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Role of early GnRH administration in sexual behavior disorders of rat pups perinatally exposed to lead

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Abstract

The effects of maternal exposure to lead (Pb) during the perinatal (1% and 0.1% Pb) periods of sexual brain differentiation were studied in adult male offspring. Maternal Pb levels were measured after treatment. Behavioral (open field and sexual behavior), physical (sexual maturation, body and organ weights), and biochemical (testosterone levels and hypothalamic monoamine and respective metabolite levels) data were assessed in perinatally exposed offspring. The effects of gonadrotopin-releasing hormone (GnRH) administration to pups at birth on puberty and sexual behavior were also investigated in offspring postnatally exposed to the metal. Results showed that perinatal administration of the two Pb concentrations did not modify maternal weight gain; 1% Pb exposure reduced offspring body weight during the 7 days of treatment while no changes were observed after 0.1% Pb exposure; neither Pb concentration altered offspring sexual maturation; the higher Pb concentration improved sexual behavior while the 0.1% concentration reduced it; exposure to 0.1% Pb caused decrease in testis weight, an increase in seminal vesicle weight and no changes in plasma testosterone levels; hypothalamic VMA levels were increased compared to the control group; GnRH administration reversed the effects of 0.1% Pb administration on male sexual behavior. These results show that perinatal exposure to Pb had a dose-dependent effect on the sexual behavior of rats and that a decrease in GnRH source in the offspring was probably involved in the reduction of their sexual performance.

Keywords: Lead; Sexual behavior; Perinate; GnRH; Rat

1. Introduction

Lead toxicity has been recognized since antiquity, and warnings about its neurotoxic properties have been issued throughout history. Early development appears to be a period of particular susceptibility to the toxic effects of lead (Pb). Years of lead use in gasoline and paints resulted in generalized environmental contamination. Today, Pb has been removed from these products but its neurotoxic effects persist as a public health problem. Pb exposure has been described as a developmental neurotoxicant [17,19,37,42], manifested by embryo/fetal death, malformations, growth impairment, and several functional deficits [7,16,17,25,58,60]. The disruptive effects of elevated lead levels on the functioning of the adult hypothalamic–pituitary–gonadal axis (HPG) are also well-established [62]. Present evidence indicates that lead exposure acts at both gonadal and hypothalamic sites to disrupt reproductive physiology and behavior [21,61,62]. The effects of lead on the developing HPG system have been studied and elevated lead levels have been consistently reported to alter the neurobehavioral development of the reproductive system. Der et al. [18] observed a delay in the onset of puberty and irregular estrous cycles in female rats treated with lead for 40 days beginning at 21 days of age. Other authors reported delays in vaginal opening in rats chronically exposed to lead during pregnancy and development [24]. Sokol and Berman [59] showed a decreased reproductive capacity of rats whose mothers were exposed during the third week of gestation due

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to a disruption of HPG function in adulthood. The overall pattern of the results obtained in these studies suggests that multiple levels of the HPG axis can be affected by exposure to Pb during the period of gestation when structures related to the HPG axis are undergoing rapid proliferation.

In the rat, the critical period for the organizational actions of gonadal hormones on sexual differentiation of the brain extends from the last week of prenatal life through the first postnatal week [39] and is thought to be initiated by the surge of plasma testosterone that occurs in the male fetus around embryonic days 17 and 18 (sensitization period). Several aspects of differentiation can be influenced during this period such as adult sensitivity to the activating effects of sex steroids on behaviors [8], morphological development of the sexually dimorphic nucleus of the preoptic area of the hypothalamus [27], and long-term regulation of gonadotropin secretion.

Hypothalamus masculinization is induced by estrogen derived from male pup testosterone during the postnatal period, mainly during the first 2 h after birth. Testosterone secretion, however, depends on gonadotropin, which in turn is stimulated by gonadrotopin-releasing hormone (GnRH). This hormone has been identified in milk of rats, cows, mares, and humans [2,5,26,32,33], and Smith and Ojeda [56] have shown that suckling transfers milk GnRH to the circulation in neonatal rats. Because the neonatal pituitary lacks the functional link from the hypothalamus [23], it was suggested that milk is the GnRH source during this period. In this respect, current evidence indicates that, in vitro, Pb inhibits noradrenaline-induced GnRH release in the median eminence, supporting the hypothesis that it is able to alter the dam's hypothalamus [11] as well as maternal GnRH release during the postnatal period.

The objective of the present study was to investigate the role of GnRH in Pb-induced reproductive disorders. In the first series of experiments, we examined if maternal perinatal exposure to low and high levels of Pb altered physical, behavioral, and biochemical parameters related to the sexual sphere of their offspring. Behavioral (sexual behavior), endocrine (testosterone levels), physical (sexual maturation, body and organ weights), and neurochemical (hypothalamic monoamine levels and their metabolites) data were assessed in the male offspring of Pb-treated dams. Second, the effects of GnRH administration on the day of birth on the sexual behavior of pups exposed to Pb through maternal milk were examined.

2. Methods

2.1. Animals

Male and female Wistar rats from our own colony, weighing 250–270 g and about 90 days of age were used. The animals were housed in polypropylene cages ($32 \times 40 \times 18$ cm) under controlled temperature (22–24°C), with a 12-L:12-D light schedule and free access to food and water. All tests were carried out during the 12-L period except for sexual behavior, which was observed during the 12-D light period. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Sexually naive female rats were mated with males previously tested as fertile (two females and one male in each cage). Pregnancy was determined by the vaginal smear test. Pregnant rats were removed and kept in separate cages.

2.2. Drugs

The Pb acetate concentrations employed were 0.1% or 1% dissolved in distilled water; 4 ml 1 N HCl was added to all bottles, including controls, to prevent the precipitation of insoluble lead salts. The bottles were examined and changed every 2 days to determine precipitation. Distilled water plus 4 ml 1 N HCl was used as control solution. The GnRH 50 μg (Wieth–Ayerst) was diluted in saline solution 0.9% and administered intraperitoneally.

2.3. Procedures

2.3.1. Measurement of blood lead levels

Blood was collected from the hepatic vein of the female rats on the seventh day of lactation and Pb levels were determined by mass spectrometry.

2.3.2. Open-field studies

The open-field apparatus was based on that described by Broadhurst [12]. The apparatus was a round wooden device 40 cm in diameter and 25 cm high for pups and 97 cm in diameter and 33 cm in height for adult animals, painted white and with the floor divided into 25 and 19 sectors, respectively. During the experiment, a 40-W white bulb located 74 cm above the floor provided continuous illumination of the field. Hand-operated counters and stopwatches were employed to score ambulation (number of floor units entered) and rearing frequency (number of times an animal stood on its hind legs). Male rats aged 21 and 75 days were placed individually in the center of the open-field arena and behavioral parameters were observed for 6 min. The open-field apparatus was then washed with 5% ethanol before introducing the next animal to preclude the possible cueing effects of odors left by previous subjects. To minimize possible influences of circadian changes on rat open-field behavior, control and experimental animals were alternated.

2.3.3. Sexual behavior studies

On PND100, control and experimental male rats were submitted to mating tests as described by Felicio et al. [22] and Chiavegatto et al. [14]. Briefly, animals were maintained under controlled temperature conditions on a

12-h inverted light–dark cycle (lights on at 10:00 p.m. for at least 21 days before the experiments). All sexual behavior tests were held 4 to 8 h after the beginning of the dark period. To investigate sexual behavior, male rats were allowed to mount ovariectomized untreated females sexually activated with exogenous estradiol (50 μg/kg sc, 54 h before the tests) and progesterone (2 mg/kg sc, 6 h before the tests). The following parameters of male sexual behavior were recorded: mounts, intromissions and ejaculatory latencies, number of mounts and intromissions, and postejaculatory mounts and intromission latencies after ejaculation. Sexual behavior was assessed by direct observation from the first mount to the first intromission after ejaculation.

2.3.4. Testes, seminal vesicle, and ductus deferens weights

On PND120, experimental and control adult animals had their testes, seminal vesicles and ductus deferens removed, separated from surrounding tissue and rinsed with 0.9% saline. The organs were blotted between two sheets of filter paper and their wet weight was determined. The organ weight/body weight ratio was calculated.

2.3.5. Determination of testosterone levels

On PND 120, control and experimental male rats were weighed and anesthesized with pentobarbital and blood was collected from the hepatic vein. Testosterone concentration was measured in plasma samples using Coat-a-count kits (Diagnostic Products, LA, CA, USA). Serum samples were assayed in duplicate and sensitivity to testosterone was found to be 0.01 ng/ml. The intra-assay variation was 0.4%.

2.3.6. Determination of hypothalamic monoamine and metabolite levels

On PND 120, control and experimental animals were decapitated and their brains were dissected on dry ice. Briefly, the hypothalamus was weighted and stored at −70°C.

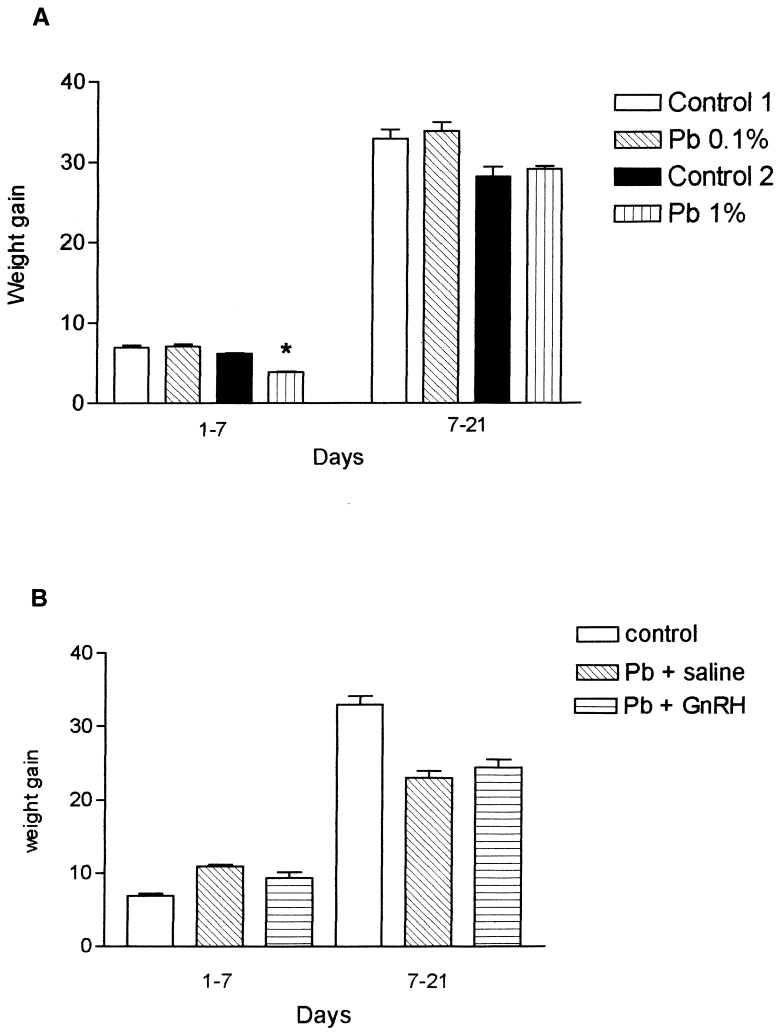

Fig. 1. Weight gain of pups treated with 0.1% and 1% Pb (A) and treated with 0.1% Pb plus GnRH (B) from PND1 to PND7 and from PND7 to PND21. Data are presented as means ± S.E.M. * $P < .05$% compared to control group (Student's t test). ( ) number of animals.

Following sample collection, perchloric acid was added to the tissues which were then homogenized by sonication 1 week before the neurochemical evaluations. Dopamine (DA) and its metabolites and 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE) and its metabolite vanilmandelic acid (VMA), and serotonin (5-HT), were measured by HPLC (Shimadsu, model 6A) using a C-1 column (Shimpak-ODS), an electrochemical detector (Shimadsu, model 6A), a sample injector with a valve for 20 μl, and an integrator (Shimadsu, model 6A Chromatopac). Dihydroxybenzamine (DHBA) was used as the internal standard. Each sample was run for 28 min. The detection limit was 2 pg for DA, DOPAC, NE, and 5-HT.

2.4. Experimental design

2.4.1. Experiment 1

Perinatal studies: Four groups of dams received Pb acetate 0.1% ($n=8$ females), 1% ($n=8$ females) Pb or control solution (Control 1, $n=8$ females; Control 2, $n=8$ females) from the last day of pregnancy (GD21) to the seventh day postpartum (PND7). Since experiments were performed at different times of the year, we used two control groups.

In the rat, the critical period for the organizational actions of gonadal hormones on sexual differentiation of the brain extends from the last week of prenatal life (GD17–18) through the first postnatal week. The rationale for choosing this schedule of Pb administration was to expose mothers and pups to the metal during the postnatal period, because its is only during this period that GnRH has effects on male differentiation; the treatment was initiated on the final day of pregnancy to assure sufficient Pb levels during the first 2 h after birth, a crucial period for brain hormone imprinting for brain masculinization [13]. The Pb treatment of mothers ended on PND7.

Maternal body weight was measured only from PND1 to PND7 to determine any maternal toxicity. Weight gain was calculated by the difference in body weight between these days.

On PND1, all litters were culled to eight pups, with four females and four males which were examined externally, sexed, and weighted. No cross-fostering was done. Male animals from each group was evaluated once a day for testis descent and also weighed on PND1, PND7, and PND21. All tests were carried out at the same time of day (9:00 to 11:00 a.m.). Although the unit analysis was the pups, at least one or two animals from each litter were used in these experiments. On PND21, the offspring were weaned and the littermates were separated and housed together by sex. From PND18 to PND28, male animals were evaluated once a day for testis descent. Animals from different litters were used for the experiments in adult age. Animals observed for physical development were also used to the neurochemical experiments. Rats observed in the open-field and in the sexual behavior tests were also employed to evaluations of the organs weight and testosterone levels. All female pups were used for another experiments of our laboratory.

2.4.2. Experiment 2

Effects of GnRH perinatal effects: pups from litters exposed to 0.1% Pb were injected intraperitoneally with 50 μg/g GnRH (15 pups from 4 litters) or saline solution (9 pups from 4 litters) at birth and observed for body weight, sexual maturation, and sexual behavior. Since experiments were performed at the same time of the neurobehavioral studies with the Pb 0.1% dose, we used the data from Control 1 to compare the GnRH effects.

2.4.3. Experiment 3

Other dams (15 females/5 per group) were mated and treated from the last day of pregnancy and up to the seventh day of lactation with the control solution, 0.1% Pb or 1% Pb or just distilled water plus 4 ml of 1 N HCl. The blood of these dams were used to evaluate the Pb blood levels.

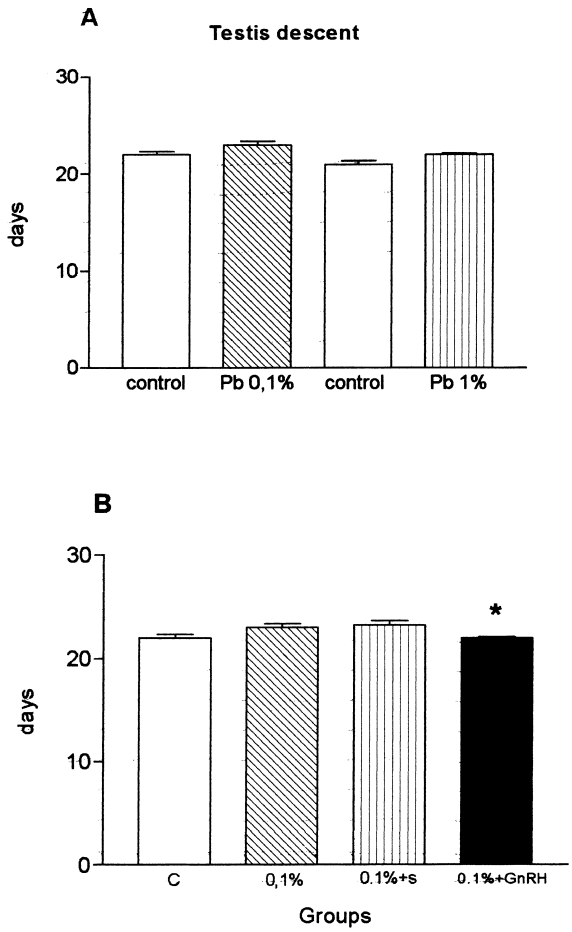

Fig. 2. Median day of testis descent of animals perinatally exposed to 0.1% and 1% Pb (A) and to 0.1% Pb plus GnRH (B). Data are presented as means ± S.E.M. $n=12–14$ animals.

2.5. Statistical analysis

Bartlett's test [28] was used to determine data homogeneity. The Student's *t* test [57] was used to compare open-field behavior, male sexual behavior (animals exposed to 0.1% Pb and 1% Pb and), plasma testosterone levels, body weight during adult age, testis, ductus deferens and seminal vesicle weights, and hypothalamic monoamine levels. ANOVA followed by the Tukey's or Dunnett's test was employed to analyze maternal and pups weight gain, sexual behavior (groups exposed to 0.1% Pb plus saline and to 0.1% Pb plus GnRH and controls), testis descent, and blood lead concentrations. In all cases, results were considered significant for $P < .05$.

3. Results

3.1. Maternal variables

3.1.1. Maternal weight gain

No group differences in weight gain were observed in control, 0.1% and 1% Pb-treated dams (Control Group $1 = 15.96 \pm 3.09$ g, $n = 8$; 0.1% Pb $= 19.40 \pm 6.71$ g, $n = 8$; 1% Pb $= 27.84 \pm 5.32$, $n = 8$), suggesting the absence of maternal toxicity [$F(2,21) = 1.353$, $P = .28$].

3.1.2. Maternal blood lead concentrations

Blood lead levels of control animals were 8.27 μ/dl (lower detection limit -7 μg/dl), indicating that the blood samples from these animals were not contaminated during the collection procedure. In all cases, the lead-treated groups had significantly higher blood lead levels than control animals [ANOVA, $F(2,12) = 17.61$, $P = .0003$]. Pb levels were 36.12 ± 9.49 μg/dl, $n = 5$, and 13.08 ± 9.42 μg/dl, $n = 5$, for dams exposed to 1% and 0.1% Pb, respectively. The Pb levels of the control group were 8.27 ± 3.16 μg/dl, $n = 5$. The Tukey–Kramer multiple comparisons test showed that the plasma lead levels of the three groups, exposed to 0.1 and 1% Pb and control groups were significantly different [$F(2,12) = 17.608$, $P = .0003$].

3.2. Litter variables

3.2.1. Litter weight gain

The weight gain of the offspring exposed to 0.1% Pb was not modified during lactation, whereas pups from dams treated with 1% Pb showed a decreased weight gain compared to the control group from PND1 to PND7 (Fig. 1A), but no further effect from PND7 to PND21. Additional comparisons between groups showed a difference between control and experimental groups from PND1 to PND7 [$F(3,31) = 3.188$, $P = .037$], with the weight gain of

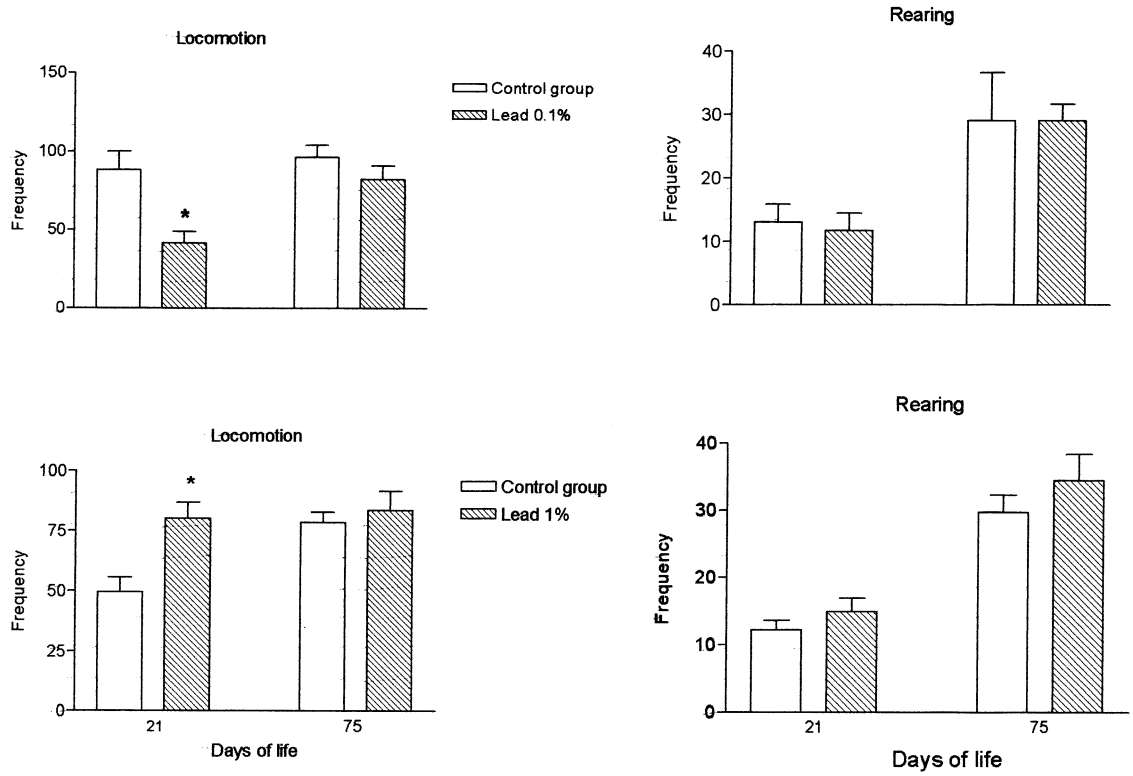

Fig. 3. Effects of 0.1% and 1% Pb on locomotion and rearing frequencies of rats observed in an open field at 21 and 75 days of age. Data are reported as the means ± S.E.M. for each parameter. * $P < .05$ compared to control group (Student's *t* test).

the 1% Pb group being reduced compared to control. No change in weight gain was observed from PND 7 to PND21 [$F(3,31) = 0.528$, $P = .662$].

Fig. 1B shows that the weight gain of animals exposed to 0.1% Pb and treated with GnRH was not modified during either observation period [PND1 to PND7 — $F(2,15) = 1.616$, $P = .231$; PND7 to PND21 — $F(2,15) = 1.560$, $P = .243$] compared to control and to animals treated with 0.1% Pb.

3.2.2. Sexual maturation

The time of testis descent was similar for controls and for the 0.1% Pb- and 1% Pb-treated groups [$F(3,31) = 0.447$, $P = .721$] (Fig. 2A).

Comparison of 0.1% Pb ($n = 9$) and Control 1 ($n = 8$) — both from Experiment 1, 0.1% Pb + saline ($n = 4$) and 0.1% Pb + GnRH ($n = 4$) groups in terms of the day of testis descent did not show differences between groups. Also, the ANOVA applied to the data for the Control 1, 0.1% Pb, 0.1% Pb + saline, and 0.1% Pb + GnRH groups did not show alteration in this parameter [$F(3,22) = 0.207$; $P = .890$] (Fig. 2B).

3.2.3. Open field

At 21 days of age, perinatal exposure to 0.1% Pb ($n = 10$) reduced locomotion frequency, and perinatal exposure to 1% Pb ($n = 10$) increased it compared to control ($n = 12$ animals/group), whereas rearing frequency was not modified by either dose. In contrast, in adult age (75 days of life) no differences were observed between locomotion and rearing frequencies of control and experimental rats (Fig. 3).

Table 1
Effects of perinatal exposure to lead on sexual behavior of male rats

	Groups			
Parameters	Control 1 (10)	0.1% Pb (8)	Control 2 (8)	1% Pb (9)
ML	0.36 ± 0.10	0.31 ± 0.06	0.44 ± 0.08	0.38 ± 0.12
IL	0.32 ± 0.28	0.31 ± 0.06	0.46 ± 0.10	0.38 ± 0.12
EL	8.84 ± 0.98	14.40 ± 2.60*	9.05 ± 1.56	7.53 ± 1.62
NM	18.60 ± 2.50	24.60 ± 1.80*	22.70 ± 3.50	15.6 ± 1.10*
NI	14.10 ± 1.60	18.70 ± 2.40	20.00 ± 3.10	14.1 ± 1.10
NMI	4.50 ± 0.90	5.90 ± 0.0	2.70 ± 3.10	1.50 ± 1.10
PML	5.05 ± 0.20	5.56 ± 0.33*	4.96 ± 0.44	4.10 ± 0.44
PIL	5.17 ± 0.19	5.74 ± 3.46	5.46 ± 0.19	4.58 ± 0.32*
FI/min	1.77 ± 0.37	2.72 ± 1.32	2.28 ± 0.64	1.86 ± 0.94

Animals were treated with 0.1% or 1% of lead administered in the drinking water from GD21 to 7 days of lactation. Sexual behavior was observed at 100 days of age. Data are presented as means ± S.D. Latencies are presented in minutes.

( ) = number of animals/group; ML = mount latency; IL = intromission latency; EL = ejaculation latency; NM = number of mounts; NI = number of intromissions; NMI = number of incomplete mounts; PML = postejaculatory mount latency; PIL = postejaculatory intromission latency; FI/min = frequency of intromissions/minutes.

* $P < .05$ compared to the respective control group (Student's t test).

Table 2
Effects of perinatal exposure to lead on sexual behavior of male rats

	Groups		
Parameters	Control 1 (10)	0.1% Pb + saline (9)	0.1% Pb + GnRH (15)
ML	0.36 ± 0.10	0.31 ± 0.20	0.35 ± 0.28
IL	0.32 ± 0.28	2.07 ± 3.75	0.44 ± 0.33
EL	8.84 ± 0.98	14.46 ± 7.80*	6.11 ± 2.07**
NM	18.60 ± 2.5	24.67 ± 5.55*	15.67 ± 5.38**
NI	14.10 ± 1.60	18.78 ± 7.24*	10.20 ± 4.60**
NIM	4.50 ± 0.90	5.89 ± 2.21	5.47 ± 0.78
PML	5.05 ± 0.20	3.76 ± 6.91	1.60 ± 8.40
PIL	5.17 ± 0.19	5.74 ± 1.04	5.73 ± 1.47
FI/min	1.77 ± 0.37	2.10 ± 1.21	1.83 ± 0.47

Animals were treated with 0.1% Pb administered in the drinking water from GD21 to 7 days of lactation. Sexual behavior was observed at 100 days of age. Data are presented as means ± S.D.

( ) = number of animals/group; ML = mount latency; IL = intromission latency; EL = ejaculation latency; NM = number of mounts; NI = number of intromissions; NMI = number of incomplete mounts; PML = postejaculatory mounts latency; PIL = postejaculatory intromission latency; FI/min = frequency of intromissions/minutes.

* $P < .05$ compared to the control group.

** $P < .05$ compared to the group treated with Pb plus saline. (ANOVA followed by Tukey's test).

3.2.4. Sexual behavior

Male rats perinatally exposed to 0.1% Pb ($n = 10$) exhibited reduced reproductive behavior compared to control ($n = 8$). A significant increase in latency to ejaculation, in the number of mounts, and in latency to postejaculatory mounts was observed in experimental males (Table 1). In contrast, rats exposed to 1% Pb ($n = 9$) showed an improvement in sexual behavior, i.e., a decrease in the number of mounts and in postejaculatory intromission (Table 1). No animal failed to exhibit sexual behavior.

Table 2 shows the sexual behavior parameters for control rats ($n = 8$) and for the 0.1% Pb + saline ($n = 9$) and 0.1% Pb + GnRH ($n = 10$) groups. GnRH treatment reversed the effects of 0.1% Pb treatment, with the data being similar to those for the control group. In addition, multiple comparison test showed that ejaculatory latency, the number of mounts, and the number of incomplete mounts were reduced in

Table 3
Plasma testosterone levels, body weight, testis, ductus deferens, and seminal vesicle wet weight of rats perinatally exposed or not to 0.1% lead

Parameters/groups	Control (8)	0.1% Pb (8)
Plasma testosterone levels (ng/l)	7.09 ± 2.17	12.39 ± 4.01
Body weight (g)	390.00 ± 39.72	425.71 ± 31.54
Testis wet weight/ body weight (g)	0.89 ± 0.06	0.79 ± 0.09*
Ductus deferens/ body weight (g)	0.22 ± 0.02	0.22 ± 0.05
Seminal vesicle/ body weight (g)	0.28 ± 0.05	0.32 ± 0.04*

Data are presented as means ± S.E.M. ( ) = number of animals/group.

* $P < .05$ compared to the control group (Student's t test).

Table 4
Hypothalamic monoamine levels in male rats treated with 0.1% lead acetate during the perinatal period

	Groups	
Monoamines/metabolite	Control (5)	0.1% Pb (5)
DA	334.66 ± 127.08	383.40 ± 146.85
DOPAC	88.65 ± 38.08	134.66 ± 40.72
NE	1751.30 ± 370.00	1841.00 ± 348.12
VMA	470.92 ± 19.37	588.77 ± 64.66*
5-HT	172.70 ± 73.97	199.17 ± 155.14
DOPAC/DA	0.27 ± 0.09	0.37 ± 0.09
VMA/NA	0.28 ± 0.05	0.32 ± 0.06

DA = dopamine; DOPAC = 3,4-dihydroxyphenylacetic acid; NE = norepinephrine; VMA = vanilmandelic acid; 5-HT = serotonin. Data are means ± S.D. ( ) = number of animals/group.

* $P < .05$ compared to the control group (Student's t test).

relation to the control group meaning an improvement in the sexual behavior. Data for the 0.1% Pb + saline group were closely similar to those for the 0.1% Pb group shown in Table 1.

3.2.5. Weight of the accessory sex organs

The testis wet weight/body weight ratio was reduced by perinatal 0.1% Pb exposure, whereas the seminal vesicle wet weight/body weight ratio was increased. No difference was observed in the ductus deferens wet weight/body weight ratio of experimental and control animals (Table 3).

3.2.6. Blood testosterone levels

Testosterone levels were not different between controls ($n = 8$) and 0.1% Pb ($n = 8$)-treated animals (Table 3).

3.2.7. Hypothalamic monoamine levels

Animals exposed to 0.1% Pb ($n = 8$) showed higher hypothalamic VMA levels than the control group ($n = 8$). There were no differences between groups in hypothalamic NA, DA, DOPAC, or 5HT levels, or in DOPAC/DA and VMA/NA ratios (Table 4).

4. Discussion

Taken together, the present results show a disruption of reproductive behavior and physiology by perinatal lead exposure, as previously shown for prenatal treatment [40]. In addition, these disturbances were dose-dependent. We also present evidence indicating that a low lead dose acted via milk GnRH inhibition during the postnatal period, inducing a long-term organizational effect on brain sexual differentiation in the offspring, while the higher dose (1%) had an excitatory effect.

Dams were treated during the perinatal period by exposure to 1% or 0.1% lead concentrations in drinking water from the last day of pregnancy to the seventh day of lactation. The circulating blood Pb levels were 36.12 μg/l in animals exposed to 1% Pb, 13.08 μg/l in animals exposed to 0.1% Pb, and 8.27 μg/l in controls.

Although the blood lead levels in the present study were higher than observed in humans, no significant effects on growth were detected either in dams or fetuses, probably because rodents are well known to be more resistant than humans to a wide range of toxins. In fact, female rats exposed from conception to weaning to 0.5 mM (0.02%), 2.0 mM (0.04%), and 4.0 mM (0.15%) of lead showed blood levels of 14.5, 46.1, and 70.8 μg/dl, respectively, and their offspring had weaning blood levels similar to those of their mothers. This regimen did not affect body weight gain of dams or offspring development and had no effect on cerebral weight or on hematological parameters of 23-day-old rats. The behavioral alterations of the offspring, i.e., a decreased habituation in the open field as well as a significant reduction in the footshock escape latency in a shuttle avoidance task, were observed with lead blood levels in the 11–50.6-μg/dl range [46].

The maternal blood lead levels observed in the present investigation had low toxicity since no effects were detected on dam weight gain and pups showed a decreased weight gain only during treatment with the higher dose. All animals appeared healthy and none of them showed signs of toxicity. In addition, the present results indicate that exposure to the two Pb concentrations did not induce maternal toxicity. These findings are important since undernutrition during pregnancy and/or lactation, as well as alterations in maternal behavior, often result in differences in physical development [29,55].

The weight gain of Pb-exposed offspring was decreased in a dose-dependent manner compared to control in the group exposed to the higher Pb concentration, while perinatal exposure to the lower concentration did not modify offspring weight gain.

The decreased weight gain after exposure to the higher Pb concentration is an expected toxic effect and might be related to an endocrine effect of Pb. Several lines of evidence have shown that prenatal exposure to Pb inhibits growth hormone secretion in human beings. There is a positive correlation between low birth weight and reduced height at puberty [3,53]. On the other hand, data showing lack of effects on offspring body weight after the low dose are in agreement with the literature. In fact, Lasley and Lane [36] and Roussouw et al. [50] showed that exposure to 0.2% Pb in the drinking water from parturition to weaning did not modify the offspring body weight.

Perinatal lead exposure as well as GnRH treatment did not alter the puberty of rats evaluated on the basis of the day of testis descent. Since sexual maturation in the male rat results from complex interactions of the hypothalamus, anterior pituitary, testes, and secondary sex organs [32,56], the lack of perinatal Pb effects on the day of testis descent suggests that the metal was not able to alter the processes involved in the initiation of puberty or that the Pb level

during the period of sexual maturation was too low to induce these alterations.

Increased locomotion frequency was observed at weaning after 1% Pb exposure, while the 0.1% Pb concentration reduced this parameter in relation to the respective control group. No differences were detected in the rearing frequencies for the groups tested. In adult age, neither locomotion nor rearing frequencies observed in the open field were modified by Pb exposure.

The open-field test was originally proposed as a measure of emotionality, with the animals being exposed to high levels of light and of noise. In our laboratory, we propose the open-field test as measure of motor activity in a situation of low light levels and withdrawal from environmental noises. In children the effects of lead on the central nervous system (CNS) have been associated with attention deficit disorder with hyperactivity, impaired cognitive function, and other CNS dysfunctions such as auditory and visual system deficits and motor function disturbances [10,15,45]. In experimental studies, behavioral effects associated with Pb exposure have been shown in a wide range of Pb exposure regimens. Spontaneous activity in Pb-exposed animals is one of the aspects of lead effects on neurobehavioral function most extensively studied. These determinations have also produced contradictory results, with reports of hyperactivity, hypoactivity, or no changes in activity related to lead exposure [4,6,24,43,44,46]. Rodrigues et al. [46] associated hyperactivity in the open field after perinatal Pb exposure with cognitive impairment as shown by failure of habituation to the open field, deficit in learning retention in a shuttle box, and an apparent impairment of footshock escape acquisition in a shuttle box.

Our results show a dose-dependent effect of Pb on open-field behavior of pups at 21 days of age. These results are probably related to the degree of neurotoxicity induced by the metal. Since no alterations in open-field parameters were observed during adult age, we assume that lead produced toxicity in young rats by a direct effect on the CNS.

Lead is a reproductive toxicant for males [9,35,49,63]. Several lines of evidence indicate that lead acts at both gonadal and hypothalamic sites to disrupt reproductive physiology and behavior [21,61,62].

The present results also show that Pb exposure induced a dose-dependent effect on male offspring sexual behavior. The lower dose decreased sexual performance while the higher dose improved it. These facts might reflect different degrees of delayed lead toxicity. In fact, clinical evidence suggests that the facilitation of sexual behavior observed in animal research represents an "ejaculatio praecox" while an inhibition of this behavior is thought to be a signal of impotence [52]. Both effects might impair several aspects of sexual performance and, consequently, animal and human reproduction.

Animals exposed to 1% Pb showed a decreased total number of mounts, with no changes in total number of incomplete mounts and decreased latency to the first intromission after ejaculation. These effects might reflect increased potency and motivation for sexual behavior. In addition, a motor interference cannot be ruled out since increased locomotion frequency in the open field was observed at 21 days of age, possibly reflecting subtle changes in motor aspects during adulthood. Thus, maternal exposure to a high Pb concentration induced maternal toxicity causing the offspring to present decreased body weight, high levels of motor activity at weaning, and improved sexual behavior during adult age. These results were primarily descriptive, and the nature of the finding presented here needs to be further investigated. However, it is known that lead exposure results in dopaminergic alterations, which in turn improves sexual behavior activity.

In contrast, exposure to 0.1% Pb disrupted sexual behavior. Animals showed both reduced potency and motivation. Furthermore, a motor interference cannot be ruled out since decreased locomotion frequency in the open field was observed at 21 days of age.

Perinatal administration of Pb may disrupt sexual behavior by two mechanisms. First, by a reduction in the release of newborn testosterone during the postnatal critical period since Pb has been reported to decrease this hormone [30,48]. Long-term exposure to Pb induces testicular atrophy in adult animals [1] and continuous exposure to this metal during animal development decreases serum testosterone [47,48]. Presently, although testosterone levels were not decreased during adulthood after exposure to 0.1% Pb, testis weight was reduced suggesting the presence of testicular atrophy. Moreover, Ronis et al. [47] showed that Pb exposure from the fifth day of pregnancy to weaning reduces testosterone levels from puberty on. No differences in testosterone levels were observed between control and experimental animals during adulthood.

A second mechanism may be interference with the content of bioactive substances in mother's milk [31]. During the postnatal critical period for male brain differentiation, milk GnRH of early lactation is thought to play an important role in this process in the rat [51]. Carlos et al. [13] showed that pups deprived of early lactation milk (ELM) exhibited, as adults, reduced fertility, decreased weight of ductus deferens and seminal vesicle, reduced levels of fructose in the seminal vesicle and prostate gland, as well as altered sexual performance. Thus, the sex disruptive effects caused by perinatal exposure to Pb in male rats might have been due to interference with the content of bioactive substances in milk. Since Pb inhibits noradrenaline-induced GnRH release in the median eminence in vitro [11], the present data support the hypothesis that Pb also acts on the hypothalamus in vivo.

In the present study, sexual behavior and testis weight were reduced in the adult offspring of 0.1% Pb-treated dams. These findings are consistent with a disruption of the androgenic milieu during the perinatal period resulting in hypothalamic hypoandrogenization. In addition, a reduction

in androgen or estrogen levels during this period has also been shown to reduce the volume of the sexual dimorphic nucleus of the medial preoptic area in adult males, as observed for certain hypothalamic areas of homosexual men.

Previous studies of developmental lead exposure have sometimes found evidence of nonlinear Pb effects. Similar facts were here observed since it was observed an improvement in general activity and sexual behavior after prenatal 1% Pb exposure while 0.1% Pb exposure reduced both behaviors. So, it is possible that different physiological mechanisms may be involved depending on the level of Pb exposure.

Norepinephrine (NE) innervation to the hypothalamus releases GnRH via prostaglandin-2, which can be blocked by Pb exposure [11]. In the present study, we observed an increased activity of this amine, since NE levels remained unchanged and the levels of its metabolite, VMA, were increased. Malmnas [38] and McIntosh and Barfield [41] showed that the inhibition of NE synthesis by FLA-63 disrupts male sexual behavior, while Kwong et al. [34] reported that male sexual behavior was improved by increased NE activity. The higher levels of NE activity observed here are in accordance with a demasculinizing effect of Pb since it has been observed that hypothalamic NE levels are higher in females than in males. In addition, Donoso et al. [20] showed that castration of male rats produced an increase in NE in the anterior hypothalamus.

In an attempt to clarify our hypothesis about the effects of Pb on GnRH sexual differentiation in rat pups, the offspring of dams exposed to 0.1% Pb was treated with GnRH during the first 2 h after birth. No change in pup weight gain was observed during lactation; in relation to testis descent, no interference was produced by GnRH administration.

The sexual behavior of animals treated with 0.1% Pb plus GnRH was very similar to that of control animals, and all alterations induced by perinatal exposure to Pb were reversed. Thus, we conclude that the reproductive effects of perinatal exposure to 0.1% Pb were due, at least in part, to a decrease in GnRH in mother's milk, which is responsible for androgenization of the hypothalamus.

In summary, the above data indicate that perinatal exposure to Pb during the second critical period of male brain sexual differentiation has long-term effects on the reproductive physiology and behavior of male rats. These data provide information about the underlying mechanism by which the lower metal concentration influenced male sexual differentiation, possibly involving changes in the HPG system by altering the composition of the mother's milk. However, the stimulatory effects of exposure to 1% Pb remain to be investigated.

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