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Influence of Prenatal and Postnatal Lead Exposure on Discrimination Learning in Rats[1]

HAROLD ZENICK, ROBERT PADICH, THERESA TOKAREK AND POLLY ARAGON

Department of Psychology, New Mexico Highlands University, Las Vegas, NM 87701

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ZENICK, H., R. PADICH, T. TOKAREK AND P. ARAGON. *Influence of prenatal and postnatal lead exposure on discrimination learning in rats.* PHARMAC. BIOCHEM. BEHAV. 8(4) 347–350, 1978. – The animals in this study were the offspring of dams, who, from 21–99 days of age, were exposed to 1000 mg/kg of lead acetate via a daily restricted watering schedule with exposure continuing throughout gestation and nursing. Control dams received distilled water under the same watering schedule. Offspring were weaned at 21 days of age and did not receive lead treatment from that point. Testing began at 30 days of age with animals receiving 10 trials/day for 10 days on a brightness discrimination task conducted in a water-escape T-maze. This task was followed by a shape discrimination problem in the same apparatus. Analysis of results revealed that the lead-exposed pups made significantly more errors than the controls but had significantly shorter swimming times on both the brightness and shape discrimination tasks. The failure to attend to relevant discriminative cues may account for the observed deficits in lead-exposed animals.

Pre and postnatal lead exposure Learning

IN an early study, Brown *et al.* [4] reported that rats injected IP with lead acetate on Days 8, 21, or 35 exhibited no significant decrement on a discrimination learning task. However, since that investigation, several reports have documented poorer learning abilities of pups exposed to lead (Pb) either directly or via the dam during gestation and/or lactation. Tasks have included a variety of two-choice discriminations [2, 3, 6, 12], active avoidance [13,14], and performance under a fixed ratio schedule of reinforcement [9]. Exposure has occurred during gestation [6], various times during nursing [3,14], nursing and postweaning [1,5], and various combinations of gestation-nursing and postweaning [9]. We have recently reported the effect on progeny following maternal and/or paternal exposure. All of the offspring performed poorer on a black-white T-maze discrimination task irrespective of which parent was exposed [2].

The maternal effect was examined further in the present study employing a different method of exposure aimed at providing a means of precisely controlling external dosage while simulating a natural route of exposure. Assessment of offspring learning was conducted on brightness and shape discrimination tasks.

METHOD

Animals

Twenty, 30-day-old CD (Charles Rivers) offspring (10 males), selected from 10 litters, were used. These pups were born and reared in our laboratory in accordance with procedures described below. At twenty-one days of age these offspring were weaned and group-caged by sex, three to four animals per cage. Dams and weaned pups were maintained on Purina Lab Chow No. 5001. The laboratory was maintained at 25.5°C with a 12-hr, light-dark cycle.

Apparatus

The apparatus was a water T-maze constructed of galvanized iron and painted with a flat gray 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. Cues were displayed only along the back wall of the maze and in the culs in the form of interchangeable Plexiglas panels. For the brightness discrimination, the panels were painted black and white. For the shape discrimination, three white circles and three white triangles (two along the back wall and one in the cul on either side of the choice point), were mounted on gray Plexiglas. The depth of the water was 19.32 cm with the temperature maintained at 25°C.

Groups and Conditions

At 21 days of age, 10 female Charles Rivers CD rats, born in our laboratory and designated potential mothers, were weaned and randomly assigned to the Pb and control conditions (5/group) and begun on their respective treatments. All mothers were caged individually. Treated animals received 1000 mg/kg of lead acetate daily dissolved in varying amounts of distilled water. Administration was via daily restricted water intake, with treatment being

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available from 6 p.m. to 8 a.m., followed by access to tap water until noon. The volume of water administered to each animal was set to insure total consumption within the 14-hr period. Furthermore, availability of tap water until noon reduced the possibility of dehydration. Control animals received equivalent amounts of distilled water.

Matings occurred between 90–100 days of age, with vaginal lavages taken to confirm the presence of sperm. Two females were placed with a single mate between the hours of 10 p.m. to 6 a.m. At these times, no water was available for consumption. Maternal treatment was continued throughout gestation and nursing. During the latter period, water spouts were situated so that only dams could gain access to the treatment.

These manipulations yielded two groups: Group Pb, offspring whose dams had been exposed to lead acetate for 70–80 days prior to mating and then throughout gestation and nursing; and Group C, offspring whose dams had never been exposed to the lead acetate treatment. No pup directly received treatment after weaning (Day 21). One male and one female were randomly selected from each of five Pb and five C litters to yield an N of 10/group for testing which began at 30 days of age.

Procedure

The first day of testing was designated pretraining with the animals required to swim down a straight, gray alley-way for three consecutive trials with an intertrial interval (ITI) of 30 sec. The pretraining period was employed to reveal any impairment in swimming ability prior to the beginning of discrimination training. Escape latency was defined as the time from the placement of the animal into the water (facing start wall) until the animal's forelegs touched the escape ladder. Discrimination training began the next day, with the task being a black-white discrimination with white being reinforced (escape ladder present) for all animals. The animals received 10 trials/day for 10 days. The correct side was determined according to a sequence randomly selected from the Gellerman list [7]. The trials were massed with an ITI of 30 sec. 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 scored as an error. All animals were run by experimenters unaware of the treatment history.

Following a two-week interval, shape discrimination training was initiated with circle being correct for half of the animals in each group and triangle being correct for the remainder. The animals received 10 trials/day for nine days. Scheduling problems in the laboratory prevented a tenth day of training. Daily weights and water consumption were recorded for the mothers, and birth, weaning, and periodic test day weights for the offspring.

RESULTS AND DISCUSSION

The method of exposure employed in the present study appeared to be more satisfactory than that of intubation employed in our earlier work [2]. In the previous study, dams were gavaged daily with Pb; and although this technique insured precise dosing, it was observed to be highly stressful to the dam. This may have had an indirect, differential effect on the Pb-exposed pups reflected in subsequently poorer T-maze performance. Furthermore, intubation resulted in the delivery of the Pb dosage as a single bolus into the stomach, altering the parameters of absorption from that which would be observed with a slower rate of intake over a greater period of time each day. The present technique not only avoided the problems encountered with intubation, but also avoided the imprecision that occurs when dosing is via ad lib water intake [10,11]. In the latter instance, the animal, not the experimenter, controls dosage as a function of varying its daily water intake. In the absence of the ability to monitor daily internal dosage through lead analysis, the present method can provide the experimenter with a means of precisely controlling external dosage while maintaining a natural route of administration.

There were no differences in litter size (M = 11.4) nor were there differences in weight, weight gain, or water consumption between Pb and control mothers. However, *t*-tests run on birth and weaning weights for the litters revealed that the Pb-exposed pups weighed significantly less at birth ($t = 6.81$, $df = 8$, $p \leq 0.01$) and weaning ($t = 4.34$, $df = 8$, $p \leq 0.05$) compared to controls. The means and standard deviations for birth and weaning litter weights are presented in Table 1. Although these differences persisted throughout the brightness discrimination task, they had disappeared by the time the shape discrimination task was initiated (approximately 50 days of age). Although these differences may have influenced the performances observed in the T-maze, weight cannot be a complete explanation, since no differences in weights were observed between treated and control mothers during the premating-exposure period or during gestation and nursing.

TABLE 1

GROUP MEAN BIRTH AND WEANING WEIGHTS FOR LEAD AND CONTROL GROUPS

Group	Weight (g)	
	Birth	Weaning
Lead	5.63±0.40	33.5±7.0
Control	6.46±0.15	41.1±4.49

A 2 (groups) × 3 (trials) repeated measures ANOVA run on pretraining latencies revealed only a significant trials effect, $F(2,36) = 12.13$, $p \leq 0.01$). Paired *t*-tests revealed that swimming times across groups were significantly faster on Trial 2 ($t = 3.51$, $df = 9$, $p \leq 0.01$) and Trial 3 ($t = 5.37$, $df = 9$, $p \leq 0.01$) than Trial 1. The difference between Trials 2 and 3 was not significant. The group mean latencies and standard deviations for Trials 1, 2, and 3 were 10.71 ± 6.42, 6.24 ± 2.89, and 4.70 ± 1.70, respectively. This finding contrasts results reported in our earlier study [2] wherein Pb-exposed offspring had significantly longer latencies than controls on pretraining trials. In that study, offspring received five trials/day for two days with an ITI of 30 min, whereas in the present study, pretraining was restricted to three trials on a single day with a 30 sec ITI. Although these procedural differences may account for the discrepancy between the two studies, the mechanism is not clear.

In analyzing the brightness discrimination days, 2 × 10 (days) repeated measures ANOVA's were run on the daily mean errors and mean latency/animal. The latency analysis revealed a significant group effect, $F(1,18) = 18.76$, $p \leq 0.01$, resulting from shorter latencies across days for the

Pb-exposed offspring (M = 10.67 ± 3.46) compared to the controls (M = 17.50 ± 8.40). There was also a significant group × days' interaction, $F(9,162) = 2.05$, $p \leq 0.05$, resulting in significantly shorter latencies for the Pb-exposed group ($p \leq 0.01$) on every day except Days 1, 2, and 7 [8]. This interaction is illustrated in Fig. 1.

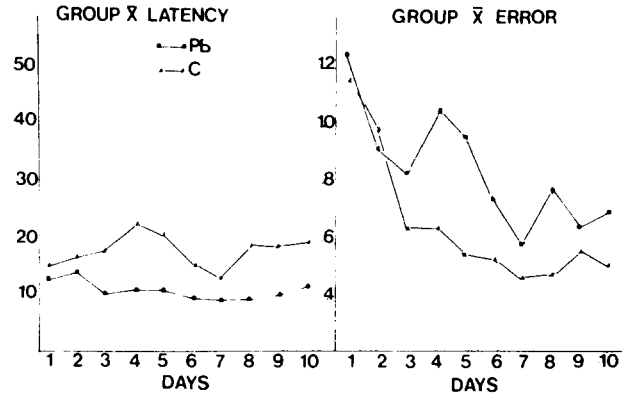

FIG. 1. Group mean latency and errors/trial/day for brightness discrimination task.

The error analysis reflected a significant group effect, $F(1,9) = 8.29$, $p \leq 0.01$, and days effect, $F(8,162) = 11.07$, $p \leq 0.05$. The days' effect was a result of a decrease in errors over days, collapsed across groups, while the group effect was a result of the controls making fewer errors ($t = 2.31$, $df = 18$, $p \leq 0.05$) collapsed across days than their Pb-exposed counterparts (M = 0.64 ± 0.13 vs 0.82 ± 0.09, respectively). The group × days interaction was not significant.

Similar trends in behavior were observed on the shape discrimination task. A 2 × 9 repeated measures ANOVA was run on the error and latency data, respectively. The error analysis revealed only a significant group effect, $F(1,18) = 9.06$, $p \leq 0.01$, with significantly fewer errors made by the controls (M = 0.64 ± 0.10) as compared to the Pb-exposed offspring (M = 0.81 ± 0.06) collapsed across days. The failure to decrease errors across days as was seen on the brightness discrimination task may have been a result of the more difficult nature of the form discrimination and/or fewer training days (Fig. 2).

The latency analysis revealed a significant group effect, $F(1) = 16.88$, $p \leq 0.01$, days effect, $F(8,144) = 2.1$, $p \leq 0.01$, and group × days interaction, $F(8,144) = 3.57$, $p \leq 0.01$. The group effect was a result of significantly shorter latencies across days for the Pb animals (M = 12.99 ± 2.91) as compared to the controls (M = 21.72 ± 5.16). The days' effect was a result of elevated latencies, across groups, on Days 3, 6, and 7. Post hoc analyses [8] revealed that the group × days' interaction was a result of significantly faster swimming times ($p \leq 0.01$) by the Pb-exposed offspring on Days 3–8 (Fig. 2).

Since it was possible for the animals to make more than a single error/trial, additional information on the animal's performance was gained by analyzing the number of errorless trials/animal/day. A 2 × 10 repeated measures ANOVA was run on this dependent measure for the brightness task and a 2 × 9 repeated measures ANOVA on the shape task.

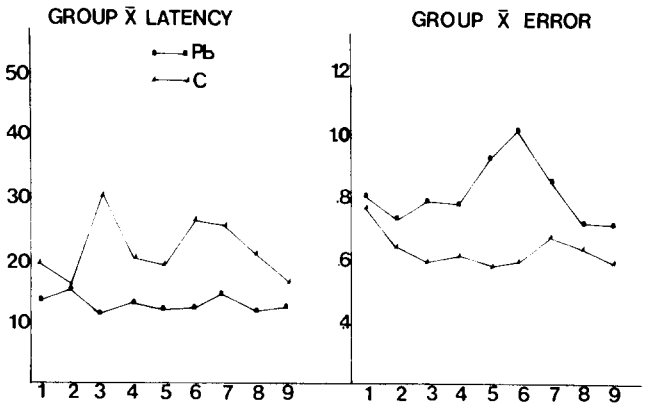

FIG. 2. Group mean latency and errors/trial/day for shape discrimination task.

The analysis of brightness task revealed a significant days' effect, $F(9,162) = 9.05$, $p \leq 0.01$, group effect, $F(1,18) = 5.23$, $p \leq 0.05$, and group × days' interaction, $F(9,162) = 2.72$, $p \leq 0.01$. The days' effect was a result of an increase in errorless trials in the control group (M = 5.39 ± 0.70) as compared to the Pb-exposed offspring (M = 4.62 ± 0.81). Post hoc analysis of the group × days' interaction [8] revealed that the controls performed significantly better ($p \leq 0.01$) on Days 8, 9, and 10 (Fig. 3).

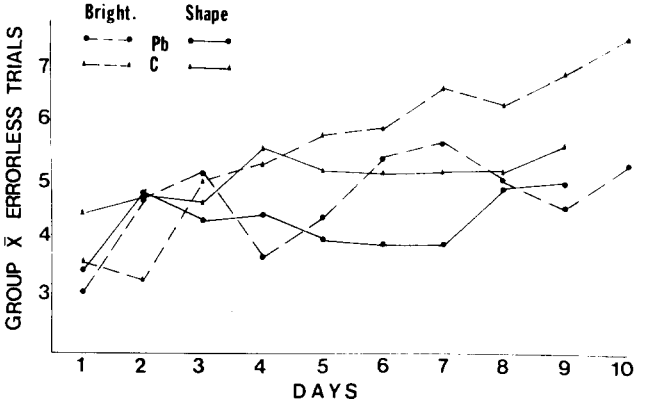

FIG. 3. Group mean errorless trials/day for brightness and shape discrimination tasks.

The analysis of the shape discrimination task revealed only a significant group effect, $F(1,18) = 17.14$, $p \leq 0.01$, with the controls having a significantly greater number of errorless trials (M = 4.42 ± 0.41) than their Pb-exposed counterparts (M = 3.70 ± 0.37). The failure to find a significant days' or group × days' interaction may be a result of the more difficult nature of this task alluded to earlier.

Whereas, the error data replicated results reported earlier from this laboratory [2], the latency trend was reversed. Sufficient differences in methodology across the two studies prevent comparison of latency data. However, one factor that may have contributed to this shift is worth noting. In the earlier study, the correct cue was presented

as an interchangeable panel that fit down the entire stem, arm and cul of the maze. Thus, the correct cue was available from the starting point and throughout the entire length of the maze. In that study, controls were observed to make their selection near the starting point out and then orient along that wall as they swam down the maze. In the present study, the cues were displayed only along the back wall and in the culs of the maze. In this instance, animals later identified as controls, were observed to swim to the choice point and often pause prior to making their selection. A similar pause was not noted in the behavior of the Pb-exposed offspring. Although it was not timed, this pause did contribute to the increased latency of the controls; however, such pausing at the choice point may have also contributed beneficially to their decreased error performance as compared to Pb-exposed offspring.

That the alteration of cues in the apparati did not alter the behavior of the Pb-exposed offspring in the maze (e.g., no pausing) is suggestive of the failure of the animal to attend and/or utilize the appropriate cues in decision-making. No dominant pattern of cue selection seemed to characterize the behavior of the Pb-exposed animals (e.g., alternation, side preferences, or using the side of the preceding trial success or failure to determine subsequent choice). An elucidation of the strategies employed by the Pb-exposed animals may be gained by a systematic manipulation of the location and properties of the correct cue instead of the traditional randomization procedure as employed in the present study.

In conclusion, the error data reaffirms earlier results regarding the deleterious effects of Pb on discrimination learning as well as supports recent findings reported by Brown [3] and Snowden *et al.* [12]. Combining the present results with past studies suggest that the observed deficits may be a function of a variety of factors including the inability to overcome initial learning deficits seen on Day 1 of training [2], retarded rate of acquisition [3,12], or a combination of these variables. Furthermore, an attentional hypothesis should be given consideration. The failure of the animal to attend to and/or maintain attention to the relevant discriminative stimuli in the task could contribute to the poorer performance. Such an attentional breakdown has also been suggested to underlie, in part, the inferior fixed ratio behavior of the Pb-exposed offspring observed in our laboratory [9].

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