

Effect of lead acetate on neurobehavioral development of rats

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Abstract

We investigated the effects of lead exposure during the pre- and postnatal period on the neurobehavioral development of female Wistar rats (70-75 days of age, 120-150 g) using a protocol of lead intoxication that does not affect weight gain. Wistar rats were submitted to lead acetate intoxication by giving their dams 1.0 mM lead acetate. Control dams received deionized water. Growth and neuromotor development were assessed by monitoring daily the following parameters in 20 litters: body weight, ear unfolding, incisor eruption, eye opening, righting, palmar grasp, negative geotaxis, cliff avoidance and startle reflex. Spontaneous alternation was assessed on postnatal day 17 using a T maze. The animals' ability to equilibrate on a beaker rim was measured on postnatal day 19. Lead intoxication was confirmed by measuring renal, hepatic and cerebral lead concentration in dams and litters. Lead treatment hastened the day of appearance of the following parameters: eye opening (control: 13.5 ± 0.6 , N = 88; lead: 12.9 ± 0.6 , N = 72; $P < 0.05$), startle reflex (control: 13.0 ± 0.8 , N = 88; lead: 12.0 ± 0.7 , N = 72; $P < 0.05$) and negative geotaxis. On the other hand, spontaneous alternation performance was hindered in lead-exposed animals (control: 37.6 ± 19.7 ; lead: $57.5 \pm 28.3\%$ of alternating animals; $P < 0.05$). These results suggest that lead exposure without concomitant undernutrition alters rat development, affecting specific subsets of motor skills.

Key words

- Lead
- Neurobehavioral development
- Undernutrition
- Heavy metal intoxication
- Teratology
- Rat

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Introduction

Lead is among the more common toxic metals present in our environment. The primary site of action of lead is the central nervous system, and exposure to the metal is associated with several neurobehavioral and psychological alterations (1-4). Neurochemical alterations induced by lead include age-specific changes in the level of binding of

glutamatergic and cholinergic ligands (5-7), competition with Ca^{2+} and direct stimulation of calmodulin (8), reduced effects of depolarization on Ca_i^{2+} levels (9), and increased liperoxidative damage in brain (10).

The developmental toxicity of lead has become a significant area of research since children are much more sensitive than adults to learning impairment following low-level lead exposure (11). Regarding this point, animal

and human studies have reached similar results, since behavioral effects induced by lead are seen in rats at approximately the same blood levels that cause deficits in humans (3,12,13).

Administration of diets with a high lead content to rodents during suckling causes growth impairment, hematological alterations, breakdown of the blood-brain barrier, and higher pup mortality (14,15). Moreover, significant effects on neuromotor development of rats have also been described. Reiter and colleagues (16) reported delay in development of righting reflex and eye opening in animals exposed to lead acetate in the drinking water through gestation and lactation. These data were confirmed by Luthman and colleagues (17), who described delayed eye opening and impairment in negative geotaxis test performance during postnatal *ip* lead acetate treatment. Grant and colleagues (18) reported significant delays in vaginal opening in female pups and in the development of surface- and free-fall-righting reflexes. Similarly, Livesey and colleagues (19) reported that animals exposed to lead from birth to 20 days of age presented retardation in the development of free-fall righting and acoustic startle reflexes. However, a statistically significant effect of lead treatment on body weight was evidenced in these studies. Since it is well known that undernutrition is causally related to neurobehavioral development retardation (20), it becomes difficult to attribute these deficits solely to lead exposure.

Studies using milder forms of lead intoxication have revealed no differences in weight gain, but significant effects on adult rat behavior (21,22), confirming the view of a deleterious role for lead. However, developmental studies without concomitant undernutrition are still lacking in the literature. Therefore, in the present study we investigated the effects of chronic lead exposure, starting at a prenatal age, on the neurobehavioral development of rats using a protocol of lead intoxication that does not affect weight gain (21).

Material and Methods

Animals were submitted to prenatal and postnatal lead acetate intoxication as previously described (21). Briefly, female Wistar rats (70-75 days of age, 120-150 g) from our breeding stock were given 1.0 mM lead acetate or distilled and deionized water as the sole drinking fluid. Rats were maintained on a natural light cycle in an air-conditioned, constant-temperature colony room. Each cage contained three rats which had free access to lab chow and water or lead acetate solution. After 4 weeks of treatment, rats were placed in breeding groups of three virgin females and one male in an opaque plastic cage (50 x 25 x 18 cm). Mating lasted 21 days, and pregnant rats were selected and placed in individual cages containing wood shavings as nesting material. Twenty litters were used, eleven of them from females treated with water and nine from females treated with lead acetate. On the day of birth (day 0), the litters were culled to eight pups (4 males and 4 females, whenever possible) and the pups examined for the occurrence of gross malformations. Animals were weighed at 0, 5, 10, 15, 20 and 30 days after delivery.

Reflex tests and observations of the maturation of physical characteristics were carried out daily at the appropriate ages by one experimenter that was not aware of the subject treatment. Testing was carried out between 10:00 and 12:30, and progress of the same individuals from each litter was followed throughout the experiment. The date of appearance of ear unfolding, eruption of upper incisors and eye opening was recorded using previous criteria (20,23-26). The reflexes assessed were righting, palmar grasp, negative geotaxis, cliff avoidance, free-fall righting and auditory startle, as described in detail by Smart and Dobbing (20). Briefly, righting was scored by measuring the time required for the animal to turn ventrally after being placed in a dorsal position from postnatal day 1 to 5; palmar grasp was evaluated

by stroking gently the palm of a forepaw with a paper clip and observing the digital flexing response. The date of disappearance of the flexing response was recorded. Negative geotaxis was scored as follows: the rat was placed head downwards on a 20° slope, and scored 0, 1, 2, or 3 if it turned to face up the slope 0°, 45°, 90°, or 135°, respectively, in a 90-s session; cliff avoidance was assessed by placing the animal on the edge of a bench, with nose and forepaws just over the edge. The postnatal day the animal moved away from the edge in three consecutive 30-s trials was recorded. Free-fall righting was recorded as the day the animal turned in mid-air to land ventrally after being dropped, back downwards, from a 35-cm height onto a cotton wool pad in three consecutive trials, and auditory startle was recorded as the day the animal responded with a sudden and brief extension of the hind limbs to the sound of a mouse trap snapping.

On postnatal day 17, animals were tested for spontaneous alternation. The apparatus was a T maze (a 35-cm base and two arms each 25 cm long). Latency to enter one arm of the maze and arm entered were recorded. The animals were tested in consecutive trials (intertrial interval, 20 s) until they entered the contralateral arm, with a maximum of 5 trials. When the latency to enter one of the sides was longer than 300 s, the animal was excluded from subsequent testing (23). Percent of failure to alternate was also calculated for each litter and compared between groups. On day 19, the ability to balance and move along the rim of a 2-liter beaker was tested, as described by Smart and Dobbing (20). Latency to escape from the rim and percent of animals that fell from the rim were calculated and compared between groups.

Cerebral, hepatic, and renal lead content were evaluated in some females before mating and in the pups after delivery in order to assess lead intoxication. Kidneys, livers and brains were selected at random, and samples were accurately weighed and placed in a

plastic container with 5 ml of nitric acid (27). The mixture was allowed to stand overnight to prevent a too vigorous reaction during microwave heating (28). Thereafter the containers were placed in a domestic microwave oven (Consul, MU31A), where samples were submitted to the following microwave program: 1) four times at 50% power for 5 s; 2) three times at 50% power for 10 s; 3) twice at 100% power for 5 s (29). After digestion, vessel content was filtered and diluted to 25 ml (final volume) in a calibrated flask with deionized water.

A Perkin-Elmer 3030-B atomic absorption spectrometer equipped with an HGA-400 graphite furnace and an AS-10 autosampler was used to determine lead at the 283.3-nm resonance line (spectral bandwidth 0.7 nm) under gas stop and maximum power conditions. A hollow lead cathode lamp operating at 10 mA was used as the light source. A standard continuous background corrector (deuterium arc bulb) was used. Pyrolytic graphite tubes with L'vov platforms were also used (30). The ashing and atomization temperatures used were 900 and 1800, respectively. A solution of palladium and magnesium was used as a chemical modifier. Measurements were made using an integrated area mode (31).

The data from the date of appearance of each reflex (except for palmar grasp, for which the day of disappearance was recorded) and physical features were analyzed by 2 (Pb or water) x 2 (sex) ANOVA using the mean litter score. Negative geotaxis data were analyzed by 2 (Pb or water) x 2 (sex) x 10 (sessions) ANOVA with the session factor treated as within subject factor using the mean litter score. Righting reflex time was analyzed as done for negative geotaxis scores except that the number of sessions was five. Body weight data were analyzed by 2 (Pb or water) x 2 (sex) x 6 (measures) ANOVA with the measure factor treated as within subject factor using the mean litter weight. Latency for the first trial in the spontaneous

alternation test and latency to rim escape were analyzed by 2 (Pb or water) x 2 (sex) ANOVA using the mean litter score. The percent of animals in each litter that failed to alternate in the spontaneous alternation test and that fell from the rim in the beaker test were analyzed by 2 (Pb or water) x 2 (sex) ANOVA. Bartlett's test for homogeneity of variances was run before ANOVA. Cerebral, hepatic and renal lead content data were analyzed by the F-test. F values are presented only if $P < 0.05$.

Results

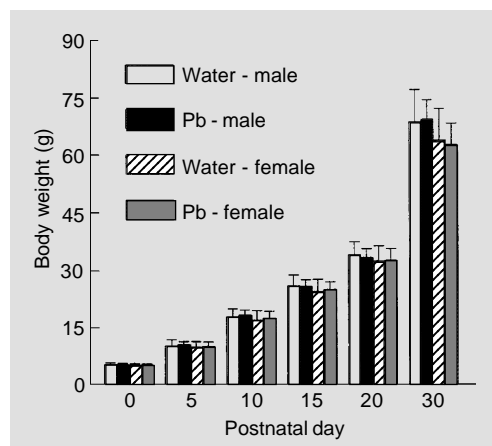
Statistical analysis of data concerning renal, cerebral and hepatic lead content in

Table 1 - Renal, cerebral and hepatic lead content of female rats before mating and of pups after delivery.

* $P < 0.05$ compared to respective control (F values are presented in Results). Results are reported as means (ppm) \pm SEM, and the number of animals is given in parentheses.

	Control	Lead acetate
Females		
Kidney	0.39 \pm 0.06 (5)	1.84 \pm 0.49* (4)
Liver	0.88 \pm 0.13 (5)	2.43 \pm 0.92* (4)
Brain	0.036 \pm 0.006 (5)	0.039 \pm 0.004 (4)
Pups		
Kidney	0.52 \pm 0.30 (6)	0.55 \pm 0.16 (6)
Liver	0.82 \pm 0.58 (6)	1.22 \pm 0.12* (6)
Brain	0.059 \pm 0.019 (6)	0.075 \pm 0.066 (6)

Figure 1 - Effect of lead acetate on body weight gain of male and female pups. Data are reported as means \pm SEM of 11 control (water; 42 males and 46 females) and 9 lead acetate-treated litters (Pb; 38 males and 34 females). ANOVA revealed a significant sex by measure interaction [$F(5,190) = 3.31$; $P < 0.01$], indicating that males exhibit a different weight gain curve compared to females.



females before mating revealed that animals which had received 1 mM lead acetate in the drinking water presented higher levels of lead in liver [$F(1,7) = 14.2$; $P < 0.005$] and kidney [$F(1,7) = 48.6$; $P < 0.001$], but no difference was observed in the brain (Table 1). These results indicate that dams were already intoxicated at mating. Statistical analysis of data about renal, cerebral and hepatic lead content in pups at delivery revealed only an increase of hepatic lead content [$F(1,10) = 5.55$; $P < 0.05$] (Table 1).

Number of living pups and body weight at birth were similar between groups (data not shown). Lead administration caused no effect on the body weight of the pups. Only a significant sex by measure interaction was observed [$F(5,190) = 3.31$; $P < 0.01$], suggesting that males exhibit a different weight gain curve compared to females (Figure 1). These results confirm previous studies showing that this protocol of lead intoxication does not affect weight gain (21,22). Statistical analysis of other parameters of physical development revealed that lead treatment caused accelerated eye opening [$F(1,18) = 4.4$; $P < 0.05$]. No difference between groups was observed in the date of ear unfolding or incisor eruption (Table 2).

Statistical analysis of the negative geotaxis data revealed a significant treatment (deionized water or lead acetate) by days interaction [$F(9,180) = 3.11$; $P < 0.005$], because lead-treated animals presented higher scores and reached a maximal negative geotaxis score before control animals (Figure 2). Statistical analysis also revealed a main effect of treatment for free-fall righting [$F(1,18) = 7.18$; $P < 0.05$] and auditory startle [$F(1,18) = 7.97$; $P < 0.05$], indicating that lead administration accelerated the appearance of such reflexes. No significant differences between groups were observed in date of appearance of palmar grasp or cliff avoidance (Table 2) or in time required for righting from a dorsal position during the first five days of life (data not shown). Analysis of beaker rim escape

latencies revealed no differences between groups in the latency to reach the platform or in the litter percent of falls from the beaker rim (data not shown). Finally, analysis of spontaneous alternation test data revealed a higher percentage of non-alternating animals in the Ac₂Pb litters (37.6 ± 19.7 for controls against 57.5 ± 28.3 for the Ac₂Pb group - $F(1,18) = 3.86$; $P < 0.05$).

Discussion

In the present study we examined the effect of chronic lead intoxication, initiated before conception, on the development of different behavioral and physical parameters in rats. As previously reported and confirmed in the present study, the addition of 1 mM lead acetate to the drinking water caused no effect on the weight of pups at delivery (21) or on litter size. These results are in agreement with previous reports from our group, which also demonstrated that brain lead levels are similar to those observed in control animals at birth (22). Furthermore, lead acetate administration had no effect on litter weight gain, and therefore undernutrition, a common confounding variable in teratology studies, can be ruled out as a causative agent of the changes observed.

Analysis of other physical development parameters revealed that lead-treated animals presented accelerated eye opening, as also observed in animals subjected to prenatal methylmercury exposure without concomitant undernutrition (32), indicating a teratogenic role for lead acetate. Similarly, the startle reflex appeared earlier in lead-treated animals. In contrast to our study, Luthman and colleagues (17) and Reiter and colleagues (16) reported a delayed eye opening in rats exposed to lead, and Livesey and colleagues (19) found no alteration in this parameter. A common finding reported in these studies was weight gain deficits in lead-treated animals.

Further evidence for a teratogenic role of

lead acetate comes from the developmental analysis of different behavioral parameters. Although presenting brain lead levels comparable to the control group at birth, lead-treated animals presented increased scores in the negative geotaxis test. This result also agrees with those reported by Vorhees (32) who studied the effects of prenatal methylmercury intoxication on development without concomitant weight reduction, and are corroborated by the presently observed earlier date of appearance of the free-fall righting reflex. Our results contrast with previous observations by Bull and colleagues (33) and Grant and colleagues (18) who showed that lead-treated animals present a delay in

Table 2 - Postnatal day of appearance of some physical parameters and reflexes.

Palmar grasp is scored as the postnatal day of disappearance of the reflex. Data are reported as mean litter scores (\pm SEM) of 11 control and 9 Ac₂Pb litters. * $P < 0.05$ compared to respective control (F values are presented in Results).

	Postnatal day	
	Control	Lead acetate
Physical parameters		
Ear unfolding	3.0 \pm 0.7	2.9 \pm 0.2
Incisor eruption	9.5 \pm 1.0	9.4 \pm 1.4
Eye opening	13.5 \pm 0.6	12.9 \pm 0.6*
Reflexes		
Cliff avoidance	4.6 \pm 0.9	4.1 \pm 0.9
Palmar grasp	8.1 \pm 1.9	7.6 \pm 1.6
Startle	13.0 \pm 0.8	12.0 \pm 0.7*
Free-fall righting	14.0 \pm 1.4	12.1 \pm 1.6*

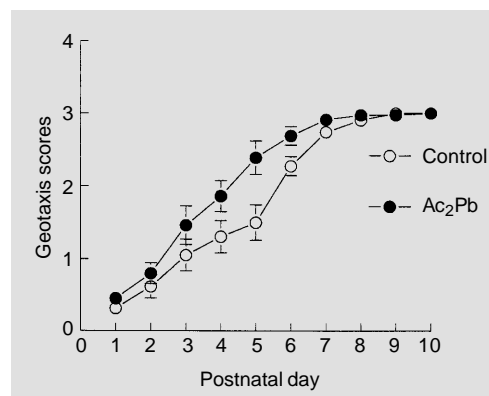


Figure 2 - Effect of lead acetate on the negative geotaxis scores. Data are reported as means \pm SEM of 11 control (42 males and 46 females) and 9 Ac₂Pb litters (38 males and 34 females). F values are presented in Results.

motor development, including free-fall righting reflex deficits (18,19). However, those studies also reported concomitant body weight gain losses. Similar contradictory results were also observed in animals exposed to methylmercury, since Rocha and colleagues (34) demonstrated that methylmercury-intoxicated animals (which presented weight gain losses) showed impaired performance in the negative geotaxis test, contrasting with data obtained by Vorhees (32). These differences among results may be attributed to different metals, onset, duration and method of imposing heavy metal intoxication, and alterations in maternal behavior, but we think that concomitant undernutrition may be a critical confounding variable in these studies.

It is interesting to point out that two of the behavioral parameters hastened by lead treatment depend on motor responses (negative geotaxis and free-fall righting reflexes). Therefore, it is reasonable to expect that hyperactivity might contribute, at least in part, to the better performance of lead-treated rats in these tasks. However, if hyperactivity were present, one should also expect its influence on other behavioral parameters that depend on motor responses, such as cliff avoidance and beaker equilibration task. These behavioral responses were not affected by lead treatment and do not corroborate the hypothesis that hyperactivity might be involved in this effect. Since spontaneous locomotor activity was not assessed in the present study, the contribution of an increased locomotor response to these results will continue to be speculative.

It is well known that the appearance of the startle reflex coincides with ear channel opening (24-26), and that hearing neuronal circuitry is already developed at this age (35,36). Therefore, earlier ear channel opening is a possible explanation for the earlier occurrence of the startle reflex observed here.

Lead-treated animals present deficits in acquisition of a radial eight arm maze task,

performing like hippocampally damaged rats (37). Furthermore, neonatally lead-intoxicated animals present behavioral deficits in the spontaneous alternation test during adulthood (38). In the present study we demonstrated that this deficit occurs at a young age in animals exposed to lead. These results are in agreement with the view that lead treatment causes alterations in hippocampal function (37-41).

In summary, the present study demonstrated that lead acetate intoxication caused significant alterations in the development of rats. The majority of the observed alterations pointed to an advance in the day of appearance of some reflexes, and were in contrast to the deficit observed in the spontaneous alternation test. Similar contradictory advancing and retarding or deleterious effects on rat and hamster development have been demonstrated by others (26,32) using different treatments, and now there seems to be a consensus that any alteration (regardless of direction) in animal development must be considered deleterious (32,42). Therefore, the lead-induced alterations in rat development observed here are important in terms of searching for similar developmental alterations in human beings exposed to low levels of lead, and reinforce the importance of identifying concomitant undernutrition as a confounding variable in experimental teratology studies. Moreover, since subclinical undernutrition may cause biochemical and behavioral alterations in humans, it is highly desirable to evaluate such a condition in animals by measuring other indicators of nutritional status, particularly in studies dealing with chronic administration of neurotoxicants.

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