Pharmacology Biochemistry & Behavior, Vol. 11, pp. 95–98. Printed in the U.S.A.

Prenatal and Postnatal Chronic Lead Intoxication and Running Wheel Activity in the Rat[1]

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Received 5 December 1978

VERLANGIERI, A. J. *Prenatal and postnatal chronic lead intoxication and running wheel activity in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(1) 95–98, 1979.—Prenatal and postnatal chronic lead intoxicaton was induced in Wistar rats with 0.1 mg/ml $PbCl_2$ in the drinking water of pregnant and lactating females. Offspring were supplied lead water at weaning. Exposure totaled 14 months. Activity was measured in the Wahman LC-33 wheel. Running activity in experimental and control rats was maximized with a 23-hr light cycle followed by a 5-hr dark period in the wheels. There was no significant difference in body weight gains or lead intake (as measured by water consumption) between any sex-matched group. Lead was found to produce overall hypoactivity in both males and females. Under auditory stress the hypoactivity in the males was normalized by combination of a hypoactivity component in the controls and a progressive hyperactivity in the lead animals. This study clearly shows a sex-dependent hypoactivity effect of chronic lead intoxication and suggests its mechanism as an interaction between lead and environmental stress.

Prenatal Postnatal Chronic lead intoxication Running wheel activity Hypoactivity Hyperactivity Environmental stress Auditory stimuli Interaction Sex-dependent effects

KNOWLEDGE of the effects of acute doses of lead on the central nervous system has caused speculation as to the ability of lesser, chronic doses of lead to produce behavioral abnormalities. Young children, whose developing central nervous systems are sensitive to the neurotoxic effects of lead, are believed to be at particularly high risk.

A study [7] of 210 institutionalized children with nonclassified mental retardation revealed that the incidence of elevated blood-lead levels was 45%, while in the 80 normal controls, the incidence was 2.5%. In a recent study [2] of the water-lead levels in the homes of retarded children, the authors concluded that lead contamination of water may be one factor in the multifactorial aetiology of mental retardation. It has been shown [6] that age-adjusted performance IQ differences between children with blood-lead levels of 40–68 μg/100 ml and controls with less than 40 μg/100 ml that no differences were found in full scale IQ, verbal IQ, behavior and hyperactivity ratings. Neurological dysfunction and motor impairment in children with an increased lead burden has been observed [8]. Hyperactive children have been shown to have significantly higher blood-lead levels and postpenicillamine urine-lead excretion than non-hyperactive children [3].

While several of these investigations suggest effects of subclinical levels of lead, absolute correlation of cause and effect is lacking. Variables such as socioeconomic status, health, nutrition, emotional status and intellectual potential are extremely difficult to control, much less determine, and may modify a particular response to and during chronic lead exposure. With these facts in mind, one must consider the animal model as a possible alternative. While generalizing from comparative animal studies is frequently difficult, animal investigations may provide a basis for understanding the role of chronic lead exposure in producing specific behavioral abnormalities.

Rats continuously exposed to lead acetate solutions and tested on a visual discrimination problem in the open field and in two shuttle avoidance test situations, produced a lower acquisition of the visual discrimination and lowered activity in the open field [4] relative to non-exposed rats. Chronic lead intoxication in rats previously trained on an operant discrimination, increased the variability of discrimination behavior [9]. Performance deficits in lead-treated rats were demonstrated under: (1) bar press responding for food, (2) two choice discriminations under negative reinforcement and (3) emotional responses in the open field [1]. Chronic ingestion of lead acetate, supplied through the drinking water, caused a behavioral disorder in mice [10]. Increased motor activity on a grid was found at 40–60 days for mice exposed to lead from birth. Pups started on a daily dose of lead acetate in the drinking water at age two weeks had depressed motor activity in a gridded room at age 9 weeks.

[1]Presented in part at the 62nd Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, NJ, April 1978.

At 19 weeks, the dogs were retested in the gridded activity room, and the earlier hypoactivity had normalized [11]. Lead-treated rats were more active in a Y-maze and tilt box, but not in running wheels [5].

The present long term study was initiated to investigate the effect of chronic pre- and postnatal lead on activity in the running wheel with maximization of circadian activity and external environmental stress.

METHOD

Twenty-four female Wistar rats (200–225 g) were housed in stainless steel metabolism cages with deionized water and feed supplied ad lib. Room temperature was maintained at 18 ± 0.5°C. The test animals were acclimated to this regime for 14 days.

After 14 days the rats were divided into two groups of twelve. The experimental group (Pb) began receiving 0.1 mg/ml $PbCl_2$ in the drinking water ad lib, while the control group (R) received no lead in the drinking water.

After two weeks the experimental group and control group were provided non-lead exposed mates. During the mating phase all animals were placed on non-lead water to prevent exposure of the males. Following impregnation (presence of sperm in the vagina) the females were separated from the males and again placed on lead-water or control non-lead water regime.

Upon parturition, offspring in the experimental group received lead indirectly through nursing and in time through the water supply before removal from lactation cages. At 28 days of age, experimental and control weanlings (total of 242) were separated according to sex and 6 of each sex were placed randomly into each of four groups: (1) lead males, (2) non-lead males, (3) lead females and (4) non-lead females. The animals were placed in metabolism cages and the lead groups continued to receive the stock lead containing drinking water and the non-lead groups distilled water.

At this time the photoperiod of the animal room was changed from a 12 hr light/dark cycle to a 23:5 hr light/dark cycle in the home animal room. The animals were then placed in running wheels on alternate days for 5 hr in the dark.

Activity was measured in the Wahman LC-33 cage in a separate animal room with sound proofing. During running the appropriate water supply was available but no feed. On alternate running days the same animal never used the same wheel in order to compensate for wheel placement in the room and differences in wheel torque and wheel counters. Daily water consumption in the home cages and running wheels was measured to determine daily lead intake.

Since lead is believed to affect the central nervous system it may be modified by environmental stress, auditory stress was used at variable times during the study. Dual automotive horns sounded at random intervals and provided the auditory stress on alternate months of the study (see Table 1).

TABLE 1

AUDITORY STRESS* SCHEDULE DURING ACTIVITY PERIOD†

Time Interval	Mode
0–6	off
6–8	on
8–9	off
9–11	on
11–56	off
56–76	on
76–79	off
9–100	on
18.5 Min	off

*112.3 DBA; 112.5 DBC; average at 18″ cage placement.
†15 cycles repeated per 5-hour activity.

TABLE 2

AVERAGE RUNNING WHEEL ACTIVITY IN MALES

	No Pb	Pb	Difference	L.S.D.
No stress	432	294	*−138(−31.9%)	32
Stress	355	316	*−39(−10.9%)	32
Difference	*−77(−17.8%)	22		
L.S.D.	32	32		

Difference expressed relative to No Pb or No Stress. L.S.D. = Least Significant Difference Expected at $p<0.05$.
*Significantly different.

TABLE 3

AVERAGE RUNNING WHEEL ACTIVITY IN FEMALES

	No Pb	Pb	Difference	L.S.D.
No stress	1720	1345	*−375(−21.8%)	110
Stress	1824	1321	*−503(−27.6%)	110
Difference	*+104(+6.0%)	−24		
L.S.D.	102	102		

Differences expressed relative to No Pb or No Stress. L.S.D. = Least Significant Difference Expected at $p<0.05$.
*Significantly different.

RESULTS AND DISCUSSION

Water consumption measurements showed no statistical differences in water consumption in both female groups or male groups (Fig. 1). A higher male consumption is noted generally. Lead intake, as measured by water consumption, was not significantly different in any of the groups. Cumulative lead intake averaged approximately 1000 mg $PbCl_2$ per animal per 50 weeks (20 mg/week). These data confirm the lead had no direct effect on palatability of the water supply.

Body weight gains (Figs. 2 and 3) showed no significant differences between lead and non-lead females or lead and non-lead males. Lead exposed animals utilized feed as efficiently as non-lead animals.

From these intake data it is reasonable to assume the experimental animals were in the same physiological status as the controls, exhibiting no untoward reactions of acute lead intoxication. General nutrition and hydration were the same in all animals and there was no indication of lead anemia.

As shown in Table 2, stress significantly reduced activity in non-lead males 17.8%, but had no effect in those males exposed to lead. However, lead alone reduced activity in males 31.9% relative to non-lead males. Lead and stress re-

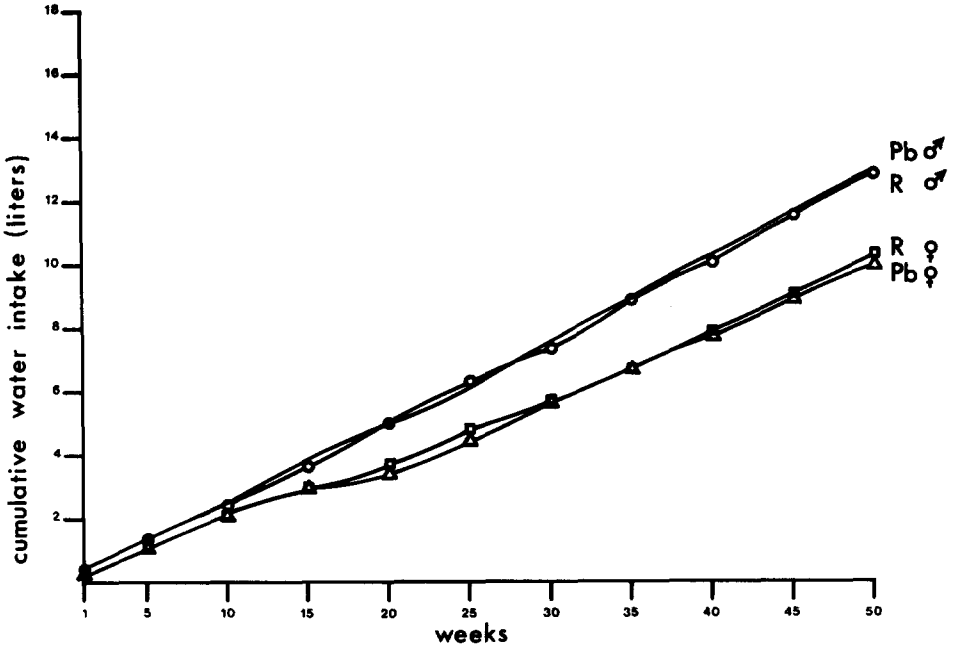

FIG. 1. Cumulative water consumption for male and female rats.

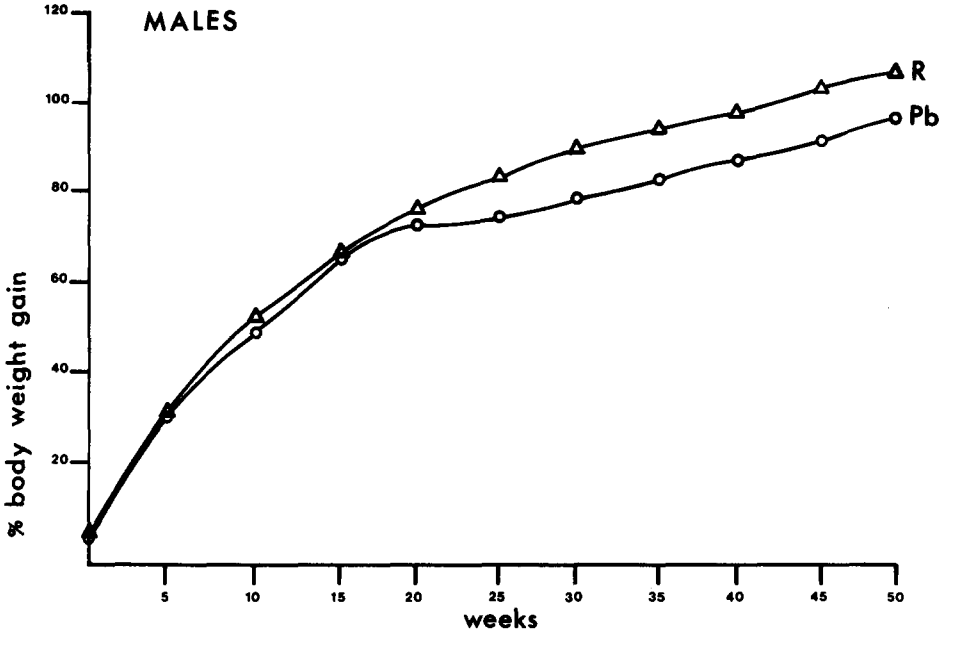

FIG. 2. Male growth profile.

duced activity only 10.9% relative to lead non-stressed animals. It appears stress is acting to normalize the lead hypoactivity. The component of this normalization is a hypoactive effect in the non-lead stressed animal relative to the lead stressed animal. In fact the lead group was not significantly effected by stress. As shown in Table 3, stress significantly increased activity in non-lead females 6.0% but had no effect in the females exposed to lead. Lead with no stress reduced activity 21.8% and stress further reduced the activity to 27.6% relative to lead non-stressed females. It is interesting to note that this comparison in males shows a normalization of hypoactivity while in the females an increase in the hypoactivity occurs. It is apparent lead intoxicated males and females do not react the same to stress, and the data show a definite interaction between environmental stress and chronic lead intoxication. It is apparent in the males that lead causes a substantial hypoactivity under no stress, and the hypoactivity is modified upward with the in-

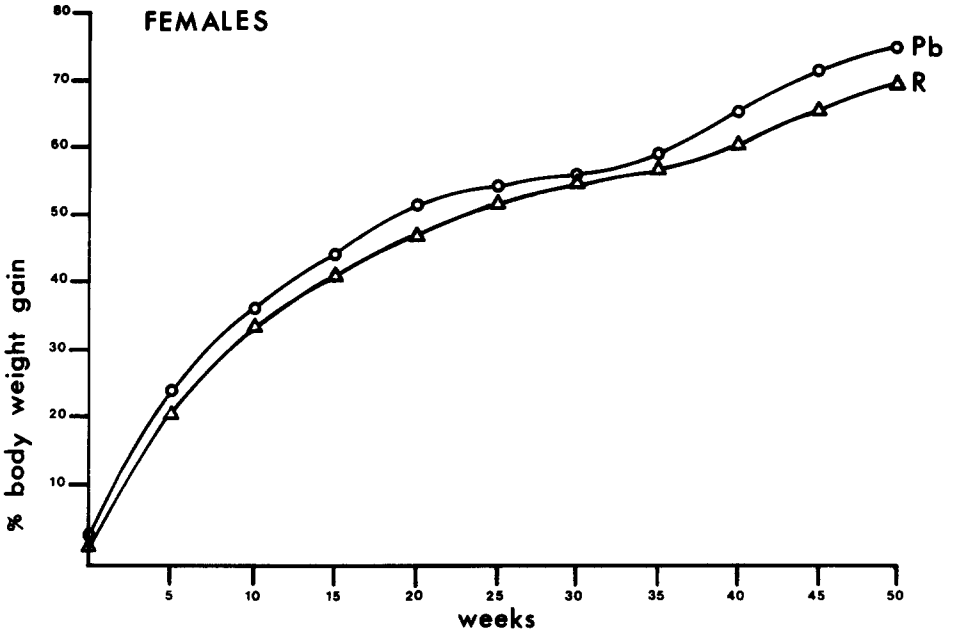

FIG. 3. Female growth profile.

troduction of stress. In this study activity approached to within 10.9% of the no stress condition and might be expected to exceed this level into hyperactivity if the study was run longer. This may actually occur under specific situations and may in part explain the hyperactivity postulates of chronic lead intoxication. This analysis may not apply to the females because they were frankly hypoactive in all comparisons in this study.

It is apparent then, from this study that lead has an overall hypoactive effect on running wheel activity in the rat under the test conditions used. The data further show that the lead intoxicated rats do not respond to auditory stress as the control rats do. The biological mechanism of these observations on running wheel activity require further study, but may in part be due to de-sensitization to stress or stimuli. The normalization of activity in non-lead males and lead males, with and without stress may be caused by a hypoactivity component without stress and a progressive hyperactivity component in the presence of stress. This study may also serve to identify a hyperactivity component not sorted out previously and attributed to lead alone rather than lead in combination with non-specific environmental or experimental stress or stimuli. These findings may well only apply to running wheel activity data collected within the design of the study. However, it appears clear that the observed effect is real and may be only one effect in many that will ultimately be determined with different experimental models or methods.

ACKNOWLEDGEMENTS

Appreciation is expressed to Mr. Jay Nelson for the every day routine necessary for the conduct of this study. The author would also like to thank Dr. William Swallow for the expert statistical analysis of the rather complex data. Paper of the Journal Series, Project 620, of the New Jersey Agricultural Experimental Station, Cook College, Rutgers University, The State University of New Jersey, New Brunswick, NJ. Supported in part by a Biological Sciences Support Grant of Rutgers University.

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