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Influence of Parental Lead Exposure on Subsequent Learning Ability of Offspring

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BRADY, K., Y. HERRERA AND H. ZENICK. *Influence of parental lead exposure on subsequent learning ability of offspring.* PHARMAC. BIOCHEM. BEHAV. 3(4) 561–565, 1975. – This study was designed to assess the learning ability of rat offspring following the exposure of one or both parents to lead acetate (Pb) from 30–90 days of age. At that time, parents were mated to yield four groups: Group Pb-Pb, both parents had received Pb; Group Pb-N, only the mother had received Pb; Group N-Pb, only the father had received Pb; and Group N-N, the control parents. Mothers were continued on their respective treatments throughout gestation and nursing. Testing of offspring began at 30 days of age, employing a black-white discrimination water T maze. Analysis of results revealed that the three Pb groups made more errors than the controls, but did not differ from one another. However, offspring in Group Pb-Pb had longer swimming times than those in Groups Pb-N and N-Pb, who, in turn, had longer swimming times than Group N-N. Thus dual parental exposure was more severe than single parental exposure, which, however, still exerted a detrimental effect compared to control performance.

Lead   Parental exposure   Learning ability in offspring

ALTHOUGH the detrimental effects of lead (Pb) exposure during development have been confirmed in a number of studies [6, 7, 8, 9, 10], behavioral examinations of Pb poisoning have yielded conflicting results. Except for Bullock, *et al.* [4] all of the behavioral studies have used inorganic Pb. In the Bullock study, [4] tetraethyl Pb, injected i.p. into rats for eight days, did not affect their ability to learn a water T maze, although the treated animals' swimming times were slower during initial trials. Brown, *et al.* [3] is the only study in which administration of an inorganic Pb, Pb acetate, had no significant effect on rats on the learning or memory of a water T maze or on the acquisition of an active avoidance response. However, this study also reported impaired swimming ability in Pb animals.

In contrast to the above studies, Brown [2] found decreased learning ability in a T maze in 8–10 wk old rats nursed three wks by mothers receiving various daily dosages of Pb acetate since partuition. Van Gelder *et al.* [16] reported that lambs of ewes exposed to high levels of Pb during gestation required significantly more days to learn a two-choice discrimination task. Furthermore, disruption of conditioned avoidance responses has been reported in goldfish [17] and adult rats and rabbits [5] exposed to Pb.

In all of the developmental-behavioral studies, only the maternal influence on subsequent offspring behavior has been examined by administering Pb during gestation and/or nursing. Furthermore, administration has been always initiated at the start of these periods, unlike the more natural setting in which parental exposure, via environment or industry, may occur over an extended period of time prior to mating. Only one study in fact, has examined the effect on reproductive ability and progeny of exposing only one or both parents to Pb [14]. Stowe and Goyer [14] found that either paternal-only or maternal-only exposure resulted in a reduction of the number of pups/litter, a reduction in mean pup birth weight, and a reduction in pup survival. These effects were magnified however in offspring whose parents both had been exposed to Pb. Stowe and Goyer [14] concluded that the parental contributions could be either gametotoxic and/or extrauterine-intrauterine in nature.

The present study was designed similarly to that of Stowe and Goyer in order to assess the learning ability of offspring-following the exposure of one or both parents to Pb over an extended period of time.

METHOD

Animals

Sixty-eight, 30 day old CFE rats were emloyed in the present study. This population resulted from the mating of

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12 CFE females with 12 males of the same strain. Food and water were provided ad lib. At weaning, the offspring were tailmarked and housed in groups according to sex and litter.

Apparatus

The apparatus was a water T maze constructed of galvanized iron and painted with a flat black enamel paint. The stem was 76.20 cm long and 15.25 cm wide. The alleyways were 30.58 cm long and 7.62 cm wide. A white panel that fit along one side of the stem and an arm of the maze was also constructed. This panel was interchangeable with either side of the maze. The depth of the water was 19.32 cm with the temperature maintained at 28.5°C.

Drugs

All treatment parents were intubated daily with 500 mg/kg of Pb acetate dissolved in distilled water and brought to a concentration of 240 mg/ml. Control parents received equivalent volumes of distilled water based upon body weight. This dosage is at the lower limit of the range used in the frequently duplicated design of Pentschew and Garro [7, 9, 10, 11, 15], which used 4% Pb added to the mother's daily diet or water. However, the intubation method of the present study avoids some of the error resulting from unconsumed diet, spillage, leakage, and the possibility that the offspring may gain access to the Pb regime, thus increasing their exposure.

Groups and Conditions

The parent population, at weaning (30 days of age), were randomly assigned to control or Pb groups and begun on their respective treatments. At 90 days of age these animals were mated to yield four groups: Group Pb-Pb, both the mother and the father had received Pb; Group Pb-N, only the mother had received Pb; Group N-Pb, only the father had received Pb; and Group N-N, neither parent had received Pb. The mothers were continued on their respective treatments throughout gestation and nursing. These matings provided 17 pups per group for testing at 30 days of age. Pups were randomly selected from each litter, with no more than 5 pups being selected from any one litter in the composition of each group.

Procedure

The task employed was a black and white discrimination task, with the white side being reinforced for all animals. During the first 2 testing days, termed pretraining, the animals received 5 trials/day swimming down a straight, all-white alleyway. The pretraining period was employed to reveal any debilitation in swimming ability prior to the beginning of the discrimination training. Escape latencies were defined as the time from the placement of the animal into the water until the animal's forelegs touched the escape ladder. The discrimination task commenced two days after the pretraining trials, with the animals receiving five trials/day for four days. The correct side was counterbalanced across the 20 trials (noncorrection procedure). The intertrial interval was 30 min. Latency and errors were recorded, with an error being defined as any turn inconsistent with escape. Thus, a turn away from the ladder or a turn back into the stem was counted as an error.

Daily weights were recorded for parents and weaning weights for offspring.

RESULTS AND DISCUSSION

Weight and weight gains did not differ for mothers or fathers across groups prior to mating, nor were there any differences in weight curves between Pb and control mothers during gestation. Furthermore, there were no significant differences in the weaning weights for the offspring across groups. These findings suggest no debilitation associated with these factors. This weight data is in agreement with the results reported by Sauerhoff and Michaelson [11], Brown [2], and Morris *et al.* [8]. However, there are studies that have reported growth [7, 9, 10, 15]; thus, the literature is inconsistent on the effects of Pb on weight and growth. It should be noted however, that the retarded growth noted in these studies is in a large part attributable to the lighter birth weights of the Pb exposed offspring.

The major findings for pretraining and acquisition are presented in Table 1: the means and standard deviations for the four groups are presented in Table 2. A 2 (fathers exposed or not) $\times$ 2 (mothers exposed or not) $\times$ 2 (days) repeated measures ANOVA run on pretraining latencies revealed three significant effects; namely, days, mother exposure (M), and father exposure (F). The significant days' effect was a result of decreased swimming times across groups from the first pretraining day ($\bar{x} = 53.48$, $\sigma = 19.92$) to the second session ($\bar{x} = 41.43$, $\sigma = 21.43$). The M effect was attributable to offspring of Pb exposed mothers swimming significantly slower ($\bar{x} = 54.03$, $\sigma = 23.50$) than offspring of unexposed mothers ($\bar{x} = 40.88$, $\sigma = 17.03$). The F effect was a result of offspring of Pb exposed fathers swimming slower ($\bar{x} = 55.61$, $\sigma = 24.32$) than offspring of unexposed fathers ($\bar{x} = 39.31$, $\sigma = 14.27$). An examination of group means revealed that offspring in Group Pb-Pb had significantly longer escape latencies ($p \leq 0.01$) than Groups Pb-N and N-Pb, whose offspring, in turn, had longer latencies ($p \leq 0.01$) than control offspring, Group N-N (Duncan's New Multiple Range Test, [12]).

A 2 $\times$ 2 $\times$ 4 (Days) repeated measures ANOVA of error data revealed four significant effects; namely, father exposure (F), mother exposure (M), an F $\times$ M interaction, and a days' effect. The days' effect showed a significant decrease in errors across groups over the four acquisition days (Fig. 1). The F effect was in the direction of more errors being made by offspring of Pb exposed fathers ($\bar{x} = 11.42$, $\sigma = 6.78$) than offspring of unexposed fathers ($\bar{x} = 8.62$, $\sigma = 5.49$). Similarly, the M effect reflected poorer performance by offspring of Pb exposed mothers ($\bar{x} = 11.61$, $\sigma = 6.43$) than offspring from unexposed mothers ($\bar{x} = 8.43$, $\sigma = 5.80$). The F $\times$ M interaction revealed that all Pb exposed offspring (Groups Pb-Pb, Pb-N, N-Pb) made significantly more errors than controls, Group N-N; however, the former groups did not differ from one another. The F $\times$ M $\times$ D interaction was not significant, indicating a similar rate of learning across groups. Thus, the poorer performance of the three Pb exposed groups suggests that they are unable to overcome initial learning deficits (Day 1, Fig. 1).

The error data from the present study is in agreement with the results of Brown [2] and Van Gelder [16] cited earlier, and in contrast with those of Brown *et al.* [3] and Bullock *et al.* [4]. Unfortunately, neither of the latter two studies report error data, thus a direct comparison of the findings of those studies with the present one is difficult. Furthermore, differences in methodologies (route of administration, dosage, age, etc.) across these studies may be

TABLE 1

F VALUES FOR ESCAPE LATENCY AND ERROR DATA DURING PRETRAINING AND ACQUISITION

	Pretraining (Escape Latency)		Acquisition Errors		Acquisition Escape Latency
	df	F	*df*	F	F
Father exposure (F)	1	24.69*	1	19.54*	13.68*
Mother exposure (M)	1	16.07*	1	25.07*	17.23*
F × M	1	<1	1	16.78*	<1
Days (D)	1	17.53*	3	48.60*	43.44*
D × F	1	<1	3	2.54	4.56*
D × M	1	<1	3	<1	1.05
D × F × M	1	3.34	3	<1	<1
Between error MS	64	365.65	64	27.31	2105.01
Within error MS	64	282.09	192	21.26	1239.82

$^*p \leq 0.01$

TABLE 2

MEANS AND STANDARD DEVIATIONS FOR PRETRAINING AND ACQUISITION DAYS

	Pretraining (Escape Latency)		Acquisition Errors		Acquisition Escape Latency	
Group	Mean	SD	Mean	SD	Mean	SD
Pb–Pb	62.65	27.61	11.71	7.28	110.06	63.0
Pb–N	45.42	14.34	11.5	5.5	92.37	37.4
N–Pb	48.56	18.33	11.13	6.28	89.85	48.15
N–N	33.21	11.45	5.74	3.68	66.38	24.5

sufficient to account for the observed differences.

A 2 X 2 X 4 repeated measures ANOVA of acquisition latencies revealed four significant effects; namely, an M effect, an F effect, a days' effect, and a F X D interaction. The days' effect reflects the decreased escape latencies across groups over the four acquisition days (Fig. 2). The M effect was a result of offspring of Pb exposed mothers having longer escape latencies ($\bar{x}$ = 101.21, σ 52.37) than offspring of unexposed mothers ($\bar{x}$ = 78.12, σ = 39.84). The F effect was a result of offspring of Pb exposed fathers having longer escape latencies ($\bar{x}$ = 99.96, σ = 56.78) than offspring of unexposed fathers ($\bar{x}$ = 79.38, σ = 34.09). Analysis of group means across days (Duncans New Multiple Range Test) revealed that offspring in Group Pb-Pb had significantly longer latencies ($p \leq 0.01$) than offspring in Groups Pb-N and N-Pb, whose offspring, in

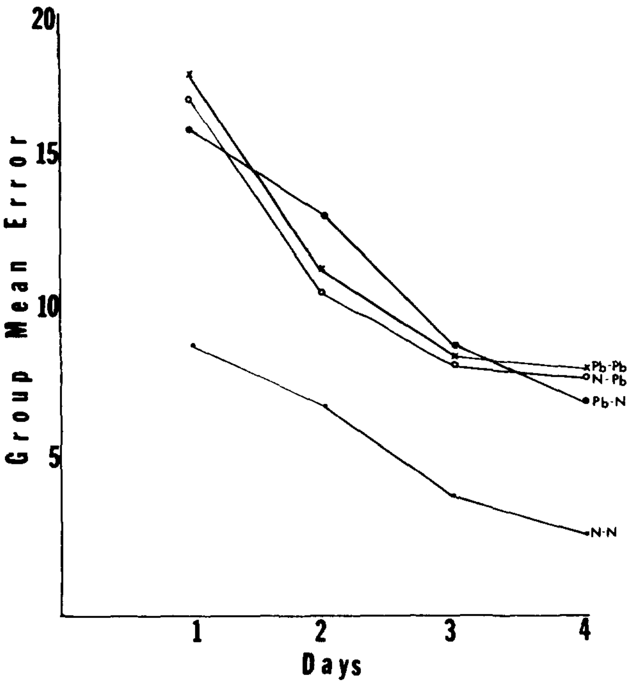

FIG. 1. Daily group mean errors during acquisition.

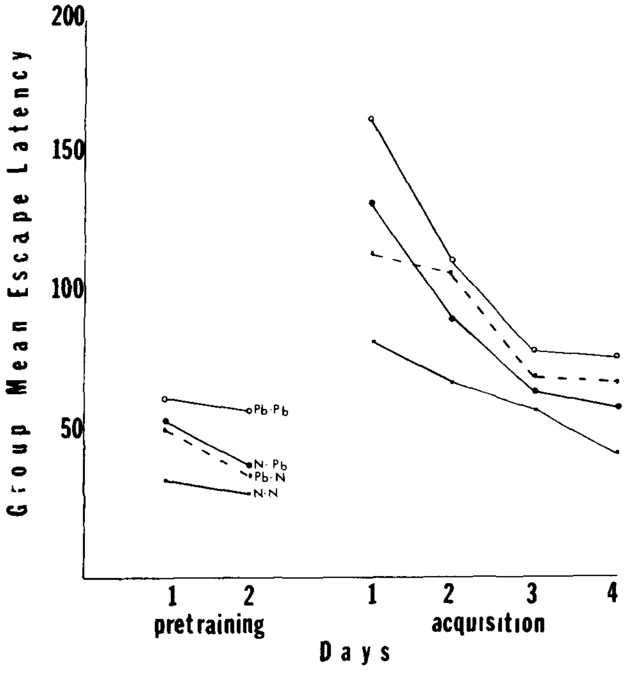

FIG. 2. Daily group mean escape latencies during pretraining and acquisition (sec).

turn, had longer latencies ($p \leq 0.01$) than controls, Group N-N. The F X D interaction was a result of offspring of Pb exposed fathers showing a greater decrease in swimming times over the first three acquisition days than offspring of unexposed fathers. However, the latter offspring still swam significantly faster on Days 1, 2, and 4 of acquisition than those offspring of Pb exposed fathers.

The increased errors of the Pb groups can not be the sole explanation for the increased swimming times, since the acquisition latency trend is identical to that seen during pretraining, where no choice was required of the animals. Rather, the increased swimming times of the Pb groups observed in this study are in accordance with the observed motor impairments noted in other studies including impaired swimming performance [3,4], hyperactivity [11,13], and excessive stereotyped behavior [13].

Although the error data does not distinguish the Pb exposed groups. latency data from both pretraining and acquisition do reveal that offspring from Group Pb-Pb are the most severely affected, indicating some cumulative effect of dual parental exposure that exceeds that of exposing only one parent. However, single parental exposure is detrimental as observed by the longer latencies and increased errors of Groups Pb-N and N-Pb as compared to the controls, Group N-N.

Numerous studies have reported adverse effects on offspring following the administration of Pb to the mother during lactation [7, 9, 10, 11]. However, in this study, offspring of both the mother-only exposure group (Pb-N) and the father-only exposure group (N-Pb) were not differentiated in terms of adverse effects. It is possible that since lower dosages of Pb were employed in this study, that the amount passing to the offspring via the mother's milk was not sufficient to induce additional, observable damage as measured by the task employed in this study. Assessment of the Pb content of the milk may substantiate this conclusion.

In addition, the cumulative effects of exposure prior to mating following by exposure during gestation and nursing may be different than the effects exerted by exposure during a single developmental period such as nursing. A partitioning out of the various developmental contributions may be achieved by cross-fostering offspring of Pb-exposed mothers to control mothers and visa-versa. Thus offspring born to mothers never exposed to Pb would be nursed on Pb mothers, allowing evaluation of the effect of nursing-only exposure. On the other hand, offspring of mothers exposed to Pb during gestation would be nursed on control mothers, allowing assessment of the gestation-only effects. These findings support Stowe and Goyer's suggestions [14] that Pb may exert a detrimental effect gametotoxically through the father, as seen in Group N-Pb, or Pb may have both a gametotoxic and/or intrauterine-extrauterine influence as reflected by Groups Pb-Pb and Pb-N's poorer performance. The possible influence of this latter mode of exposure is supported by evidence that Pb crosses the placental barrier [1] (intrauterine effect) and that Pb appears in the milk of lactating females [9,14] (extra-uterine effect).

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