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Learning Deficits in Lead-Injected Rats[1]

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SNOWDON, C. T. *Learning deficits in lead-injected rats.* PHARMAC. BIOCHEM. BEHAV. 1(6)599–603, 1973. --Weanling and adult rats injected with one of three concentrations of lead acetate for 37 days failed to demonstrate any learning impairments as measured by a Hebb-Williams maze series relative to water injected controls. Rats at the highest dose level showed clear symptoms of lead poisoning. Pregnant females injected during pregnancy with an asymptomatic dose of lead acetate showed a 100% abortion rate, while 75% of water injected controls delivered litters. Rats whose mothers were injected with asymptomatic doses of lead acetate throughout nursing developed more slowly, weighed less, and demonstrated learning deficits relative to controls. The behavioral and physiological effects of lead may be greatest during the earliest developmental stages.

Learning deficits Prenatal Postnatal

THE USE of laboratory animals, such as the rat, as experimental models for the behavioral effects of lead poisoning has been limited by the failure to find evidence in animals of the intellectual impairment commonly found among young human victims of lead poisoning [6, 14]. The previous experimental studies with rats have used either injections of 1.5 mg/100 g body weight of tetraethyl lead for an eight day period in adult female rats [5] or 10 mg/100 g body weight of lead acetate for a three or four day period in weanling male rats [4]. Both experiments used a water escape maze as the means of evaluating learning deficits and both failed to find differences between lead injected and control animals, even though some of their animals displayed symptoms of lead poisoning.

The use of smaller doses of lead administered over a longer time course might better approximate the chronic lead poisoning of young humans and the water escape maze might not prove to be the most sensitive indicator of learning deficits. Indeed, Davenport and Dorcey [8] have reported that in experiments evaluating the effects of thiouracil injections in rats, only the Hebb-Williams maze series out of nine behavioral measures of learning used detected learning impairments in the treated animals. In the work to be reported here the use of a prolonged series of small doses of lead injection and use of a Hebb-Williams maze series was expected to better elucidate learning deficits in lead injected animals. In addition we thought it valuable to examine the effects of lead exposure at each of four developmental time periods: prenatally, during nursing, post weaning and adulthood.

EXPERIMENT 1: EFFECTS OF LEAD ACETATE INJECTIONS ON LEARNING IN WEANLING AND ADULT RATS

Method

Animals. The animals were 56 adult and 56 weanling rats obtained from the Sprague-Dawley Colony in Madison, Wisconsin.

Apparatus. The apparatus used was a semi-automated version of the Hebb-Williams maze modified from the symmetrical maze designed by Davenport *et al.* [7]. The maze consisted of a 76 cm sq. field enclosed by wooden walls 7.5 cm high. Start–goal alleys, which were 42.5 cm long, extended from the field at diagonally opposite ends. Wooden barriers (7.5 cm high and 1.9 cm thick) of varying lengths divided the field into symmetrical maze patterns. A bolt imbedded in one edge of the barrier was inserted through the expanded aluminum flooring and fastened to hold the barrier in place. All wooden surfaces were painted flat black.

Lehigh Valley pellet feeders dispensed one 45 mg Noyes pellet per trial into a shallow aluminum dish at the far end of each start–goal alley. A galvanized steel plate electrically isolated from the floor of the alley was placed in front of each feeding dish. This was wired to a contact detection circuit [see 12]. When an animal made contact with this plate, pneumatic doors closed and an intertrial timer was started. At the end of the 10 sec ITI the doors were opened and a pellet dispensed to the opposite goal box. Observers trained in the scoring system of Davenport *et al.* [7] scored the animal's errors and recorded total errors and time to the

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nearest sec after each trial. The animals were presented with 4 practice problems, one each day, and then each of the twelve test problems of Davenport *et al.* one each day. Animals were tested until they reached a criterion of 4 out of 5 errorless trials or until they ran 48 trials, whichever came first.

Procedure. On arrival in the laboratory the animals were randomly assigned to one of 4 injection groups: Distilled water, 0.5, 0.8, or 1.2 mg of lead acetate per 100 g body weight intraperitoneal injections were begun on the day of entry into the laboratory and continued for 21 days, when testing was started, and through the subsequent 16 days of testing - a total of 37 days of injections. In the weanlings injections were begun at 22 days of age, with testing beginning on the 43rd day. The adults weighed a mean of 260 g at the start of injections and were approximately 100 days of age. Each group contained 14 animals, 7 males and 7 females.

During the initial 21 days in the laboratory the adult rats were slowly deprived to 85% body weight. The weanling rats were not deprived to a specific body weight percentage but were placed on a one hour per day feeding schedule starting four days before the onset of testing. Following the last day of testing each rat was placed into a metabolism cage and 24 hr urine samples were collected under mineral oil. All samples were immediately pipetted into glass vials and frozen, and were subsequently analyzed for the amount of delta-aminolevulinic acid (ALA) after the method of Davis [10,11]. Urinary ALA levels have been reported to correlate well with the levels of blood lead and thus to provide an estimate of metabolically active body lead. There has been a dispute over the precision of this test [2,3,9,20], but it appeared to be an adequate gross estimate of the presence of body lead, even though it may not always correlate exactly with the actual body lead content.

Results and Discussion

The animals which received the highest doses of lead acetate (1.2 mg/100 g/day) showed signs of lead poisoning. Only 8 out of 14 adults and 10 of 14 weanlings survived to complete the entire test series. Most of the animals in this group developed a hunched posture which elevated the ventral surface of the body above the cage floor. They were extremely sensitive to the handling of the abdomen and to intraperitoneal injections. Those animals which died showed a severe ataxia and weight loss in the days preceding death. These symptoms were seen only among animals receiving 1.2 mg injections and not among the other injection groups. The animals which died were excluded from the analysis of the learning data.

Despite the successful production of symptoms with high doses and the use of a more sensitive learning situation – the closed-field maze series, there were no significant differences in learning. Table 1 presents the means of all groups on trials to criterion, total number of errors, seconds per trial, and urinary ALA values. The only measure to reach significance was an increased urinary ALA level with lead injections ($F = 4.72$, $df = 3/46$, $p<0.01$, adults; $F = 3.65$, $df = 3/48$, $p<0.025$, weanlings).

In order to examine the possibility that there might be retention deficits despite the lack of differences in initial learning, 34 of the weanling rats were retested on the maze problems six weeks after the completion of the first series. The animals were distributed in conditions as follows: 10 were in the distilled water group; 9 each in the groups with 0.5 and 0.8 mg of lead acetate/100 g injections, and 6 with

TABLE 1

MEASURES OF LEARNING AND BODY LEAD IN ADULT AND WEANLING RATS INJECTED WITH VARIOUS DOSES OF LEAD ACETATE (MEAN ± S.E.M.)

Measure	Injection Condition (mg/100g) 0.0	0.5	0.8	1.2
		ADULTS		
Mean trials to criterion	230.9 ± 9.8	238.1 ± 11.9	219.0 ± 10.3	237.1 ± 11.6
Mean total errors	333.6 ± 17.3	331.4 ± 21.1	311.3 ± 17.6	332.6 ± 23.5
Mean sec/trial	14.3 ± 1.1	16.6 ± 1.6	15.6 ± 0.8	18.7 ± 2.0
Mean urinary ALA (mg %)	0.25 ± 0.07	1.92* ± 0.62	2.04‡ ± 0.37	2.69‡ ± 0.58
N	14	14	14	8
		WEANLINGS		
Mean trials to criterion	237.3 ± 7.9	231.3 ± 13.9	220.1 ± 11.6	256.0 ± 11.1
Mean total errors	337.9 ± 17.0	343.2 ± 28.7	313.1 ± 89.9	371.8 ± 25.2
Mean sec/trial	13.8 ± 0.9	14.5 ± 1.4	15.1 ± 2.2	16.7 ± 2.7
Mean urinary ALA (mg %)	0.40 ± 0.08	2.98‡ ± 0.90	3.69‡ ± 1.02	3.66* ± 1.19
N	14	14	14	10

*Significantly different from controls, $p<0.02$, t-test ‡$p<0.01$ ‡$p<0.001$

1.2 mg/100 g injections. No animal received injections following the completion of the first maze learning series. Analyses of the savings scores indicated that there were no differences between groups in retention (t's< 1.18 for trials to criterion, df's = 14 17, p's<0.20; t's< 1.53 for total errors, df's = 14–17, p's>0.10). Thus neither symptomatic nor asymptomatic doses of lead administered chronically had an effect on the learning ability of adult and weanling rats, and similarly did not affect the retention ability of weanling rats when tested on the closed field maze series.

Thus the results of this study parallel those of Brown *et al.* [4] and Bullock *et al.* [5] that lead injected into weanling or adult rats failed to produce a learning impairment despite the fact that symptoms of poisoning could be produced. The superiority of the closed-field maze series to other measurements of learning found by Davenport and Dorcey [8] did not serve to demonstrate a deficit here.

Since lead poisoning seems to affect younger children more than older children and adults, possibly the failure to find learning deficits with lead injections both in the present study and in the others reported [4,5] was because exposure to lead was administered after weaning. In order to evaluate this hypothesis a further experiment was undertaken to examine the effects of prenatal and preweaning lead exposure.

EXPERIMENT 2: EFFECTS OF LEAD INJECTIONS ON PREGNANT AND LACTATING FEMALES ON THE LEARNING ABILITY OF THEIR OFFSPRING

Method

Animals. The animals were 33 female Holtzman rats obtained from the supplier on the first day that a sperm plug was present. It was necessary to change the strain of rats used at this point since the Sprague-Dawley Company would not determine pregnancy in their rats with any reasonable precision.

Procedure. The pregnant females were divided into two groups: 17 received daily injections of 0.8 mg of lead acetate/100 g body weight and 16 received equal volumes of distilled water. Injections were begun on the day of arrival into the laboratory and were continued daily for 21 days. The dose of 0.8 mg/100 g was selected since it was the highest dose used previously which had produced no overt symptoms of poisoning or other visible pathological changes. Twelve females which were injected with distilled water during pregnancy were divided into two groups at partuition. Five mothers were injected with the same dose of distilled water throughout the 21 days past partuition; the other seven received doses of 0.8 mg/100 g of lead acetate during this period. Twenty-six of the offspring of the water injected mothers and 36 of the offspring of lead injected mothers were tested on the Hebb-Williams maze problems with practice sessions beginning at Day 43 of age. At the end of maze testing these animals were placed in a metabolism cage, with urine collected under mineral oil. The urine sample was pipetted into glass vials, frozen immediately, and subsequently analyzed for the presence of delta-aminolevulinic acid levels as before [10,11].

Apparatus. The apparatus used for the closed-field maze testing of subjects in this experiment was a modification of that used in Experiment 1. A mosaic floor made of 176 electrically isolated plates was constructed with each plate running to a contact of a MAC panel. A variety of plug boards were wired to activate the plates that marked the entries to erroneous pathways for each of the twelve test problems. These in turn were wired to a contact-sensitive amplifier which recorded on a printing counter the number of errors made on each trial. A separate amplifier system using contact sensitive plates in the goal boxes recorded the running time for each trial. Thus the closed-field maze series was completely automated. No human observer was needed and thus no human bias could be introduced into the scoring as might have been possible in Experiment 1. A complete description of the maze, the amplifier cricuitry and validation of the automated scoring system can be found in [12]. The testing procedure was identical to Experiment 1. Four practice problems were given one each day and then the twelve test problems of Davenport *et al.* [7] also one each day. One 45 mg Noyes pellet served as reinforcement. The same criterion of 4 of 5 errorless trials or 48 trials was used.

The automated version of the maze does produce an increased number of errors relative to hand scoring, but the trial to trial correlations between observer and machine scoring are above 0.80 for most problems, and the animal to animal correlations are above 0.90. Thus the automated maze is measuring the same performance and the same differences between animals despite the higher incidence of errors.

Results and Discussion

Effect of lead injections during pregnancy. None of the 17 females which were injected with 0.8 mg of lead acetate/100 g/day delivered any offspring. In contrast 12 of the 16 animals injected with distilled water delivered with a mean litter size of 8.6 pups. Several days after delivery was due several of the lead injected females were sacrificed and their uteri examined. In most there were signs of fetal resorbtion. The lead injected females demonstrated no overt signs of poisoning. They did not show the typical large weight gain of pregnancy, but they did gain a mean of 12.0 g over the course in injections, a weight gain consistent with that of non-pregnant females over a similar period. Thus a dose of lead that failed to produce any signs of symptoms in adult and weanling rats previously, that had no obvious effect on the inseminated females was sufficient to induce a 100% abortion rate.

There are reports that lead oxides were widely used in England as abortifacients in the late 1800's and early 1900's [c.f. 1], and the one study that has compared pregnant women factory worker exposed to lead versus those not exposed to lead found a decreased success of pregnancy among the lead exposed women [1]. Thus the data from inseminated rats agrees with human data – that levels of lead exposure which apparently have little or no effect on the mother can have a devastating effect on the fetuses.

Postnatal lead injection. The lead injections appeared to produce no symptoms in the nursing mothers. The mean weight gain for the lead injected females was 13.3 g over the 21 days of injections, while the weight gain of the water injected females was 18.0 g. This difference in weight was not significant (U = 11, n = 5/7, p = 0.172). However, all pups of lead injected mothers showed signs of developmental retardation. They were consistently 1–2 days slower in the time of eye opening, and their mean body weight at weaning was 64.5% that of the water injected offspring. This agrees with the finding of reduced body weight and body size found in mice [17].

The summary of the results from the learning study is

TABLE 2

MEASURES OF LEARNING AND BODY LEAD IN RATS WHOSE MOTHERS WERE INJECTED WITH DISTILLED WATER OR 0.8 mg LEAD ACETATE/100 g/DAY (MEAN ± S.E.M.)

Measures	Controls	Lead Exposed
Mean trials to criterion	235.7 ± 9.23	260.2 ± 16.55
Mean total errors	450.8 ± 25.65	529.2* ± 27.85
Mean sec/trial	17.1 ± 0.7	16.2 ± 1.96
Mean Urinary ALA (mg %)	0.17 ± 0.05	0.29* ± 0.05
N	26	36

*Significantly different from control value, $p<0.05$

presented in Table 2. The analyses of the results indicated that rats whose mothers were injected with lead acetate during nursing made significantly more errors than did offspring of water injected mothers (Mean = 529.2 error-lead exposed; 450.8-controls, $t = 2.00$, $df = 60$, $p = 0.05$). While lead exposed animals did take more trials to reach criterion, this difference barely missed reaching significance (Mean = 260.2 trials - lead exposed; 235.7 trials - controls, $t = 1.63$, $df = 60$, $0.10<p<0.05$). Comparison of running times per trial indicated that there were no differences between the animals (Mean = 16.18 sec/trial - lead exposed; 17.11 sec/trial - controls, $t = 0.388$, $df = 60$, $p>0.20$). Thus the increased number of errors made by offspring of lead injected mothers was not due to a motor deficit or a reduced level of motivation for food reward which might have been induced by lead exposure. Urinary ALA determinations made at the end of testing indicated barely significant levels of ALA in the treated rats, but a significant difference relative to controls ($t = 2.17$, $df = 60$, $p<0.05$).These ALA levels are, however, considerably lower than those found immediately following lead injections in Experiment 1.

The present study did not address the question of whether and how the lead injected into the mother operated to produce a learning disorder in the offspring. However, in several other studies in which lead was administered to nursing females evidence of neuropathology in the offspring of both rats [16, 18] and mice [17] was found, and delta-aminolevulinic acid activity has been observed in suckling rats whose mothers were exposed to lead [15]. Thus it seems likely that lead is transferred by maternal milk to the nursing offspring and that lead does act to produce nervous system pathologies. However, in these studies the amount of lead to which the mothers were exposed was considerably greater than in the present study. In several of these investigations [16, 18] the animals were exposed to lead by adding 4% lead acetate to the maternal diet. Assuming that a nursing female ingests approximately 12.5 g of chow per day this represents an intake of 200 mg/100 g/day. Assuming the figures available from studies of human lead ingestion [13, 19], approximately 4 10% of ingested lead will be absorbed into the body. This would represent an absorbtion of 8 20 mg/100 g per day of lead. This is a range that is 10 25 times the amount that the nursing females were administered here. Thus relatively small amounts of lead that produce no symptoms in female rats, can when administered during nursing produce offspring which have retarded growth and development, and which evidence learning impairments when tested three to five weeks after weaning.

The work presented here shows that symptoms of lead poisoning can be produced in weanling and adult rats without producing any obvious behavioral or learning impairments. On the other hand asymptomatic doses of lead presented at earlier developmental stages have a profound effect on the offspring without producing any obvious symptomatology in the mothers. Asymptomatic doses of lead produced a 100% abortion rate in sperm positive pregnant females and produced animals with retarded development, reduced growth, and learning impairments in the offspring of treated lactating females.

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