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Differential effects of adult and perinatal lead exposure on morphine-induced locomotor activity in rats

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Abstract

The effects of adult and perinatal lead treatment on the development of locomotor sensitization produced with repeated morphine administration was investigated. In Experiment 1, adult male rats received a diet containing 250 ppm lead acetate or a control diet for 43 days. Animals then received 10 mg/kg morphine sulfate or water vehicle (ip) and locomotor activity was monitored for 14 consecutive days. While both control and lead-exposed animals demonstrated a locomotor sensitization to morphine, the magnitude of the increased locomotor response was reduced in lead-treated animals. Subsequent analysis of blood-lead in the adult lead-exposed animals indicated residue levels ranging between 20 and 30 μg/dl. In Experiment 2, adult female rats were treated daily with 0, 8, or 16 mg lead via gavage for 30 days before breeding with non-exposed males. Lead exposure in dams continued through gestation and until pups were weaned at postnatal day (PND) 21. At PND 60, male offspring received morphine or vehicle challenges identical to those described in Experiment 1. Animals perinatally exposed to dams receiving 16 mg lead daily demonstrated an enhanced behavioral response to morphine relative to control animals. Analysis of offspring blood indicated lead levels below detectable limits (<1 μg/dl) for all animals. The results suggest exposure to lead at environmentally relevant levels produces long-lasting changes in drug-induced behavior, and the developmental period in which lead exposure occurs is a significant contributor to the manifestation of these effects.

Keywords: Development; Lead; Locomotor; Morphine; Sensitization

1. Introduction

Initially linked to lead-based paints and atmospheric dispersal from auto emissions, lead prevalence is now widespread with exposure vectors that include food, water, and soil [4,30]. The extant literature on lead contamination indicates that the metal is associated with disturbance of a variety of neurobehavioral functions, including toxicant-related deficits in intellectual and cognitive performance (e.g., Ref. [2]; but see Ref. [23]). Expanding the range of behavioral phenomena potentially affected by lead, an emerging animal literature suggests that exposure to lead produces an alteration in the behavioral properties of commonly abused hedonic drugs. At clinically relevant levels (≈20 μg/dl lead in blood), adult exposure to this heavy metal produced a tolerance to the rate-altering properties of cocaine on an operant task [5] and blocked the increase in locomotor activity that typically follows acute administration of cocaine [11]. Regarding repeated presentation of cocaine, the development of locomotor sensitization was attenuated in rats that received a lead-contaminated water supply as adults relative to animals that received an unadulterated water supply [32].

Developmental (perinatal) exposure to lead also has been associated with an alteration in cocaine-induced behaviors. Pups exposed perinatally to dams receiving 8 or 16 mg lead daily, when tested as adults, displayed a decreased response to the locomotor-activating properties of an acute presentation of cocaine, relative to control animals. With repeated cocaine administration, lead-exposed and control pups both exhibited a locomotor sensitization effect, but the magnitude of sensitization was greater in pups exposed to lead than control pups [33]. These behavioral results were present although levels of lead in blood had fallen below detectable limits (< 1 μg/dl).

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The directional differences in locomotor sensitization to cocaine with adult lead exposure (attenuation) and perinatal lead exposure (augmentation) are not altogether surprising given the differential neurochemical effects produced by lead presented during different stages of development. At the adult level, lead has been shown to decrease dopaminergic and glutamatergic function integral to the expression of cocaine sensitization [12,13,28,37], yet the opposite neurochemical pattern has been reported when lead is exposed during early development [44]. Because other receptor systems such as the opioids may similarly be affected differently by developmental or adult lead exposure (cf. Ref. [24]), it must be considered that the pattern of change in opiate responsiveness produced by lead would depend on the stage of the life cycle where the toxicant was exposed. To test this possibility, the present study examined the effects of adult lead exposure (Experiment 1), or perinatal lead exposure (Experiment 2), on the locomotor-stimulating effects of repeated administration of morphine [21].

2. Method

2.1. Experiment 1 — Adult lead exposure

2.1.1. Animals

The subjects used in this study were 32 male Sprague–Dawley rats (Holtzman, Madison, WI) that were approximately 50-days-old at the time of their arrival to the laboratory. Body weights ranged from 175 to 199 g. Sixteen animals received ad libitum access to a purified rodent diet (AIN-93G). The remaining 16 animals received ad libitum access to an identical diet, except that it contained 250 ppm lead acetate. Both were specially prepared by Dyets (Bethlehem, PA). Continuous access to tap water was available in the home cage.

Throughout the experiment, animals were single-housed in hanging polycarbonate cages. These cages were located in a temperature- and humidity-controlled animal colony with a 12/12-h light/dark cycle. Food consumption and body weights were recorded weekly throughout the experiment. Behavioral training and testing sessions commenced at 0700 h during the light portion of the cycle.

The Department of Psychology animal holding and testing facility at Texas A&M University is approved by the Association for Assessment and Accreditation of Laboratory Animal 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. The health of the animals was monitored throughout the duration of the project by the campus veterinarian.

2.1.2. Apparatus

The behavioral apparatus used was an automated Digiscan-16 system. The system included four optical beam activity monitors (Model RXYZCM-16; Omnitech Electronics, Columbus OH) composed of 16 vertical and 16 horizontal infrared sensors. Each monitor surrounded an acrylic activity monitor cage ($40 \times 40 \times 30.5$ cm), which was completely enclosed and had 0.5 cm air holes drilled into the top panel. The monitors and cages were located in a sound-proof room with a 40-dB white noise generator operating continuously. A multiplexor-analyzer (Model DCM-4; Omnitech Electronics) in an adjacent room monitored beam breaks from the optical beam activity monitors and tracked the simultaneous interruption of beams. The multiplexor-analyzer updated the animal's position in the acrylic cage every 10 ms using a 100% real-time conversion system. Computerized integration of the data obtained from the monitor afforded the recording of general activity using total distance (in cm) as the dependent measure. It has been suggested that of the 14 measures available from the Digiscan-16 system, total distance provides the most appropriate index of general locomotor activity [40]. A selector switch on the multiplexor-analyzer was set to print updated totals for each test cage at 5 min intervals.

2.1.3. Drugs

Morphine sulfate was provided gratis by the Research Technology Branch of the National Institute on Drug Abuse. The drug was dissolved in a filtered distilled water vehicle, and the dosage is expressed as the salt. The volume for all injections (ip) was 1 ml/kg.

2.1.4. Procedure

On Day 43 of exposure to their respective dietary regimens, Control and lead-exposed animals were assigned to one of two drug administration groups (water vehicle, morphine), such that four total groups were formed (Control-Vehicle, Control-Morphine, Lead-Vehicle, Lead-Morphine). On each of 14 testing days, rats were administered vehicle or 10 mg/kg morphine sulfate and immediately placed in the test chamber apparatus. The subjects remained in the apparatus each day for 80 min and lights were turned off while activity was monitored. Test chamber assignments were counterbalanced for exposure condition and type of injection.

On Day 15 of testing, all animals within each group were given vehicle injections. Previous investigations of locomotor stimulants have shown that contextual cues contribute to the augmented responding associated with repeated drug administration [38]. Insofar as administering the injections, placement in the test chamber, turning off the test room lights, and other pre-injection events serve as conditional stimuli, it is reasonable to assume that such events could play an additive role in behavioral sensitization. Although it has been determined that these factors play a nominal role in behavioral sensitization to morphine [18,34], it was of interest to determine the potential degree of involvement of conditioning (context) elements in any disturbances registered in lead-treated animals.

In the present experiment, all animals received 0, 10, and 20 mg/kg morphine sulfate on successive days (Test Days 15–17). This permitted the characterization of the profile of the locomotor-altering properties of morphine in animals that had previously received the drug (Control-Morphine and Lead-Morphine) and drug-naive animals (Control-Vehicle and Lead-Vehicle).

Twenty-four hours after the final day of testing, animals were rendered unconscious with 60 mg/kg sodium pentobarbital (ip). Animals were decapitated and 3–5 ml trunk blood was collected. Following collection of blood samples, lead residues in blood were measured via atomic absorption spectrophotometry [35]. 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 insure 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.

2.2. Experiment 2 — Developmental lead exposure

2.2.1. Animals

Upon arrival to the laboratory, adult female Sprague–Dawley rats (Charles River, Houston, TX) were weighed and randomly assigned to one of three exposure groups (0, 8, and 16 mg lead). Rats were housed individually in hanging polycarbonate cages, and maintained on a 12 h/12 h light/dark cycle (lights on at 0500 h). Standard rat chow (Teklad) and tap water were available ad libitum. 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 and stored for later analysis [35].

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 postnatal day (PND) 7, litters were culled to 8–10 pups. All male pups remained in the litter, and enough female pups remained to maintain consistent litter size across all dams.

At PND 21, pups were weaned and transported to a different colony room. Pups were double-housed and had ad libitum access to ordinary laboratory chow and tap water throughout the remainder of the experiment. Body weights were recorded weekly.

2.2.2. Apparatus

The apparatus used for Experiment 2 was the same as used for Experiment 1.

2.2.3. Drugs

Morphine sulfate was prepared and administered to pups in Experiment 2 exactly as described for Experiment 1.

2.2.4. Procedure

At PND 59, pups perinatally exposed to dams receiving 0, 8, or 16 mg lead were weighed and assigned randomly to one of two drug administration groups (water vehicle, morphine), such that six total groups were formed (Control-Vehicle, Control-Morphine, 8 mg Lead-Vehicle, 8 mg Lead-Morphine, 16 mg Lead-Vehicle, 16 mg Lead-Morphine). Behavioral testing commenced on PND 60 and the procedures were precisely as described for Experiment 1. For 14 consecutive days (Test Days 1–14), pups received an injection of 10 mg/kg morphine sulfate or water vehicle and were placed in the apparatus for 80 min. On Test Days 15–17, dose–effect testing was conducted with all animals receiving 0, 10, or 20 mg/kg morphine sulfate before placement in the apparatus for 80 min.

2.3. Data analysis

All data are expressed as the mean, plus or minus the standard error of the mean. For Experiment 1, body weights and food consumption were recorded weekly from the arrival of animals to the laboratory through euthanasia. A 2 Exposure Group (control, lead) × 6 Week (1–6) repeated measures analysis of variance (RANOVA) was performed on the weekly body weight data from arrival to the laboratory through commencement of behavioral testing. A separate, identical analysis was performed on food intake data. When appropriate, Newman–Keuls post-hoc tests were employed throughout the study to determine significant group differences. For Experiment 2, dam body weights and food consumption were recorded weekly, and pup body weights were recorded at PND 30 and PND 60 (commencement of behavioral testing). To avoid problems associated with litter effects (cf. Ref. [15]), the data from pups from the same litter were averaged and treated as a single statistical unit ($N = 5–6$ litters/group).

Because the interval (5-min bins) variable failed to yield locomotor response patterns different from those evident in the overall session data, only the latter are reported here. For Experiment 1, data were analyzed through a 4 Groups (Control-Vehicle, Control-Morphine, Lead-Vehicle, Lead-Morphine) × 14 Test Days RANOVA. Simple effects analyses and Newman–Keuls post-hoc tests were used when appropriate to determine significant group differences ($p < 0.05$). Total distance scores from the Days 15–17

morphine injections (0, 10, and 20 mg/kg morphine) were analyzed with a 4 Groups × 3 Test Days RANOVA. For Experiment 2, data were analyzed through an identical procedure, except that six groups (Control-Vehicle, Control-Morphine, 8 mg Lead-Vehicle, 8 mg Lead-Morphine, 16 mg Lead-Vehicle, 16 mg Lead-Morphine) served as the between-subjects variable for the separate Days 1–14 and Days 15–17 analyses.

3. Results

3.1. Experiment 1 — Adult lead exposure

3.1.1. Food intake and body weights

A significant main effect of Exposure Group was found ($F(1,30) = 7.04$, $p < 0.05$) as animals receiving the control diet (mean = 182 g, SEM = ± 2 g) consumed more each week on the average than animals receiving the lead diet (mean = 171 g, SEM ± 3 g). A significant interaction of Exposure Group × Weeks was also found ($F(5,150) = 3.32$, $p < 0.01$). Post-hoc tests indicated that control animals consumed more than lead-exposed animals in weeks 1, 5, and 6; and lead-exposed animals consumed more in week 3. On the measure body weight, a significant main effect of Exposure Group was found ($F(1,30) = 5.67$, $p < 0.05$) as control animals (mean = 389 g, SEM = ± 11 g) had greater body weights than lead-exposed animals (mean = 362 g, SEM = ± 9 g).

3.1.2. Behavioral data

A significant Groups × Test Day interaction was found ($F(39,364) = 4.32$, $p < 0.01$). Subsequent post-hoc analyses were conducted to examine the effects of lead exposure on morphine-related changes in locomotor activity (see Fig. 1). On Test Day 1, Groups Control-Morphine and Lead-Morphine, which did not differ, both exhibited lower total distance scores than Groups Control-Vehicle and Lead-Vehicle, which did not differ ($p < 0.05$). With repeated administration of 10 mg/kg morphine, there was an increase in locomotor activity for control and lead-exposed animals relative to animals in either metal exposure group that received only vehicle. It was determined further that animals in the Control-Morphine group showed greater increases in locomotor activity than animals in the Lead-Morphine group on Test Days 7, 10, 11, 12, and 14. No significant differences were present among animals in the Control-Vehicle and Lead-Vehicle groups.

Within the groups of animals that received repeated morphine administration, there was an increase in locomotor activity (sensitization) relative to the acute presentation of the drug (Test Day 1). Simple effects analyses and post-hoc tests revealed a significant effect of Test Day for animals in the Control-Morphine group ($F(13,91) = 5.90$, $p < 0.001$). Post-hoc tests indicated a significant elevation in locomotor activity relative to Test Day 1 on Test Days 2, 3, and 5–14. For animals in the Lead-Morphine group, a significant main effect of Test Day was also found ($F(13,91) = 1.86$, $p < 0.05$). However, relative to Test Day

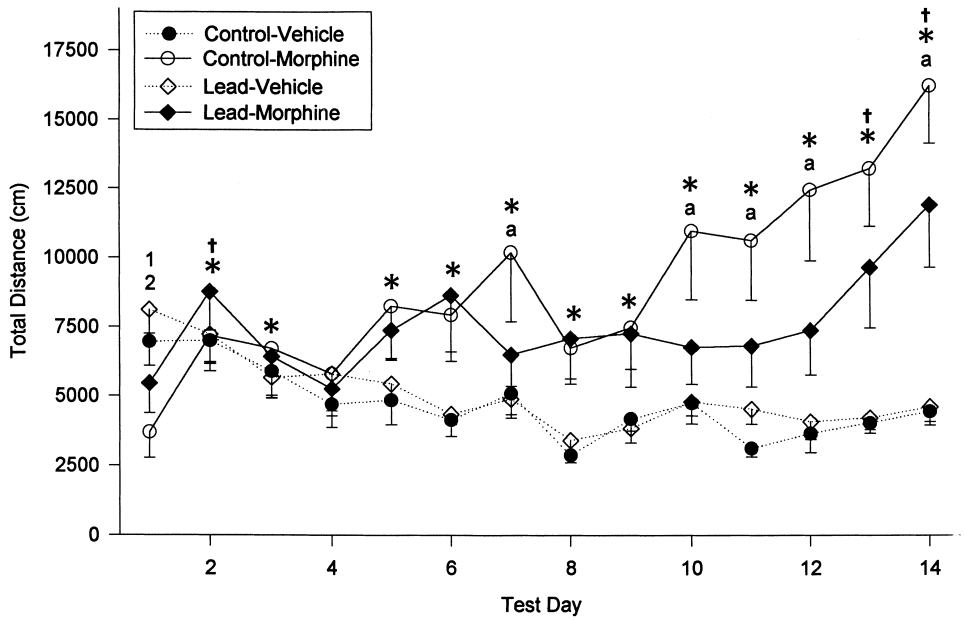

Fig. 1. Mean (±SEM) total distance traveled (cm) per daily session on Test Days 1 through 14 following a vehicle-only or 10 mg/kg morphine injection for animals that received a diet containing 250 ppm lead or a control diet. The following symbols denote significant group separation ($p < 0.05$): " * " = Control-Morphine on that Test Day > Control-Morphine on Test Day 1 (acute morphine challenge); "†" = Lead-Morphine on that Test Day > Lead-Morphine on Test Day 1; "a" = Control-Morphine > Lead-Morphine; "1" = Control-Vehicle > Control-Morphine; "2" = Lead-Vehicle > Lead-Morphine.

1, a significant elevation was evident on Test Days 2, 13, and 14 only.

On Test Days 15–17, dose–effect testing was conducted with 0, 10, and 20 mg/kg morphine administered to animals in all four groups. A 4 Groups × 3 Test Days RANOVA was performed on total distance traveled data. A significant Groups × Test Day interaction was found ($F(6,56) = 6.06$, $p < 0.001$). Subsequent post-hoc analyses were conducted to examine possible differential effects on the locomotor activity elicited by the dose–effect testing in the adult exposure case.

There was no statistically significant difference among groups on Test Day 15 ($p < 0.05$), suggesting that the contextual and environmental stimuli associated with the experimental procedure alone were not sufficient to elicit the behavioral sensitization demonstrated on Test Day 14 in Control-Morphine and Lead-Morphine group animals (see Fig. 2). On Test Day 16, however, group separation was evident, and post-hoc tests indicated Control-Morphine and Lead-Morphine group animals had total distance scores greater than animals in the Control-Vehicle and Lead-Vehicle groups ($p < 0.01$). No differences were found among animals in the Control-Morphine and Lead-Morphine or Control-Vehicle and Lead-Vehicle groups. On Test Day 17 group differences again were found. Post-hoc tests indicated the same pattern of group differences as reported for Test Day 16 ($p < 0.01$).

3.1.3. Blood-lead concentrations

Animals receiving the lead-contaminated diet (mean = 26.2 μg/dl, SEM = ± 3.1 μg/dl) had blood-lead levels significantly greater ($t(30) = 27.96$, $p < 0.001$) than animals receiving the unadulterated control diet (mean = 0.3 μg/dl, SEM = ± 0.1 μg/dl).

3.2. Experiment 2 — Developmental lead exposure

3.2.1. Maternal and pup food intake and body weights

No significant differences in body weight were present among female rats at the commencement of lead exposure (control mean = 221 g, SEM = ± 9 g; 8 mg lead mean = 226 g, SEM = ± 10 g; 16 mg lead mean = 219 g, SEM = ± 6 g; $F(2,19) = 0.97$, $p = 0.74$) or breeding (control mean = 276 g, SEM = ± 15 g; 8 mg lead mean = 250 g, SEM = ± 13 g; 16 mg lead mean = 259 g, SEM = ± 17 g; $F(2,19) = 1.05$, $p = 0.22$). Further, no differences were noted in dam weekly food consumption throughout the study (control mean = 127 g, SEM = ± 16 g; 8 mg lead mean = 120 g, SEM = ± 14 g; 16 mg lead mean = 136 g, SEM = ± 17 g; $F(2,19) = 1.27$, $p = 0.36$).

With regard to the breeding procedures, there was a decrease in litter size between the 16-mg lead-exposed dams (mean = 9.8 pups, SEM = ± 1.6 pups) and either control (mean = 13.7 pups, SEM = ± 1.8 pups) or the 8-mg lead-exposed dams (mean = 12.4 pups, SEM = ± 0.9 pups),

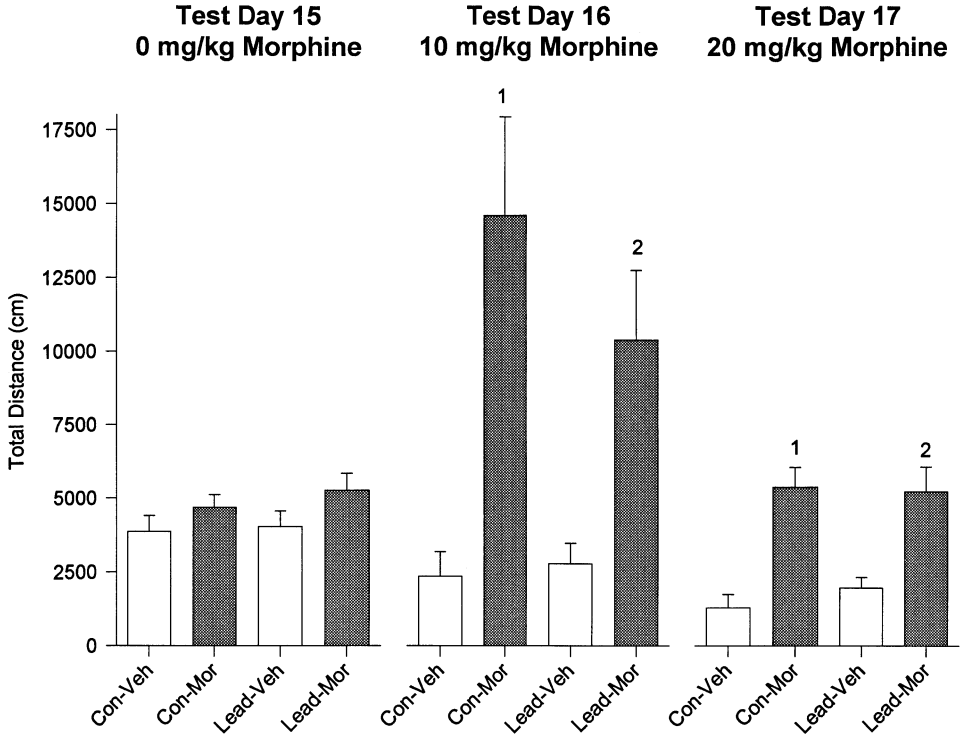

Fig. 2. After behavioral sensitization testing, the mean (±SEM) total distance traveled (cm) for animals that received a diet containing 250 ppm lead or a control diet. Ascending test doses of 0, 10, and 20 mg/kg morphine were presented daily to every animal. The following symbol denotes significant separation by Test Day for a given group ($p < 0.05$): "1" = Control-Vehicle < Control-Morphine: "2" = Lead-Vehicle < Lead-Morphine.

although the statistical analysis did not reach acceptable levels for statistical significance ($F(2,18) = 0.65$, $p = 0.47$).

An analysis of pup body weights on PND 30 indicated a significant decrease in body weights ($F(2,33) = 3.08$, $p < 0.05$) in 8 mg (mean = 118 g, SEM = ± 7 g) and 16 mg (mean = 113 g, SEM = ± 9 g) lead pups relative to control pups (mean = 138 g, SEM = ± 8 g). When behavioral testing commenced on PND 60, there were no significant differences in body weights ($F(2,33) = 1.07$, $p = 0.14$) among groups (control mean = 178 g, SEM = ± 16 g; 8 mg lead mean = 166 g, SEM = ± 11 g; 16 mg lead mean = 160 g, SEM = ± 10 g).

3.2.2. Behavioral data

In Experiment 1 of this report, the acute presentation of morphine was associated with a decrease in locomotor activity relative to animals that received an administration of vehicle-only. In the present experiment, however, there was no significant difference among Groups on Test Day 1 ($F(5,35) = 0.25$, $p = 0.93$; see Fig. 3). Furthermore, in Experiment 1, animals that were exposed to lead as an adult had an increase in locomotor activity following morphine administration, but the magnitude of the increase was less than that present in control animals that were administered morphine. In Experiment 2, however, animals in the 16-mg Lead-Morphine group had total distance traveled scores greater than Control-Morphine animals on several test days during sensitization training with 10 mg/kg morphine (see Fig. 3); $F(65,312) = 1.91$, $p < 0.01$. Further, there was a significant elevation in the 16-mg Lead-Morphine group relative to the 8-mg Lead-Morphine group on numerous test days during sensitization training (Fig. 3).

Within the groups of animals that received repeated morphine administration on Test Days 1–14, there was an increase in locomotor activity relative to the acute presentation of the drug (Test Day 1). Simple effects analyses and post-hoc tests determined a significant effect of Test Day for animals in the Control-Morphine group ($F(13,52) = 3.32$, $p < 0.01$). Post-hoc tests indicated a significant elevation in locomotor activity relative to Test Day 1 at multiple points during sensitization training (Fig. 3).

On Test Days 15–17, dose–effect testing was conducted with 0, 10, and 20 mg/kg morphine administered to animals in all six groups. A significant Group × Test Day interaction was found ($F(10,48) = 7.16$, $p < 0.001$). Subsequent post-hoc analyses were conducted to examine possible differential effects of the respective lead exposure regimens on morphine-related changes in locomotor activity.

Consistent with the results from Experiment 1, there was no statistically significant difference among groups on Test Day 15 ($p > 0.05$; see Fig. 4). On Test Day 16, however, group separation was evident, and post-hoc tests indicated a significant elevation in locomotor activity in the Control-Morphine and 16-mg Lead-Morphine groups relative to

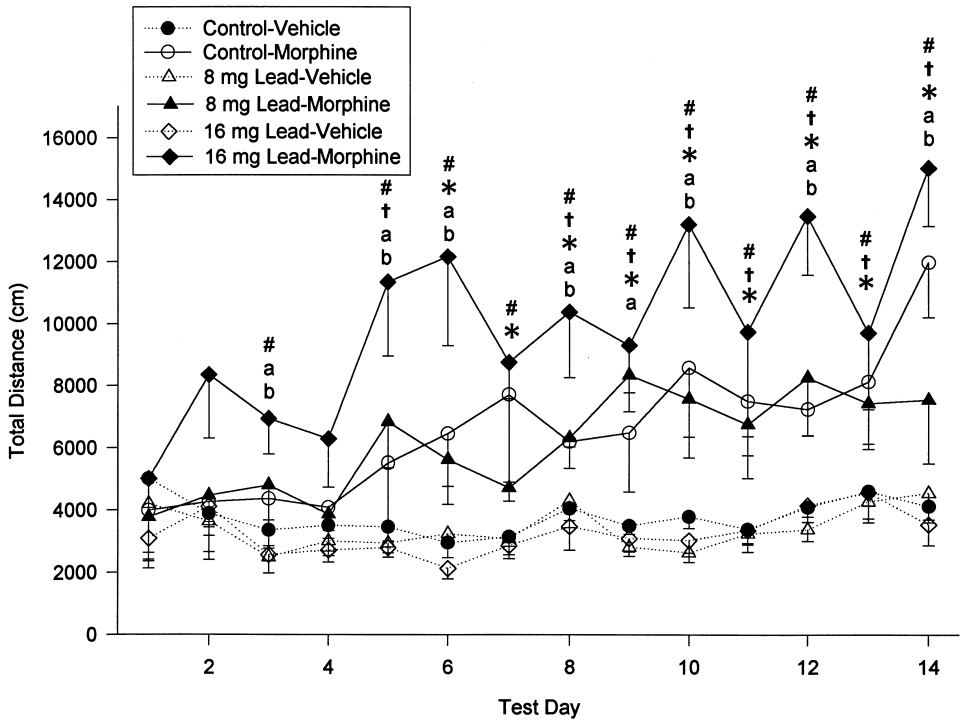

Fig. 3. Mean (±SEM) total distance traveled (cm) per daily session on Test Days 1 through 14 following a vehicle-only or 10 mg/kg morphine injection for animals perinatally exposed to dams receiving 0 (control), 8, or 16 mg lead. The following symbols denote significant group separation ($p < 0.05$): "*" = Control-Morphine on that Test Day > Control-Morphine on Test Day 1 (acute morphine challenge); "†" = 8 mg Lead-Morphine on that Test Day > 8 mg Lead-Morphine on Test Day 1; "#" = 16 mg Lead-Morphine on that Test Day > 16 mg Lead-Morphine on Test Day 1; "a" = Control-Morphine < 16 mg Lead-Morphine; "b" = 8 mg Lead-Morphine < 16 mg Lead-Morphine.

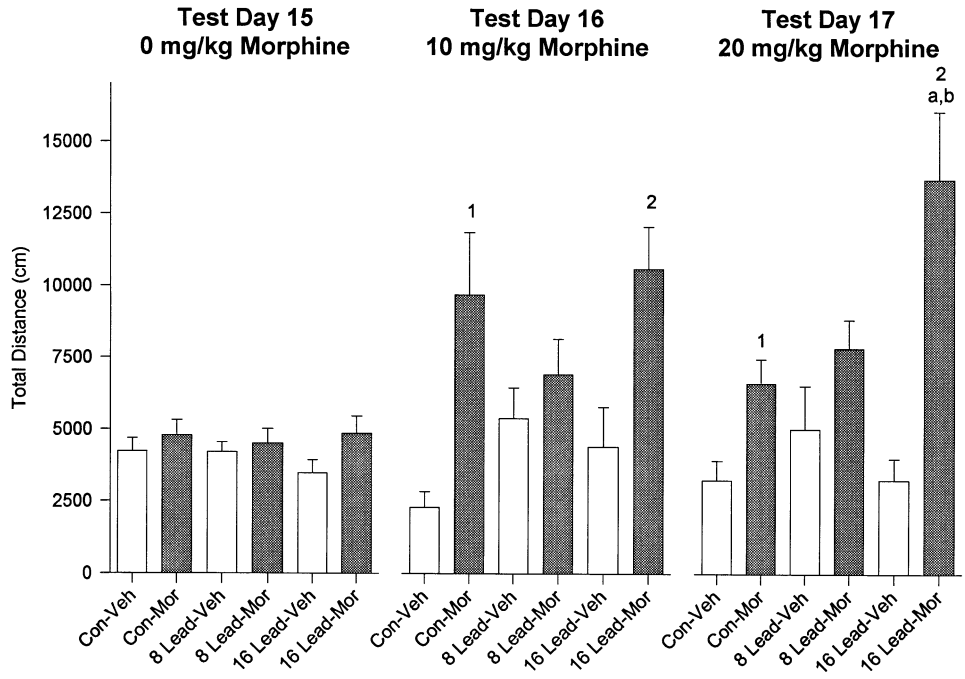

Fig. 4. After behavioral sensitization testing, the mean (±SEM) total distance traveled (cm) for animals perinatally exposed to dams receiving 0 (control), 8, or 16 mg lead. Ascending test doses of 0, 10, and 20 mg/kg morphine were presented daily to every animal. The following symbol denotes significant separation by Test Day for a given group ($p < 0.05$): "1" = Control-Vehicle < Control-Morphine; "2" = 16 mg Lead-Vehicle < 16 mg Lead-Morphine; "a" = Control-Morphine < 16 mg Lead-Morphine; "b" = 8 mg Lead-Morphine < 16 mg Lead-Morphine.

each of the three groups of animals that had received vehicle on Test Days 1–14 ($p < 0.05$). The 8-mg Lead-Morphine group was not different from any other group. Finally, on Test Day 17 group differences were found. The 16-mg Lead-Morphine group had total distance scores significantly greater than the Control-Morphine and 8-mg Lead-Morphine groups ($p < 0.05$). Differences were not indicated between the Control-Morphine and 8-mg Lead-Morphine groups, nor were differences evident among the groups of animals that had received vehicle on Test Days 1–14.

3.2.3. Blood-lead concentrations

Analysis of dam blood-lead concentrations indicated no differences among lead exposure groups before commencement of metal treatment (all animals below detectable limits of 1 μg/dl). At breeding, following 30 days of gavage with the appropriate lead concentration, there was a significant elevation ($F(2,12) = 5.05$, $p < 0.01$) in blood-lead levels in dams treated with 16 mg lead (mean = 32.5 μg/dl, SEM = ±6.5 μg/dl) and 8 mg lead (mean = 16.5 μg/dl, SEM = ±5.2 μg/dl) relative to control dams (all animals below 1 μg/dl). At parturition, this elevation in blood-lead levels persisted ($F(2,10) = 15.23$, $p < 0.01$), as dams from the 16-mg lead (mean = 34.1 μg/dl, SEM = ±5.5 μg/dl) and 8-mg lead groups (mean = 22.7 μg/dl, SEM = ±7.0 μg/dl) had increased levels relative to control dams (mean < 1 μg/dl). Finally, at weaning (PND 21), there was a significant elevation ($F(2,12) = 8.80$, $p < 0.01$) in 16-mg lead (mean = 37.0 μg/dl, SEM = ±4.7 μg/dl) and 8-mg lead dams (mean = 24.3 μg/dl, SEM = ±5.5 μg/dl) relative to control dams (all animals below 1 μg/dl).

Regarding the pups, previous studies that employed experimental procedures identical to those reported for Experiment 2 [33] indicated that on PND 1 pups from dams gavaged with 16-mg lead had blood-lead levels approximately 22.0 μg/dl and pups from dams gavaged with 8-mg lead had blood-lead levels approximately 12.5 μg/dl. By PND 30, control and 8-mg lead group pups had blood-lead levels below 1 μg/dl and levels in 16-mg lead pups were approximately 4.8 μg/dl. In Experiment 2, blood-lead levels in pups from each exposure group were below 1 μg/dl when blood samples were collected at approximately PND 80.

4. Discussion

Consistent with previous investigations [21,34], in both experiments reported here the repeated administration of 10 mg/kg morphine sulfate across 14 consecutive days induced the development of a locomotor sensitization effect relative to the initial presentation of the drug. In Experiment 1, relative to controls, the development and/or expression of morphine sensitization was attenuated in animals exposed to 250 ppm lead acetate as adults. With respect to perinatal lead exposure (Experiment 2), pups from dams exposed to 16 mg lead showed an enhanced response to morphine

administration relative to control animals. This differential sensitivity to morphine in lead-exposed animals occurred when blood-lead concentrations were at clinically relevant levels in the adult exposure case (20–30 μg/dl, Experiment 1), or below detectable limits in offspring (<1 μg/dl, Experiment 2).

Despite the directionally opposite nature of the adult vs. developmental data, the pattern of results from Experiments 1 and 2 are consistent with previous lead investigations in which cocaine was administered repeatedly. Presentation of cocaine across multiple consecutive test days is associated with an increase in locomotor activity relative to the initial presentation of the drug. Adult lead exposure produced attenuation in the development of cocaine locomotor sensitization [32], but with perinatal lead exposure, a heightened sensitization was present in lead-exposed pups, relative to control animals [33]. It appears this subsensitivity/supersensitivity dichotomy with adult/developmental lead exposure persists across different classes of abused drugs (psychomotor stimulants, opiates).

4.1. Experiment 1 — Adult lead exposure

Although in Experiment 1 there was an attenuation in the development of locomotor sensitization, it is important to note that there was no complete inhibition of a morphine-induced increase in locomotor activity in lead-exposed animals. Only a delay in the development of statistically significant sensitization was present in these animals, such that more test days were required to produce the phenomenon. The results from dose–effect testing (Test Days 15–17) suggest that the subsensitivity to morphine in lead-treated adult animals might be surmountable after repeated drug treatment.

An alternative explanation to the suggestion of a reduced sensitivity to morphine in adult lead-treated animals is a heightened sensitivity to the drug following lead exposure. It is possible that on Test Days 1–14 Lead-Morphine animals were more sensitive to the drug than Control-Morphine animals, and this was demonstrated through an increase in stereotypy and an attendant decrease in locomotor activity. However, the fact that repeated morphine administration systematically increased responding over the course of testing in lead-exposed animals, rather than further diminished it, would seem to argue against such an interpretation.

It is presumed that the behavioral differences produced with lead exposure are a result of some central action of the metal on neural function/morphology. With respect to the present investigations on lead/opiate interactions, the current belief is that the development of morphine locomotor sensitization occurs coincident to increases in the activation of dopamine fibers projecting from the ventral tegmental area (VTA) to the nucleus accumbens [17,41]. The VTA is an area rich in opioid receptors [29], including the μ-opioid receptor. The VTA also is the site of many gamma-amino-butyric acid (GABA) neurons [3], which are linked to dopamine cells in the VTA [31]. In the absence of substantial VTA μ-opioid receptor activation, GABA interneurons modulate glutamate-stimulated dopaminergic activity, ultimately constraining the basal firing rate of dopamine projection neurons [22]. With the application of morphine, however, there is an inhibition of the inhibitory effect of GABA interneurons, resulting in greater glutamate involvement in the region of the VTA, and a dopamine increase the nucleus accumbens [20].

Regarding this putative neural model for morphine sensitization, it is possible that each component of the cascade may be susceptible to disturbance by adult lead exposure. Low-level lead exposure produces a change in dopamine synthesis [16], release [19], and pre- and post-synaptic binding within the nucleus accumbens [37]. The NMDA (glutamate) receptor is also altered with adult (postweaning) lead exposure [6,12,26] and this might be related to changes in dopaminergic system functioning [7,25]. Certainly, additional experiments must be conducted to investigate the effects of adult lead exposure on opiate pharmacodynamics and pharmacokinetics. In particular, reliable interactions among lead exposure and opioid binding and receptor properties have not yet been characterized. Further, it is unknown whether lead exposure alters the absorption and distribution of morphine from the periphery into the central nervous system, such that levels of the drug are equivalent between control and lead-treated animals.

4.2. Experiment 2 — Perinatal lead exposure

As indicated, the results from Experiment 2 were directionally opposite those of Experiment 1, such that perinatal lead exposure was associated with a supersensitivity (rather than a subsensitivity) to morphine in lead-exposed animals. In Experiment 2, the morphine supersensitivity persisted in pups from the 16-mg Lead-Morphine group through dose–effect testing. While Control-Morphine pups had a non-monotonic pattern of activity across Test Days 15–17, for pups in the 16-mg Lead-Morphine group there was elevated locomotor activity following both 10 (Test Day 16) and 20 mg/kg morphine (Test Day 17) relative to 0 mg/kg morphine (Test Day 15). The pattern for development of locomotor sensitization for pups in the 8-mg Lead-Morphine group was similar to control animals.

Just as adult exposure to lead results in changes in the neural systems associated with morphine sensitization (dopaminergic, glutamatergic, GABAergic), exposure to lead during development also is associated with changes in these systems. Early postnatal lead exposure has been shown to produce an increase in the number of dopamine receptors and receptor sensitivity [8,42]. This could be related to a reported decrease in basal dopamine levels following early postnatal lead treatment [1]. Glutamatergic neurons also appear to be susceptible to developmental lead exposure, such that there is a decrease in NMDA receptor sensitivity

[13] and synaptic plasticity [10]. Finally, prenatal and postnatal lead exposure has been shown to alter opiate-induced behaviors, perhaps through a disruption of endogenous opioids and the binding properties of μ- and δ-opioid receptors [24].

It is clear that both adult and developmental lead exposure is associated with a change in the neural systems associated with morphine sensitization. However, precisely why the pattern of behavioral results differed so dramatically in Experiments 1 and 2 is yet to be determined. This dichotomy is obviously related to the developmental period in which lead exposure occurs, but at this juncture, identification of differential changes in neuroanatomical or chemical substrates responsible for such diverse effects necessarily remains a matter for conjecture. Also, issues such as differing routes of exposure (adults were exposed to dietary lead, pups were exposed to lead via a lactating dam), body weight differences, and other contrasts in experimental conditions must be considered as possible determinants of the diverse behavioral patterns observed in this investigation.

In any event, as relates to morphine-induced changes in locomotor activity, it is clear that early lead exposure has appreciably different consequences than lead exposure later in life. Or at least this would seem to be the case under the testing conditions imposed here. Perhaps other methodological conditions would yield quite different results. For instance, it is known that female rats self-administer cocaine and heroin more readily than male rats [27], and it must be considered that such gender differences could interact with lead/morphine effects (only males were tested in this study). Also, the present investigation used only sexually naive dams that had not previously littered, so questions about the generalizability of the present findings to broader populations of experienced breeders arise. Along these lines, it would further be of interest to examine the selective impact of prenatal (gestation) vs. postnatal (lactation) lead exposure on morphine responsiveness (animals were perinatally exposed to lead in this study).

4.3. Implications

It is evident from in vitro and in situ studies that lead presented to an adult or developing organism impacts neural loci associated with morphine sensitization [1,6–8,13, 16,19,24,25,37,42]. Confirmatory behavioral studies have determined a role for dopamine, glutamate, and opioid receptor types in sensitization through careful manipulation of selective receptor ligands. For example, the role of the NMDA receptor in morphine sensitization was suggested through studies in which the selective NMDA receptor antagonist dizocilpine (MK-801) blocked the expression, but not the development, of morphine sensitization [18,43]. In the present in vivo studies, it was not possible to present the non-selective antagonist (lead) at certain test days or remove the influence of the metal. As a result, it is unknown whether adult or developmental lead exposure has a preferential impact on either the development or the expression of morphine sensitization. This is significant because the development of sensitization is thought to be correlated with changes in the motivational properties of abused drugs [39].

The potential importance of the present findings derives from the implications of the results for drug seeking and taking in human populations. Measures of drug use patterns suggest that children and adults who are born 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 suburban areas [9]. Although those who live outside the city are not immune to opiate abuse, it is clear that there is increased availability and higher rates of drug use in the inner-city [14]. In addition to concerns of drug use for inner-city residents, studies of blood-lead levels report consistently that socio-demographic factors, such as poverty, are correlated positively with increases in blood-lead levels [36]. Perhaps this is because those with low-income levels are more likely to live in urban areas that retain lead through residual paint and past automobile pollution [30]. Although it would be inappropriate to assert that lead toxicity is a principal determinant of drug selection and use patterns, it is reasonable to suspect that lead exposure may alter drug responsiveness and therein contribute to the overall use profile.

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References

[1] Antonio MT, Corpas I, Leret ML. Neurochemical changes in newborn rat's brain after gestational cadmium and lead exposure. Toxicol Lett 1999;104:1–9.

[2] Bellinger DC, Stiles KM, Needleman HL. Low-level lead exposure, intelligence, and academic achievement: a long-term follow-up study. Pediatrics 1992;90:855–61.

[3] Bowery NG, Hudson AL, Price GW. $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 1987;20:365–83.

[4] Brody DJ, Pirkle JL, Kramer RA, Flegal KM, Matte TD, Gunter EW, Paschal DC. Blood lead levels in the US population: phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1991). JAMA 1994;272:277–83.

[5] 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.

[6] Cory-Slechta DA. MK-801 subsensitivity following postweaning lead exposure. Neurotoxicology 1995;16:83–95.

[7] 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.

[8] Cory-Slechta DA, Pokora MJ, Widzowski DV. Postnatal lead exposure induces supersensitivity to the stimulus properties of a D_2–D_3 agonist. Brain Res 1992;598:162–72.

[9] 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.
[10] Gilbert ME, Mack CM, Lasley SM. Chronic developmental lead exposure increases the threshold for long-term potentiation in rat dentate gyrus in vivo. Brain Res 1996;736:118–24.
[11] Grover CA, Nation JR, Bratton GR. Chronic exposure to lead attenuates cocaine-induced behavioral activation. Pharmacol Biochem Behav 1993;44:221–5.
[12] Guilarte TR. Glutamatergic system and developmental lead neurotoxicity. Neurotoxicology 1997;18:665–72.
[13] Guilarte TR, Miceli RC, Jett DA. Neurochemical aspects of hippocampal and cortical Pb^{2+} neurotoxicity. Neurotoxicology 1994;15::459–466.
[14] Harwell TS, Spence MR, Sands A, Iguchi MY. Substance use in an inner-city family planning population. J Reprod Med 1996;41: 704–10.
[15] Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol Teratol 1992;14:221–8.
[16] Jadhav AL, Ramesh GT. Pb-induced alterations in tyrosine hydroxylase activity in rat brain. Mol Cell Biochem 1997;175:137–41.
[17] Jeziorski M, White FJ. Dopamine receptor antagonists prevent expression, but not development, of morphine sensitization. Eur J Pharmacol 1995;275:235–44.
[18] Jeziorski M, White FJ, Wolf ME. MK-801 prevents the development of behavioral sensitization during repeated morphine administration. Synapse 1994;16:137–47.
[19] 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.
[20] Kalivas PW. Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. Drug Alcohol Depend 1995;37:95–100.
[21] Kalivas PW, Duffy P. Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons. J Pharmacol Exp Ther 1987;241:204–12.
[22] Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 1991;16:223–44.
[23] Kaufman AS. Do low levels of lead produce IQ loss in children? A careful examination of the literature. Arch Clin Neuropsychol, in press.
[24] Kitchen I. Lead toxicity and alterations in opioid systems. Neurotoxicology 1993;14:115–24.
[25] Lasley SM. Regulation of dopaminergic activity, but not tyrosine hydroxylase is diminished after chronic inorganic lead exposure. Neurotoxicology 1992;13:625–36.
[26] Lasley SM, Green MC, Gilbert ME. Influence of exposure period on in vivo hippocampal glutamate and GABA release in rats. Neurotoxicology 1999;20:619–29.
[27] Lynch WJ, Carrol ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology 1999;144:77–82.
[28] Ma T, Chen H-H, Ho I. Effects of chronic lead (Pb) exposure on neurobehavioral function and dopaminergic neurotransmitter receptors in rats. Toxicol Lett 1999;105:11–121.
[29] Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ. Anatomy of CNS opioid receptors. Trends Neurosci 1988;11:308–14.
[30] Mielke HW. Lead in the inner cities. Am Sci 1999;87:62–73.
[31] Nagai T, McGeer PL, McGeer EG. Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. J Comp Neurol 1983;218:220–38.
[32] 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.
[33] Nation JR, Miller DK, Bratton GR. Developmental lead exposure alters the stimulatory properties of cocaine at PND 30 and PND 90. Neuropsychopharmacology, in press.
[34] Nation JR, Miller DK, Livermore CL. Chronic exposure to cadmium attenuates behavioral sensitization to morphine. Psychopharmacology 1997;131:248–54.
[35] 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.
[36] Pirkle JL, Brody DJ, Gunter EW, Kramer RA, Paschal DC, Flegal KM, 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.
[37] 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.
[38] Post RM, Lockfeld A, Squillace KM, Contel NR. Drug–environment interaction: context dependency of cocaine-induced behavioral sensitization. Life Sci 1981;28:755–60.
[39] Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993;18:247–91.
[40] Sandberg PR, Zoloty SA, Willis R, Ticarich CD, Rhoads K, Nagy RP, Mitchell SG, Laforest AR, Jenks JA, Harkabus LJ, Gurson D, Finnefrock JA, Bednerik EJ. Digiscan activity: automated measurement of thigmotactic and stereotypic behavior in rats. Pharmacol Biochem Behav 1987;27:569–72.
[41] Vezina P, Kalivas PW, Stewart J. Sensitization occurs to the locomotor effects of morphine and the specific mu opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but not to the nucleus accumbens. Brain Res 1987;417:51–8.
[42] Widzowski DV, Finkelstein JN, Pokora MJ, Cory-Slechta DA. Time course of postnatal lead-induced changes in dopamine receptors and their relationship to changes in dopamine sensitivity. Neurotoxicology 1994;15:853–65.
[43] Wolf ME, Jeziorski M. Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res 1993;613:291–4.
[44] 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.