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Developmental lead exposure in rats: is a behavioral sequel extended at F2 generation?

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Abstract

Lead toxicity was studied in rats exposed from conception until weaning and assessed by monitoring offspring behavior in both the open field and elevated plus maze and by determining tissue lead in an assessment schedule extended to first (F1) and second (F2) generations. Dams utilized for the F1 generation were submitted to 750 ppm of lead (acetate) in drinking water during pregnancy and lactation. For F1 pups, behavioral alterations were not detected in the elevated plus maze, while in the open field, spontaneous locomotor activity as well as time of both grooming and rearing increased, while freezing time decreased in 30- and 90-day-old rats. Lead content was higher in tissues of 1- and 30-day-old pups. However, in 90-day-old rats, lead was detected only in the femur. F2 generation was lead-free but still presented alterations in both locomotor activity and grooming in 30- and 90-day-old pups. It appears that developmental lead exposure may cause behavioral effects during the developmental stage of the F1 generation, which remains throughout the animal's adult life as a sequel, regardless of lead accumulation, and is extended to the F2 generation of rats.

Keywords: Lead; Rat; Behavioral assessment; Development

1. Introduction

Lead is a well-known environmental contaminant, occurring in different concentrations in the air, water supplies, soils, and foods. Thus, lead exposure is a major risk for the general population (World Health Organization, 1989). Chronic lead intoxication, as a consequence of environmental and occupational exposure, occurs in highly industrialized regions and usually causes hematological, endocrine, gastrointestinal, and neurological dysfunctions in adults and children (Hammond and Dietrich, 1990; Lockitch, 1993). Clinical studies support the observation that there is an association between increased lead contamination and behavioral disorders (Beattie et al., 1975).

Acute and chronic lead exposure has been shown to affect the nervous system and cause various behavioral and neurotoxicological disorders. Some of these effects include impairments in the learning process, memory consolidation, attention, visual perception, manual dexterity, response speed, nerve demyelination, encephalopathy, hyperactivity, motor skills, and exploratory behavior (Cory-Slechta, 1995; Draski et al., 1989; Luthman et al., 1992; Murphy and Regan, 1999; Tang et al., 1995).

The concept of developmental-stage significance is also becoming increasingly important in toxicological risk assessment. The developmental stage of the individual has not always been considered at the time of exposure to a toxicant. This is particularly important when one considers the fact that a higher percentage of lead is absorbed from the gastrointestinal tract of infants than in adults (Graeter and Mortensen, 1996).

Lead is excreted into human and animal milk (Sternovsky and Wessolowsky, 1985; Schramel et al., 1988), although the placental limits lead passage, since large maternal fetal concentration gradients exist (Mc Clain and Becker, 1975). Human studies and animal experiments have widely documented that inorganic lead crosses the placental barrier and accumulates in fetal tissues including the brain (Dietrich, 1991; Goyer, 1990; Klein et al., 1994).

In experimental animal studies, behavioral effects associated with lead exposure have been shown in a wide range

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of lead-exposure regimens. Most of these studies deal with lead exposure during gestation and/or lactation (Rodrigues et al., 1996) corresponding to the stages in which the brain is undergoing intense development (Bayer et al., 1993) and therefore is more susceptible to lead toxicity. In spite of the many studies assessing the behavioral effects of lead exposure during the period of rapid brain growth, these reports present conflicting data and the precise effect of lead on the brain is not clear.

Since it was demonstrated that maternal inorganic lead exposure produces encephalopathy in suckling rodents (Pentschew and Garro, 1966), there has been continuing concern about the neurodevelopmental consequences associated with low-level exposure to this toxicant. The majority of prospective human and animal studies has confirmed this concern by correlating elevated perinatal blood levels with behavioral and cognitive alterations that endure throughout postnatal development, adolescence, and adulthood (Cory-Slechta et al., 1985, 1992; Finkelstein et al., 1998). However, assessment of neurological and behavioral effects on future generations following lead exposure has not been conducted.

The absence of systematic studies on the long-term consequences of perinatal lead exposure seems risk, especially in the case of pregnant women, their fetuses, and suckling babies. In the case of pregnancy, the lack of routine biological environmental monitoring at the workplace and other preventive measures for the workers' protection in certain professions, such as hairdressers, contribute to real lead toxicity risks for these people.

The goals of our study include: (1) examination of the behavioral effects of lead on open-field and elevated plus maze tests; (2) to estimate tissue lead burdens in lead-exposed dams, 1-, 30-, and 90-day-old lead-exposed pups; (3) to extend behavioral examinations to include the 30-, and 90-day-old pups of the second (F2) generation.

2. Methods

The experimental protocol was approved by the Institutional Committee for the Ethical Use of Animals in Experiments.

2.1. Animals and treatment

Seventy-day-old female Wistar rats weighing 130–150 g from our institutional colony were kept under standard conditions (up to five rats per cage, 25 ± 2°C, 70% humidity, 12-h light–dark cycle starting at 6 a.m. with light and food and water ad libitum). After 3 weeks of acclimation, rats were bred in-house and breeding groups consisted of three virgin females and one non-lead-exposed male.

2.1.1. First (F1) generation

Forty-nine pregnant rats were placed in individual cages and supplied with nesting materials, food ad libitum, and one of the following solutions: deionized drinking water ($n = 25$) or 750 ppm of lead (lead acetate trihydrate, Merck, Germany) in deionized drinking water ($n = 24$), until the 21st postnatal day (PN 21). All solutions, including control, were acidified with 0.3 mM acetic acid. At birth, litters of acceptable size were weighed within 24 h, adjusted to six females per mother, and maintained with their mothers until the PN 21. Because we were interested in testing the F2 generation offspring, only females were used in this study. Two litters of control and one of lead-exposed group were excluded from the experiment due to small litter sizes. During lactation, body weight were monitored and animals were continuously observed for intoxication symptoms, such as salivation, muscle tonus, diarrhea, etc.

2.1.2. Second (F2) generation

Rats was offspring of females coming of litters of the F1 generation (control derived and lead-exposed derived), which in adult age were utilized to compose breeding groups from supply offspring of the F2 generation. Rats, in breeding groups consisted of three virgin females and one male and pregnant rats, control-derived and lead-exposed-derived, and were lead-free at the time of pregnancy, were placed in individual cages and supplied with nesting materials, food ad libitum, and deionized drinking water acidified with 0.3 mM acetic acid (to mimic the conditions of F1 generation) until PN 21. At birth, litters of acceptable size were weighed within 24 h, adjusted to six females per mother, and maintained with their mothers until the PN 21. During lactation, body weight were monitored and animals were continuously observed.

General parameters was evaluated on F1 and F2 generations. Mothers were weighed during pregnancy and suckling. On the day of birth (Day 1), the pups were examined for the occurrence of gross malformations. Animals were weighed at 1, 7, 14, 21, 30, 75, and 90 days after delivery. Reflex (time for acquiring the righting and negative geotaxis reflex, time of disappearance of palmar grasp reflex) tests and observation of the maturation of physical characteristics (incisor eruption, eye opening) were carried out daily at the appropriate ages by one experimenter that was not aware of the subject treatment, using previous criteria (Smart and Dobbing, 1971).

2.2. Behavioral tests

Behavioral evaluations of pups were performed at the ages of 30- and 90-days for F1 and F2 generations using open-field and elevated plus maze tests in which the animals were tested at one time without previous habituation. One day prior to testing, a single pup chosen at random from each litter, to obviate possible biasing effects due to genetic homogeneity within litters, were grouped and transferred from their home cage into a sound-attenuated, temperature-controlled room, illuminated by dim red lights. The period of behavioral observation was defined between

9 a.m. and 11 p.m. To minimize the possible influences of circadian changes during the period of tests, control and lead-exposed animals were alternated at each observation. To prevent observational bias, the testers were blind to the exposure group.

2.3. Open field

The open-field behavior was assessed using a wood box measuring 97 × 32.5 cm (diameter × height), similar to that described previously (Broadhurst, 1960). This was divided into three concentric circles, which were subdivided by painted black lines into 18 similar spaces. For open-field observations, each rat was placed in the center of the arena and, for the next 3 min, was scored on the following parameters: ambulation frequency (number of floor units entered with the four paws), rearing frequency (number of times the animals stood on its hind legs), freezing duration (total time the animal was in an immobile state, often in a crouching posture with wide open eyes and irregular respiration, after it had remained motionless for at least 1 s), and grooming duration (total time the animal used to groom). The following grooming behaviors were considered: forepaw vibration, paw licking, washing of nose, face, and head, body licking, genital grooming, scratching, and head-shaking. The open field was cleaned with 5% ethanol before each animal was introduced.

2.4. Elevated plus maze

The elevated plus maze behavior was conducted as described previously (Pellow and File, 1986) and was assessed using an apparatus consisting of two open and two enclosed arms of equal length and width (50 × 10 cm). The open arms had a 1-cm high Plexiglas edge while the enclosed arms are not entirely enclosed, but rather have walls that extend 40 cm high. The plus maze was elevated 50 cm above the floor. Each rat was placed in the center of the elevated plus maze facing one of the open arms, and number of entries with the four paws, and time spent (seconds) in the open or closed arms were recorded during a 3-min test period. The elevated plus maze test is based on the principle that exposure to an elevated and open arm maze leads to an approach conflict that is considerably stronger than that evoked by exposure to an enclosed maze arm. Thus, the total entries and time spent in both open and closed arms provides a measure of anxiety- or fear-induced inhibition of normal exploratory activity (Pellow and File, 1986). The elevated plus maze was carefully cleaned with 5% ethanol before each animal was introduced.

2.5. Lead determination

Blood from the dams on the first day of mating and the first day of weaning was used for lead analyses. Lead analyses were conducted on the blood and brain from the PN 1 pups (because of a limited amount of material, a pool from the same litter was used for all lead analyses for PN 1 pups), and from the blood, brain, liver, kidney, and femur for PN 30 and PN 90 pups for F1 and F2 generations. Pups of different litters were randomly pooled. Analysis of lead levels was performed using an atomic absorption spectrophotometer Hitachi — model Z-5700 graphite furnace atomizer (Yeger et al., 1971), with adaptations.

2.6. Statistical analysis

Data from physical parameters and reflexes of the offspring, lead content in tissues of both offspring and mothers, and behavioral alterations were analyzed by unpaired two-way Student's t test to compare lead-exposed groups with their respective controls. Data from body weight gain of pups were evaluated by repeated measures of analysis of variance (ANOVA) with the mean litter weight as the within subjects factor (Snedecor and Cochran, 1980). Differences between groups were considered significant if $P < .05$.

3. Results

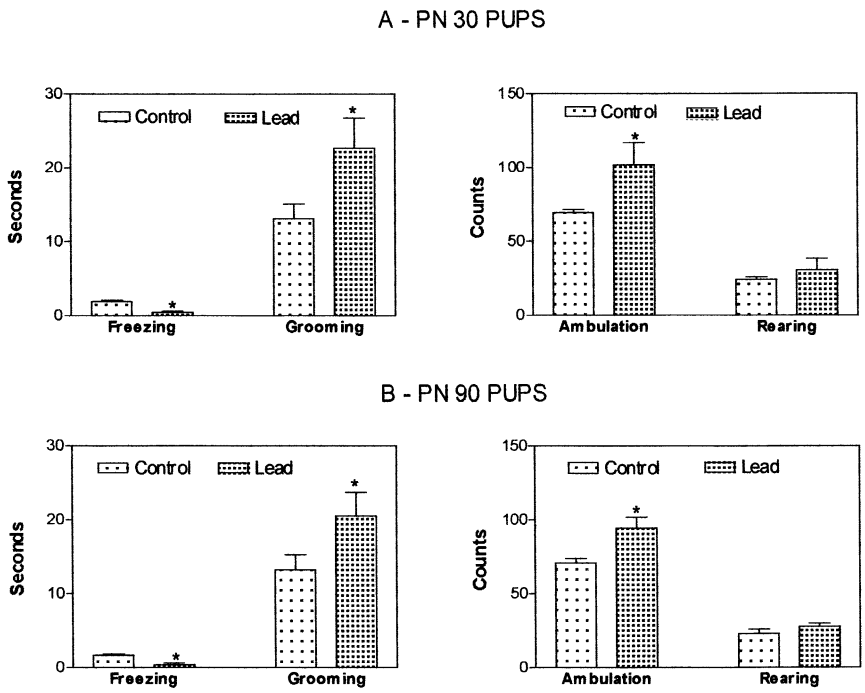
3.1. General parameters of dams and pups

During the F1 generation, the lead exposure did not affect body weight gain of females during pregnancy and suckling (data not shown). The only effect observed was a transitory reduction in lead acetate solution intake during the first week, probably due to the different flavor of the solution. The rats reliably gained weight as they aged and no differences were evident between groups in rate of growth as a result of lead exposure (Table 1). No differences between lead-exposed and control pups were observed from the appearance of physical parameters, such as incisor eruption, eye opening, and in the time for acquiring the righting and negative geotaxis reflex. The time of disap-

Table 1
Body weight of pups suckled by control and lead-exposed dams

	PN 1	PN 7	PN 14	PN 21	PN 30	PN 75	PN 90
Control	6.87 ± 1.00	14.82 ± 4.37	31.75 ± 2.50	44.55 ± 3.20	66.50 ± 6.92	150.70 ± 16.10	268.00 ± 24.00
Lead	6.60 ± 1.20	19.40 ± 1.20	32.50 ± 0.89	47.70 ± 3.90	68.00 ± 8.12	149.30 ± 16.92	262.60 ± 32.70

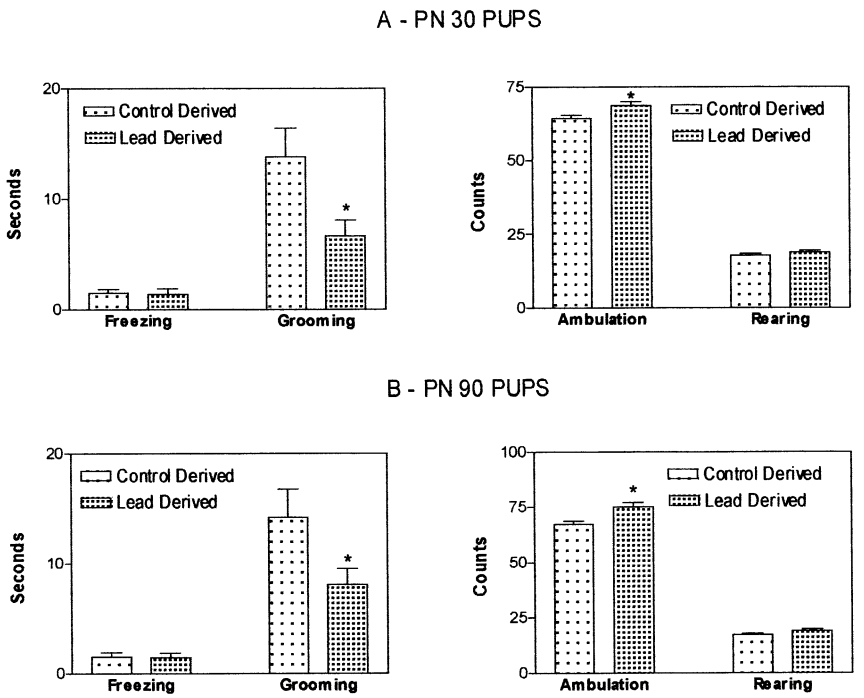
Data are expressed as mean ± S.E.M. of four to seven litters. Pups were exposed to lead from pregnancy to weaning, by giving the dams 750 ppm of lead in drinking water. Comparisons using ANOVA.



Data are expressed as mean ± S.E.M. of 10-13 animals. Pups were exposed to lead from pregnancy to weaning, by giving the dams 750 ppm of lead in drinking water. *p<0.05 = significantly different from respective control, using unpaired two-way Sudent's *t*-test.

Fig. 1. Open-field behavior of lead-exposed pups for F1 generation.

pearance of palmar grasp reflex was slightly but nonsignificantly increased in lead-exposed pups (data not shown). In the F2 generation, the pregnancy period was normal and pups of control and experimental groups did not show



Data are expressed as mean ± S.E.M. of 15-18 animals. Rats was offspring of females coming of the first generation which were lead-free at the time of pregnancy. *p<0.05 = significantly different from respective control, using unpaired two-way Sudent's *t*-test.

Fig. 2. Open-field behavior of pups for F2 generation.

Table 2
Lead content in tissues from dams and their weaning pups for F1 generation

	Non-lead-exposed	Lead-exposed
Dams' blood (first day mating)	0.142 ± 0.029 (5)	45.61 ± 6.11* (5)
Dams' blood (PN 21)	0.153 ± 0.014 (7)	47.84 ± 2.73* (7)
Pups' blood (PN 1)	ND	28.34 ± 5.11*
Pups' brain (PN 1)	ND	0.46 ± 0.06*

Data are expressed as mean ± S.E.M. The number of dams is shown in parentheses. Blood and brain were pooled from five to nine PN 1 pups of six litters. Lead concentration in blood is expressed as micrograms per deciliter (μg/dl) and in brain as micrograms per gram (μg/g) wet weight. ND = Not detected.

* $P < 0.05$, significantly different from respective control, using unpaired two-way Student's t test.

alterations in growth, appearance of physical parameters, or reflex (data not shown).

3.2. Elevated plus maze behavior

The elevated plus maze behavior of 30- and 90-day-old lead-exposed pups did not show statistically significant alterations in F1 and F2 generations. However, F1 generation lead-exposed pups showed a tendency to spend more time in the closed arm than the controls [$t(23) = 1.82$, $P = 0.082$] (data not shown).

3.3. Open-field behavior

The study on open-field behavior of the pups of the F1 generation (Fig. 1) showed the following behavioral changes: in the PN 30 lead-exposed pups (Fig. 1A), significantly decreased freezing [$t(23) = 7.02$, $P < 0.0001$], significantly increased ambulation [$t(23) = 2.20$, $P = 0.0381$], and significantly increased grooming [$t(23) = 2.20$, $P = 0.0382$]. However, the observed increase in rearing behavior was not statistically significant [$t(23) = 0.78$, $P = 0.4445$]. In the PN 90 lead-exposed pups (Fig. 1B), significantly decreased freezing [$t(18) = 4.37$, $P = 0.0004$], significantly increased ambulation [$t(18) = 3.00$, $P = 0.008$], and a trend toward increased grooming [$t(18) = 1.93$, $P = 0.07$]. Rearing behavior, similar to that observed in PN 30 pups, also was not statistically significant [$t(18) = 1.22$, $P = 0.24$].

The study on open-field behavior of the pups of the F2 generation (Fig. 2) showed the following significant behavioral changes in relation to control: in the PN 30 pups (Fig. 2A), a slight, but not significant increase in rearing [$t(31) = 1.29$, $P = 0.20$], significantly increased ambulation [$t(31) = 2.45$, $P < 0.02$], and surprisingly, significantly reduced grooming [$t(31) = 2.50$, $P = 0.02$]. The amount of time spent freezing was not significantly different from controls [$t(31) = 0.12$, $P = 0.90$]. In PN 90 pups (Fig. 2B), significant increases in ambulation [$t(31) = 2.46$, $P = 0.02$], significant reductions in grooming [$t(31) = 2.18$, $P = 0.04$], and no effect on freezing [$t(31) = 0.05$, $P = 0.96$] were observed. A slight increase in rearing was observed in the PN 90 pups, but this difference was not significant [$t(31) = 1.43$, $P = 0.16$].

3.4. Lead content

Blood lead levels in F1 dams on the first day of mating and at weaning and lead levels in brain and blood of the 1-day-old pups are shown in Table 2. Blood lead levels of the exposed dams were significantly higher than those of the controls on the first day of mating [$t(8) = 7.44$, $P < 0.0001$] and on PN 21 [$t(12) = 17.48$, $P < 0.0001$], confirming the intoxication. In 1-day-old lead-exposed pups, brain [$t(10) = 5.99$, $P < 0.0001$] and blood [$t(10) = 5.53$, $P < 0.0003$] lead levels were significantly higher.

Table 3 shows that lead levels were significantly increased in blood [$t(23) = 20.76$, $P < 0.0001$], kidney [$t(23) = 9.39$, $P < .0001$], femur [$t(22) = 6.89$, $P < 0.0001$], liver [$t(23) = 10.98$, $P < 0.0001$], and the brain of 30-day-old pups, but in 90-day-old pups, lead was significantly increased only in the femur [$t(18) = 4.97$, $P < 0.0001$]. These results demonstrate the efficiency of elimination of the lead absorbed during the exposure period.

Lead was not detected in the blood of dams from the F2 generation and in tissues of their 1-, 30-, and 90-day-old pups (data not shown).

Table 3
Lead content in tissues from lead-exposed pups of F1 generation

	PN 30		PN 90	
	Control	Lead	Control	Lead
Blood	0.117 ± 0.010 (13)	25.01 ± 1.25* (12)	0.157 ± 0.016 (10)	0.113 ± 0.013 (10)
Kidney	0.07 ± 0.02 (13)	2.33 ± 0.25* (12)	0.07 ± 0.03 (10)	0.06 ± 0.04 (10)
Femur	0.38 ± 0.07 (13)	80.74 ± 12.73* (11)	0.32 ± 0.05 (10)	5.10 ± 0.96* (10)
Liver	0.04 ± 0.02 (13)	0.43 ± 0.03* (12)	0.05 ± 0.01 (10)	0.06 ± 0.006 (9)
Brain	ND (13)	0.40 ± 0.05* (13)	ND (10)	ND (10)

Data are expressed as mean ± S.E.M. The number of animals is shown in parentheses. Pups were exposed to lead from pregnancy to weaning by giving the dams 750 ppm of lead in drinking water. Lead concentration in blood is expressed as micrograms per deciliter (μg/dl), and in kidney, femur, liver, or brain as micrograms per gram (μg/g) wet weight. ND = Not detected.

* $P < 0.05$, significantly different from respective control, using unpaired two-way Student's t test.

4. Discussion

While existing animal reports point to the possibility that perinatal lead exposure produces altered behavior in the rat that can persist into maturity, the duration of its effect remains to be determined. The purposes of this study were to investigate lead neurotoxic effects over two generations in a perinatally lead-exposed model in rats using two relatively simple behavioral tests, and to determine tissue lead accumulation.

In contrast to earlier studies, the regimen of lead exposure used in the current study affected neither body weight gain of dams during pregnancy and lactation nor the number of pups per litter. In agreement with more recent data in which the procedure for lead administration was via the dam to suckling pups, our results also indicate that lead exposure did not affect body weight gain during suckling (Rodrigues et al., 1996; Mello et al., 1998). This is probably due to the low level of exposure used in the current study since there is evidence to suggest that lead at high dosage levels results in poorer quality milk, so that all lead-affected rats are suffering nutritional, dose-related deficiencies (Barret and Livesey, 1985).

The high tissue lead levels in PN 1 and PN 30 pups (from F1 generation) may be related to both lead transferred across the placental barrier and maternal milk, which would suggest a correlation between maternal exposure and offspring tissue lead concentrations. In contrast, other studies in which lead was administered in drinking water to mothers showed lead accumulation in blood, but not in the brain of 1-day-old pups, and in both the blood and cerebral cortex of 23-day-old rats (Rodrigues et al., 1996). These differences might be explained by the different treatment schedules, including time of exposure, dose, etc.

Table 2 shows that, despite a minimum amount of blood lead level in control mothers, probably by a contamination of diet, the metal was not detected in the blood and brain of these 1-day-old pups. Also, in the lead-exposed group, the blood of the 1-day-old pups had 37% less lead than mothers. This is due to the protective properties of the placental barrier against lead intoxication of the fetus, suggesting that the lead only crosses this barrier when the blood lead level is very high.

If we consider lead neurotoxicity as lead accumulation in the central nervous system, our results with lead dosages on tissues of PN 1 and PN 30 pups (from F1 generation) show that the lead exposure protocol used in this study is a very good model of perinatal brain intoxication. Human studies revealed that the functional state of the nervous system can be compromised even by a low level of lead exposure of the fetus and newborn (Hammond and Dietrich, 1990). Exposure to lead has been shown to disrupt developmental processes in the brain and to result in impaired brain function (Luthman et al., 1992). In our study, blood lead levels of PN 30 rats are similar to those associated with behavioral deficits in children (Davis et al., 1990) and behavioral alterations in experimental animals (Nagymajtényi et al., 1998).

The nature of the behavioral response of rats to the stress or fear of a novel environment, such as an elevated plus maze or open field, depends on the test apparatus used, age of subjects, previous experience, type and level of stimuli, etc. (Barret and Livesey, 1985). In our study with F1 generation, lead-exposed 1-day-old pups have a considerable quantity of lead in the brain, which remains high at 30 days and normalize at 90 day of age. It is reasonable that the behavioral alterations are very evident in PN 30 pups because of lead in the brain, but not in PN 90 pups, which was lead-free at this time. Surprisingly, in the elevated plus maze task PN 30 and PN 90 lead-exposed pups did not show lead-related alterations in behavioral status and, in the open-field task, altered behavior was observed in 30- and 90-day-old pups. It should be noted that the alterations of open-field behavior seen in the PN 30 and PN 90 rats suggest that the behavioral effects provoked by perinatal exposure to lead extend beyond the time exposure is terminated.

The present findings demonstrate significantly increased locomotor activity and grooming behavior in F1 offspring. Previous reports have shown lead-increased grooming and hyperactivity in developmental offspring (Nagymajtényi et al., 1998). Since it has already been shown that GABA may play a role in the expression of grooming behavior (Barros et al., 1994), lead alterations at the level of this neurotransmitter system might be involved. The increased spontaneous locomotor activity observed in the open field contradicts previous studies that found hyperactivity only in the presence of lead-induced body weight loss in rodents (Munoz et al., 1989) as body weight gain in our experimental animals was the same as that of the controls.

In F1 offspring our data showed a nonsignificant increase in rearing behavior and a significant decrease in freezing behavior. As rearing and ambulation are positively correlated behaviors, as reported previously (Satinder, 1968), the observed changes of locomotor activity and rearing behaviors in developmentally lead-exposed rats, will be in agreement with this premise. In the context of the statement that freezing behavior is a direct measure of emotionality (Archer, 1973), our observations of F1 offspring behavior suggest a negative influence of lead on the emotional status of animals.

Alterations at the synapse level and vulnerability of neurotransmitter systems, specifically elevated noradrenaline, dopamine, GABA/glutamate, serotonin, and acetylcholine were found in rats orally treated with lead acetate (Shailesh-Kumar and Desiraju, 1990; Bressler and Goldstein, 1991). Abnormal changes in the neurotransmitter system of the young pups can produce behavioral alterations in these animals, e.g., excessive hyperactivity due to changes of the dopaminergic system (Wirtshafter et al., 1988).

Two questions should be prompted at this point: (a) are behavioral alterations dependent on the presence of the lead

in the brain or are they the consequence of distribution in the cerebral areas during the stage of embryonic development, remaining as a sequel in older age without regard to changes in lead presence? and (b) could behavioral alterations observed in mature F1 females be transmitted to an F2 generation of offspring?

Data on the regional distribution of lead in the brain are conflicting. Earlier studies have shown that the hippocampus, amygdala, and cerebellum have a selective lead accumulation in the rat brain (Campbell et al., 1982; Fjerdingstad et al., 1974; Sandhir et al., 1994). However, a recent study (Widzowski and Cory-Slechta, 1994) reported no selective lead accumulation in brain regions of adult rats exposed to lead. As this metal induces abnormalities in blood–brain barrier permeability (Struzynska et al., 1997) in animals exposed, developmentally, it is possible that lead accumulates only in specific regions of the embryonic brain, and that in developing pups, lead determination would not show this regional accumulation. Functional alterations as a consequence of lead exposure in developmental animals may be subtle and require a more sufficient demand on the system for detection (Ferguson et al., 1998).

In the F2 generation, it would be reasonable to expect that the rats that were from previously exposed litters would exhibit behavior similar to controls since the mothers were free of lead at the time of breeding. The observed freezing behavior was in accordance with this hypothesis, but the locomotor and rearing behaviors are altered in the same way as in the F1 generation. The only difference being that the differences were attenuated (lower increases) in the F2 generation. The strong reduction observed in expression of grooming behavior for F2 offspring is not biologically understood at this time.

These findings are somewhat similar to those obtained in adult monkeys (Gilbert and Rice, 1987), showing that behavioral effects may persist or even worsen over time in older animals, even in the absence of continued lead exposure and with a subsequent dramatic reduction in blood lead levels. Other authors have reported an impairment in active avoidance (Altmann et al., 1993) and persisting neuroplastic deficits associated with memory consolidation (Murphy and Regan, 1999) in perinatally lead-exposed rats that were tested at an adult age. In addition, studies using antagonists of the NMDA receptor (Irifune et al., 1995) have identified strong interactions between the dopaminergic and glutamatergic systems in the regulation of motor activity in mammals.

Rats studied after periods of immobilization showed behavioral consequences of restraint stress in the elevated plus maze performance (Padovan and Guimarães, 2000). This is attributed to plastic changes in the central nervous system, due to induction of c-*fos* or c-*jun* gene in several brain regions, including the hippocampus (Lino de Oliveira et al., 1997). Research suggests that c-*fos* gene acts as a "third messenger," leading to alteration of target-gene expression related to some stress-induced, long-lasting changes in animal and human behavior (Post, 1992). Previous research suggests that exposure to lead may selectively interfere with critical developmental gene expression (Zawia and Harry, 1996).

In addition, the NMDA receptor seems to be involved in the induction of c-*fos* mRNA expression (Titze-de-Almeida et al., 1994). The NMDA receptor has been associated with neuronal development and plasticity. Data supplied by Giménez-Lhort et al. (1996) have suggested that the NMDA receptor could be a target mediating the motor effect elicited by polyamines (Giménez-Lhort et al., 1996). Polyamines (putrescine, spermidine, and spermine) are intimately involved in the orderly structuring of the central nervous system in the developing brain, and as intermediates in developmental neurotoxic events, play a role in behavior (Shaw, 1979; Bell and Slotkin, 1986). Polyamines, especially putrescine, are modified after brain insults and seem to be involved in events concerning both the production of brain damage and neuronal repair (De Vera et al., 1997).

Lead has been found to directly interfere in the polyamine metabolism by modulating the activity of the rate-limiting enzyme of the pathway via ornithine decarboxylase (Zawia et al., 1994). Alteration in polyamine-regulated macromolecular synthesis by lead may result in alterations in the cytoarchitectural development during critical stages of brain development (Adhami et al., 1996). Changes in physiological concentrations of polyamines by lead during the growth-spurt period may interfere with the migration and maturation of neuronal and glial cells resulting in a perturbed neurobehavioral consequences. Perhaps, at the level of the central nervous system, these can be some ways of transferring a lead neurotoxic sequel independent of lead presence.

Our observations confirm the paradigm that lead intoxication is more dangerous to the embryonic brain when the barrier sensitivity is lower and that a behavioral sequel provoked by lead exposure in early life can be observed in the adult, as well as in the absence of continuing lead exposure. It would be premature to offer an explanation on the transmission of behavioral effects of lead to F2 generation offspring. In spite of the F2 generation's showing altered behavior in open field, i.e., increased locomotor activity and increased rearing, they were attenuated (lower increases in locomotor activity and rearing). Does this indicate that a cellular mechanism of "conscience or memory" exists in the F2 generation, which manifests a neurobehavioral sequel produced in their mothers by a neurotoxic agent such as lead?

This is a small pilot study that needs to be confirmed and extended. We suggest that other studies, in terms of neurochemical effects on developmental lead exposure, should be extended for two or more offspring generations.

Although it is difficult to relate or transfer the results of animal experiments directly to man, the alterations shown in this study were observed in the absence of signs of overt

toxicity and with blood lead levels between 23 and 26 μg/dl in young rats, which are the same levels observed in children chronically exposed to lead during development (Davis et al., 1990).

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