever and responses during an inf 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 methamphetamine self-administration was a mean of 50 inf 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] (e.g., Carroll and Lac, 1997). The methamphetamine dose (0.02 mg/kg/inf) was chosen based on data from previous studies that show this dose is marginally reinforcing, and does not produce satiation or motoric impairments (Roth and Carroll, 2004).

In order to confirm patency during acquisition training, catheters were flushed twice daily with 0.20 mls of a heparinized saline solution; once prior to and once following each daily testing

session. Catheters of questionable patency were flushed with 0.05 mls of sodium pentobarbital (7.50 mg/kg) followed by 0.20 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. inf of 7.50 mg/kg sodium pentobarbital. Again, catheter patency was verified by rapid onset of brief anesthesia. Each of the 7 control and 8 lead-exposed animals included in this report tested positive for open lines.

Although initial individual pup body weights were higher for control than lead-exposed animals, no significant group differences in body weight were evident at the commencement of testing operations (Group 0-mg= 317.30g±(5.30); Group 16-mg= 309.45g±(7.11); $p>.05$).

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

2.1.7 Tissue 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 collection of blood and tissue samples, lead residues were measured by inductively coupled plasma - mass spectroscopy on a Perkin Elmer DRC 2 instrument. The ^{208}Pb isotope and ^{209}Bi were used as internal standards. Weighted linear calibration was performed with a blank and three external standards (0.05, 20, and 200 parts per billion) and was verified by analyzing NIST SRM 1640 (trace elements in water). Data were acquired in peak hopping mode, using the autolens feature and three replicate reads per determination. Verification of the calibration and baseline were performed after every group of 10 samples and at the end of the analytical run.

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

3. Results

3.1 Acquisition of Methamphetamine Self-administration

Figure 1 illustrates the cumulative percentage of nonexposed (Group 0-mg) and lead-exposed (Group 16-mg) rats meeting criterion (50 lever presses). Five of the eight (62.5%) animals in Group 0-mg reached acquisition by day 17 of testing, approximately half-way through the 35-day testing period; whereas none of the Group 16-mg animals had reached criterion for acquisition at that point in testing. Ultimately, fewer lead-exposed animals achieved the requirements for acquisition of methamphetamine self-administration with a cumulative percentage of 85.71% (6 out of 7), in comparison to 100% acquisition rate in the control group (8 out of 8) by the end of testing. As is visually apparent in Figure 1, rates of acquisition of methamphetamine self-administration were more rapid for control animals, in comparison to lead-exposed animals (survival analysis; Kaplan-Meier Breslow statistic [$X^2 = 5.30$, $p<.05$]). Once animals reached acquisition, stable responding was maintained.

3.2 Tissue Analyses

Table 1 presents the mean (SEM) blood lead concentrations, a conventional marker of lead toxicity, for nonexposed (Group 0-mg) and metal-exposed (Group 16-mg) dams at breeding, 10 days of gestation, parturition, and weaning. Blood lead residue values are shown for littermates at PND 1 and PND 21, as well as for test animals at the termination of the

experiment. Lead concentrations in tissue (i.e. brain, kidney, liver, and tibia) are indicated for dams at weaning (PND 21) and for littermates of test animals at weaning.

By the completion of acquisition testing in Experiment 1, significant traces of lead in metal-exposed test animals were observable only in tibia ($p < .05$; data not shown). Blood lead levels of lead-exposed animals had returned to control levels at the termination of testing, i.e., in both groups lead levels were below detectable limits ($< .5 \mu\text{g/dl}$).

4. Experiment 2

4.1 Methods

4.1.1 Animals—Littermates of test animals used in Experiment 1 were used as test animals in Experiment 2 (Group 0-mg [$n=9$]; Group 16-mg [$n=7$]). All other aspects of the procedure were as described for Experiment 1.

4.1.2 Surgical Procedures—Catheter implantation and other surgical procedures were as described in Experiment 1.

4.1.3 Apparatus—The apparatus was the same as described for Experiment 1. The retractable lever remained extended and an i.v. inf 0.04 mg/kg of methamphetamine was contingent upon lever pressing.

4.1.4 Procedures—At PND 90, animals were shaped to lever-press (self-administer) methamphetamine thus avoiding possible confounds associated with differential acquisition. As adults, the male offspring were tested in daily 2-hr sessions for steady baseline i.v. self-administration of methamphetamine (0.04 mg/kg per inf) on a fixed-ratio schedule where two lever presses resulted in drug delivery (FR-2 schedule of reinforcement). After steady-state responding was established ($< 20\%$ fluctuation across 3 daily sessions), methamphetamine reinstatement responding was assessed for each group within an extinction paradigm that included daily 5-hr sessions. During the initial 1 hr of reinstatement testing, the previous baseline contingencies were in place, i.e. animals operated under an FR-2 schedule for an inf of 0.04 mg/kg methamphetamine. During hr 2, hr 3, and hr 4 of testing saline infusions were substituted for methamphetamine inf. After responding was extinguished during hr 4, reinstatement of responding was tested by administering an i.p. priming injection of either saline, 0.50, 1.00, or 1.50 mg/kg methamphetamine. Following these injections, lever responding for a saline inf was monitored during hr 5. Between the respective reinstatement test sessions animals were given two days of 0.04 mg/kg/inf methamphetamine baseline testing on a FR-2 schedule of reinforcement in order to reestablish baseline responding and to measure possible shifts in tolerance that may have occurred following chronic exposure to the psychostimulant. The order of reinstatement testing was counterbalanced by dose.

Drugs were obtained as in Experiment 1 and test animal tissues were collected and analyzed as detailed in Experiment 1. Body weights for animals during reinstatement testing did not differ between groups (mean body weights = $364.39\text{g} \pm (5.31)$ and $372.75\text{g} \pm (7.20)$ for Groups 0-mg and 16-mg, respectively; $p > .05$).

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

5. Results

5.1 Reinstatement Data

Statistical analyses of behavioral profiles included a 2 Groups (0-mg, 16-mg) X 2 Levers (inactive, active) X 4 Reinstatement Doses (saline, 0.50, 1.00, 1.50 mg/kg) repeated measures ANOVA performed on number of responses for saline only on baseline days prior to reinstatement testing (hr 5). Note that the variable Reinstatement Doses refers here to the methamphetamine dose that was to be received during hr 5 following the respective baseline tests. Although more active than inactive lever responses were exhibited, the results of the baseline analysis failed to indicate significant differences by Group or Reinstatement Doses (all $F_s < 1$). Consequently, it was established that lever responding at the baseline dose of 0.04 mg/kg methamphetamine was stable across reinstatement testing (Group 0-mg = $52.03 \pm (10.6)$; Group 16-mg = $52.18 \pm (7.16)$).

Figure 2 (left panel) presents active (right) lever responses for Group 0-mg and Group 16-mg during the initial 1-hr session at each dose of reinstatement. During this initial period baseline conditions were in place, i.e. FR-2 responding resulted in an infusion of 0.04 mg/kg methamphetamine. Shown in the additional panels of Figure 2 are the response records of the two exposure groups across the 3 hr of extinction (hrs 2–4) testing where completion of the FR-2 resulted in illumination of the stimulus light, but only a heparinized saline solution was delivered. Separate 2 Groups X 2 Levers X 4 Reinstatement Doses repeated measures ANOVAs were performed on the hourly data, with Groups serving as the between factor and Lever and Reinstatement Doses serving as within factors. The results of the analysis of hr 1 failed to show a significant main effect of Groups, and the Groups X Reinstatement Doses interaction was found to be nonsignificant (all $F_s < 1$). Both groups responded significantly more on the active lever that resulted in methamphetamine deliveries, relative to inactive lever responding ($F(1, 14) = 272.59$, $p < .01$). These findings basically replicate the aforementioned baseline results. It is visually apparent from Figure 2 that during hrs 3 and 4 active lever responding had virtually ceased for both exposure conditions.

The methamphetamine percent of baseline reinstatement data for hr 5 (reinstatement testing) for Groups 0-mg and 16-mg are presented in Figure 3 for each reinstatement dose. A 2 Groups (0-mg, 16-mg) X 4 Reinstatement Doses (saline, 0.50, 1.00, 1.50 mg/kg) repeated measures ANOVA was performed on the percent of baseline measure. Clearly, there was a dose-dependent increase in reinstatement on the active (right) lever for both control and lead-exposed conditions as supported by a main effect for Reinstatement Doses ($F(3, 42) = 10.90$, $p < 0.01$). More importantly regarding the rationale that formed the basis for conducting Experiment 2, a main effect of Groups was found ($F(1, 14) = 10.07$, $p < .01$). Subsequent comparisons revealed that this effect was due to lower percent of baseline responding on the part of Group 16-mg animals relative to Group 0-mg animals, and this effect was prominently due to lower percent of baseline responding by lead-exposed animals at the reinstatement dose of 1.50 mg/kg methamphetamine ($F(3, 42) = 4.59$, $p < .01$).

5.2 Tissue Analyses

The relevant dam and littermate tissue data for animals in Experiment 2 are the same as presented in Experiment 1 (see table 1). As in Experiment 1, by the end of reinstatement testing, significant traces of lead in metal-exposed test animals were observable only in tibia ($p < .05$). Blood lead levels of lead-exposed animals had returned to control levels by the completion of testing, i.e., in both groups lead levels were below detectable limits ($< .5 \mu\text{g/dl}$).

6. Discussion

The findings from Experiment 1 showed that the initiation of acquisition was delayed in rats born to dams gavaged daily with 16-mg lead throughout gestation and lactation when a 0.02 mg/kg/inf of methamphetamine served as the reinforcement outcome. There was potential support from Experiment 2 where it was found that priming cues (injections of methamphetamine [i.p.]) administered after extinction were less likely to occasion a return to drug seeking (relapse) in the 16-mg group relative to the 0-mg control group. That is, after acquiring self-administration responding under conditions of a FR-2 contingency employing methamphetamine as a reinforcer (0.04 mg/kg/inf), test animals were presented with an extinction (inf of saline only) format prior to experiencing i.p. injections of either saline, 0.50, 1.00, or 1.50 mg/kg methamphetamine. This reinstatement procedure yielded results showing lead-exposed animals were less likely to return to lever pressing at the 1.5 mg/kg i.p. priming dose.

These data present interpretive issues, i.e., either developmental lead exposure reduces sensitivity to the reinforcing effects of methamphetamine when animals are tested as adults, or alternatively that higher doses of methamphetamine are more aversive to lead-exposed animals. Although the acquisition data are compelling in showing the rate of acquisition of methamphetamine self-administration is slower when training involves a 0.02 mg/kg/inf of methamphetamine as a reinforcer, greater caution must be taken with respect to interpreting the reinstatement data. In the latter case, only a single priming dose yielded significant group separation, and in the absence of a more complete dose/effect profile it is difficult to render convincing statements about the directionality of lead-related shifts in reinstatement sensitivity. Perhaps most instructive in this regard, we note that a separate report from this laboratory indicates that the same developmental lead exposure regimen employed here resulted in a vertical shift in the methamphetamine self-administration dose-effect curve, and dramatically lower completed ratios across a variety of methamphetamine doses in a progressive ratio preparation (Rocha et al., 2007). Converging evidence, then, would seem to favor a diminished methamphetamine sensitivity argument regarding the present findings.

It is worth noting that the blood lead levels reported for dams, while high, fall within a clinically relevant range for adult low-income minorities living in urban areas (Brody et al., 1994; Pirkle et al., 1998). Although human data on blood lead levels immediately after birth are relatively scant, the fact that our animal lead levels had returned to control levels at the time of testing is most informative, and suggestive of longterm disturbance in the absence of conventional markers of toxicity.

The interactive patterns evident for perinatal lead exposure and methamphetamine agree with earlier findings associated with heroin self-administration (Rocha et al., 2004). Specifically, developmental lead exposure produced a downward shift in the dose-effect curve across most doses of heroin (ranging from 0.56 µg/kg to 36 µg/kg per inf) in a self-administration paradigm. Congruent with these findings perinatal lead exposure resulted in decreased progressive ratio responding (lower breaking points) across all heroin doses (ranging from 2.25 µg/kg to 18 µg/kg per inf). In contrast to the present findings and the earlier heroin data, the reinforcing efficacy of cocaine is apparently amplified by perinatal lead exposure in an acquisition preparation identical to that used here in Experiment 1 (Rocha et al., 2005), and the reinstatement procedure used here in Experiment 2 was associated with enhanced cocaine relapse responding in lead-exposed animals following post-extinction administration of various priming injections of cocaine (Nation et al., 2003). Moreover, a leftward displacement has been observed in the cocaine dose-effect curve among animals developmentally exposed to lead (Nation et al., 2004).

The varied effects of perinatal lead exposure on the phenomenology of self-administration of various psychoactive drugs is not altogether surprising given the complexity of the patterns of neuroadaptation associated with chronic drug taking (Self, 2004; Wolf et al., 2004). What is at issue is identifying the precise effects of lead exposure on mechanisms underlying functional changes in sensitivity to drugs possessing abuse liability. Although there is a sizable literature on the effects of postweaning lead exposure on relevant drug-related neural systems (refer to Cory-Slechta, 1995), our understanding of the effects of preweaning lead exposure on neural mechanisms central to defining drug reactivity is limited (Devoto et al., 2001). Even more importantly, almost no information exists regarding the potential enduring mechanistic changes caused by early lead exposure in instances where the exposure regimen has been discontinued, as was the case in the present investigations. Surely, the present behavioral findings argue that perinatal lead exposure produces long-lasting perturbations in neural mechanisms that regulate methamphetamine intake and the propensity for relapse. However, the contrasting profile for developmental lead exposure on other psychoactive drugs (i.e., enhanced sensitivity to the reinforcing effects of cocaine) underscores the possibility that differing drugs may operate on separate neural circuits and that early lead exposure may selectively impact these systems. Because, as noted, the differential effects of perinatal lead exposure on drug substrates have not been characterized, mechanistic studies are currently underway in our laboratory in an effort to establish an adequate information base and therein better understand why such dramatic differences, albeit reliable, would be manifest.

The findings reported here of possible lead-based antagonism of methamphetamine drug-taking and -seeking must be viewed cautiously. Attenuation of the psychoactive properties of drugs with abuse potential may functionally translate into a form of tolerance or counteradaptation (cf. Koob and Le Moal, 1997; Nation et al., 1996). Under such conditions drug self-administration has been shown to increase (Corrigall and Coen, 1991; Koob et al., 1987), perhaps in a compensatory effort to regulate the level of subjective affect (Koob and Le Moal, 1997). Accordingly, it must be considered that the possible antagonism of methamphetamine by developmental lead exposure may actually dispose a drug user to take in greater amounts of the drug once drug-taking behaviors are reliably repeated. In this regard, the present findings could be instructive with respect to identifying possible external (environmental) risk factors associated with the movement from methamphetamine use to methamphetamine abuse.

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Figure 1.

Cumulative percentage (%) of Group 0-mg ($n=7$) and Group 16-mg ($n=8$) rats meeting the criterion for the acquisition of methamphetamine (0.02 mg/kg) self-administration within the 35-day limit. Open symbols and closed symbols represent Group 0-mg and Group 16-mg conditions, respectively (Experiment 1).

Figure 2.

Mean (SEM) number of active (right) lever responses for Groups 0-mg ($n=9$) and 16-mg ($n=7$) during hrs 1–4 at each priming dose of reinstatement. Methamphetamine (0.04 mg/kg/inf) was available during hr 1 and saline infusions were delivered during hrs 2–4 (Experiment 2). Note that the values on the horizontal axis refer to the assignments of the methamphetamine priming dose to be received by each group during reinstatement testing (hr 5)

Figure 3.

The mean percent of baseline (hr 1 methamphetamine [i.v.]) responding for Groups 0-mg ($n=9$) and 16-mg ($n=7$) during hr 5 (reinstatement testing) following different priming doses of methamphetamine (i.p.). The symbol * above the bar indicates that Group 16-mg administered more saline infusions (made more responses on the active lever) following the prime than their control counterparts (Group 0-mg); $p<.05$ (Experiment 2).

Table 1

Mean (SEM) blood and tissue lead concentration values for dams, littermates and test animals.

Blood Lead Concentration (µg/dl, wet weight)		
	Group 0-mg	Group 16-mg
<u>Dams</u>		
Breeding	1.1 (.001)	40.6 (.03) *
Gestation Day 10	1.2 (.002)	37.3 (.02) *
Parturition (PND 1)	1.9 (.003)	52.9 (.05) *
Weaning (PND 21)	.30 (.001)	19.3 (.02) *
<u>Littermates</u>		
PND 1	1.8 (.003)	74.5 (.05) *
PND 21	1.9 (.004)	14.6 (.03) *
<u>Test Animals</u>		
Termination	< .5	< .5
Tissue Concentrations of Dams at Weaning (µg/g, wet weight)		
	Group Lead-0	Group Lead-16
Brain	.018 (.002)	.584 (.125) *
Kidney	.065 (.043)	8.244 (.507) *
Liver	.019 (.003)	.433 (.037) *
Tibia	.012 (.038)	88.620 (4.74) *
Tissue Concentrations of Littermates at Weaning (µg/g, wet weight)		
	Group Lead-0	Group Lead-16
Brain	.014 (.001)	.126 (.013) *
Kidney	.040 (.005)	1.03 (.207) *
Liver	.026 (.004)	.441 (.131) *
Tibia	.162 (.025)	16.01 (3.36) *

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