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The Effects of Developmental and/or Direct Lead Exposure on FR Behavior in the Rat[1]

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PADICH, R. AND H. ZENICK. *The effects of developmental and/or direct lead exposure on FR behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 6(4) 371–375, 1977. – In the present study, 21-day old female rats were exposed to daily doses of 750 mg/kg of lead acetate via a restricted water intake regimen for 70–80 days prior to mating. Treatment was then continued throughout gestation and nursing. At weaning, litters from half of the treated and control mothers were placed on treatment for the remainder of the experiment. This manipulation yielded four groups for testing: Group Pb/Pb, developmental and direct, postweaning exposure; Group Pb/C, developmental exposure only; Group C/Pb, direct exposure only; and Group C/C, no exposure to lead acetate treatment. Beginning at 42–49 days of age, offspring were shaped to bar press on a Fixed Ratio 20 (FR20) schedule of reinforcement and then received 20 sessions each 20 min in length. Analyses revealed that Group Pb/Pb received significantly fewer reinforcements/min across sessions than the other three groups and also took significantly longer to emit each 20 response block. Contrary to previous reports in the literature, it is suggested that rats may not be impervious to postweaning lead exposure, particularly when there is a history of developmental exposure.

Development lead   Fixed ratio schedules

THERE HAVE been a number of studies that have examined the effects of early postnatal lead exposure on rodent behavior. The lead treatment has most often occurred either during nursing only [3, 4, 18, 19, 20] or during nursing with continued postweaning exposure for the duration of testing [7, 9, 10, 14, 15, 16, 17]. These approaches may essentially be viewed as analogs to the model of pica of lead-containing materials by the human infant [9].

However, in addition to the pica threat it is conceivable that another condition which is sufficient to insure damage occurs when offspring are born to mothers living in elevated lead environments. In such an instance, one is examining the effects of prolonged maternal exposure that may include premating exposure as well as gestation and nursing [2, 5, 11]. In addition to this developmental exposure, the opportunity also exists for the offspring to be directly exposed, postweaning. Attempts by our laboratory to simulate such a model have been reported in an earlier study in which one or both parent animals were exposed to lead for an extended period of time prior to mating with mothers continued on treatment during gestation and nursing [2]. Offspring were subsequently tested at 30 days of age, employing a black-white discrimination water T maze. Analyses revealed that offspring of lead-exposed parents made more errors and had longer latencies than control counterparts.

In the present experiment, the effect of prolonged maternal exposure was examined further. In addition, at weaning offspring from half of the lead and control mothers were directly administered lead for the remainder of the experiment. Thus the effects of developmental exposure (gestation-nursing) were contrasted with direct exposure (postweaning) or the combined developmental-direct exposure.

Efforts to assess the effects of developmental lead exposure in the rodent have primarily concentrated on two areas, namely, measures of activity [7, 9, 10, 11, 14, 15, 16, 17] and/or discrimination learning [2, 3, 4, 5, 19, 20]. Few efforts have been made to examine behavior maintained under various schedules of reinforcement [1,12]. Yet, such measures have proven to be invaluable to the psychopharmacologist and have an obvious role for the behavioral toxicologist. Thus our laboratory has undertaken a series of studies to examine the effects of lead exposure on schedule-maintained behavior. In the present study, the ability of offspring to maintain bar press behavior on a Fixed Ratio (FR) schedule of reinforcement was assessed. Since there is not only a dearth of information on the effects of lead on schedule behavior, but also a lack of data on the acquisition of such behaviors in the developing rodent, a relatively simple schedule was selected for initial analysis. It seemed essential to know if the offspring could acquire and maintain performance on this schedule before

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attempting to assess behaviors maintained under more complex schedules.

METHOD

Animals

Forty, 42–49 day old CD offspring (20 male) were used. These pups were born and reared in our laboratory in accordance with procedures described below. At 21 days of age, these offspring were weaned and then caged individually for the duration of the experiment. Mothers and pups were maintained on Purina Lab Chow No. 500. The laboratory was maintained at 78° with a 12 hr light-dark cycle.

Groups and Conditions

At 21 days of age 20 female, CD rats, born in our laboratory and designated potential mothers, were weaned and randomly assigned to the lead (10 mothers) or control conditions and began on their respective treatments. All mothers were caged individually.

Treated mothers received 750 mg/kg of lead acetate (PbAc) daily, dissolved in varying amounts of distilled water. Administration was via daily restricted water intake, with treatment being 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 mothers received equivalent amounts of distilled water.

Matings occurred between 90–100 days of age, with vaginal lavages taken to confirm the presence of sperm. Maternal treatment was continued throughout gestation and nursing. During these periods, water sprouts were situated so that only the mothers could gain access to the treatment.

The pups were weaned at 21 days of age. Offspring from five of the mothers that had received PbAc and from five of the control mothers were maintained on PbAc treatment for the duration of the experiment. The remaining pups received distilled water. The restricted and ad lib watering schedules for these offspring followed the same protocol described earlier for the mothers. These manipulations yielded four groups: Group Pb/Pb, offspring exposed to lead during gestation-nursing and directly, postweaning; Group Pb/C, offspring exposed during gestation-nursing only; Group C/Pb, offspring exposed directly, postweaning only; and Group C/C offspring not exposed to the PbAc treatment.

A male and female pup were randomly selected from each of the five litters in each group to yield an N of 10 offspring/group for operant conditioning which began 42–49 days of age.

Apparatus

All conditioning was conducted in Lafayette operant chambers. Each unit was enclosed in a sound proof box, ventilated with an induction fan. Programming was accomplished with a Gerbrands G4010 Ratio Programmer and G4615 Control Panel. Data were recorded on a Harvard Cumulative Recorder, Model C-3 and a Lafayette event recorder for later analyses.

Procedure

Conditioning was delayed until 42–49 days of age, to allow the offspring to gain sufficient size and stamina to perform the task. Although training might have begun earlier, it would have required extensive modification of both equipment and conditioning techniques employed in this laboratory. Animals were gradually reduced to 80% of their free-feeding weight and then maintained at that level relative to their freefeeding, appropriate sex, littermates. Feeding occurred at the completion of each experimental trial. Animals were run three times a week, Monday–Saturday, with each daily trial lasting 20 min. Since at the start of training the animals were still exhibiting rapid growth, a supplemental ration was provided Saturday or Sunday (depending on the animal's schedule) to insure continued growth.

The animals were initially trained to bar press under a continuous reinforcement schedule (CRF) with reinforcement being Noyes pellets (4.0 mm × 3.3 mm × 45 mg). Upon attainment of the bar press response, animals were then allowed to make 300 reinforced responses across sessions with time required to attain this criterion recorded. This manipulation allowed for a quasi-experimental check on motivational, anorexic, or other interfering factors. In the absence of these factors, the time to reach criterion should have been equivalent across groups.

Following this manipulation, the offspring were shaped to an FR20 schedule and subsequently received 20 daily sessions each 20 min in length. The FR20 criterion was selected following extensive pilot work with FR10, FR15, and FR20 schedules. In the former two instances, there were no differences between groups in the number of reinforcements/min. However, observation of Group Pb/Pb revealed a difference in the behavior patterns exhibited by these offspring while in the Skinner box. Animals in this group would consistently disengage from bar pressing to engage in a number of bizarre behaviors not conducive to maximizing reinforcement (e.g., jumping at their reflection, manipulating cage light, etc.). It was hypothesized that if the Pb/Pb offspring persisted in emitting behaviors that competed with bar pressing, then at some point as schedule criterion increased, there would be a significant reduction in the number of reinforcements attained as compared to the other groups. Preliminary investigations had indicated that such differences were manifested on an FR20 schedule. Thus this schedule criterion was employed in the present study. In order to more accurately measure this effect, the number of reinforcements/minute/subject was recorded for each session and the length of the postreinforcement pause (interval between reinforcement and the next response), and the rate time (time to emit each 20 response block) were recorded on FR20 sessions 10 and 20.

RESULTS AND DISCUSSION

A comparison of the PbAc and control groups revealed that there were no differences in litter size or time of occurrence of developmental landmarks such as eye opening, appearance of hair, or coordinated walking. There were no differences in daily water consumption between PbAc or control mothers or offspring under the partial deprivation schedule. This is important in light of the problems encountered with other modes of exposure such as the unnaturalness of injection, the stress of intubation, the inaccuracy and spillage encountered in diet mixtures, and the inability to precisely control dosage when administration is via ad lib water intake. In the latter instance, the animal determines the dosage as a function of varying its daily water intake. With the present technique, there were only a few instances where the animals failed to consume the treatment in a carefully measured amount of distilled water. Thus the daily dosage was constant and solely a function of body weight.

Weight and weight gains did not differ for mothers. However, *t*-tests run on group mean litter weights at birth revealed that lead offspring were significantly smaller than controls (t = 2.865, df = 18, $p < 0.01$). These differences were not seen by weaning. Group mean weights and standard deviations are presented in Table 1. The contribution of these differences to FR performance is discussed below.

TABLE 1

GROUP MEAN ($\overline{X}$) WEIGHTS AND STANDARD DEVIATIONS (SD) FOR BIRTH AND WEANING

Maternal Exposure	Birth		Wean	
	$\overline{X}$	SD	$\overline{X}$	SD
Lead Acetate	5.58	0.52	31.41	4.10
Control	6.17	0.43	34.03	3.84

A 2 (gestation-nursing, GN) × 2 (postweaning, P) ANOVA run on minutes-to-criterion (300 bar presses, CRF) indicated no significant differences across the four groups. These data suggest that, at least at the CRF demand, the animals were equally motivated to perform the task, and PbAc did not appear to be exerting any anorexic effect.

A 2 × 2 × 20 (sessions) repeated measures ANOVA was run on the mean reinforcements/minute/subject. These F values are reported in Table 2. Analyses revealed significant main effects for sessions, gestation-nursing exposure (GN), postweaning exposure (P), and the attendant interactions. The sessions effect was a result of an increase in the number of reinforcements emitted across the 20 sessions, collapsed across groups. *Post hoc* comparisons [8] of the significant GN, P, and interaction effects revealed that these differences were a result of Group Pb/Pb receiving fewer reinforcements/minute across sessions 9–20. Furthermore, Group Pb/Pb failed to exhibit the significant increase in reinforcements/minute across sessions that was witnessed in the other groups. These trends are graphed in Fig. 1.

As was observed in pilot work, the Pb/Pb offspring repeatedly interrupted bar pressing to engage in competing behaviors, and, as hypothesized, with a sufficiently demanding schedule criterion, there was a significant reduction in the number of reinforcements attained. This evaluation of the Pb/Pb group's behavior was further supported by analyses of rate time and post-reinforcement pauses (PRP). A 2 × 2 × 2 (Sessions 10 and 20) repeated measures ANOVA run on rate time revealed a significant P effect, $F(1,36) = 27.14, p < 0.001$) and a significant GN × P interaction, $F(1,36) = 9.76, p < 0.01$). Similar analysis of PRP data revealed no significant differences.

Post hoc [8] comparisons revealed that Group Pb/Pb had a significantly longer rate time for the blocks of 20 presses than Groups C/C and Pb/C for Session 10 and

TABLE 2

ANALYSIS OF VARIANCE OF REINFORCEMENTS/MINUTE

	df	MS	F
Gestation-Nursing (GN)	1	25.64	10.34*
Postweaning (P)	1	21.63	8.72*
GN x P	1	22.74	9.17*
Between Error	36	2.48	
Sessions (S)	19	154.78	34.50*
S x GN	19	12.69	2.91*
S x P	19	27.38	6.28*
S x GN x P	19	13.25	3.04*
Within Error	684	4.36	

*$p < 0.01$.

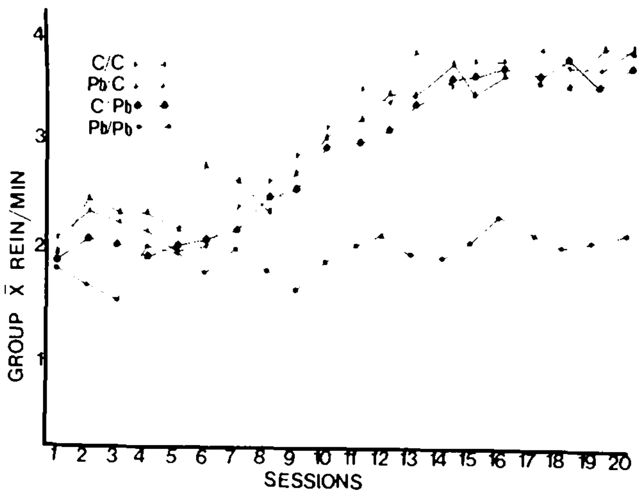

FIG. 1. Group mean reinforcements/minute across 20 sessions under a FR20 schedule of reinforcement.

longer rate times than Groups C/C, Pb/C, and C/Pb for Session 20. The means and standard deviations for rate time and PRP are presented in Table 3. These findings support observations made regarding the Pb/Pb animal's repeated abatement from bar pressing while pursuing criterion.

The possibility of differences in motivational levels as an explanation for these findings must be considered. Although the groups did not differ in the CRF minutes-to-criterion measure, the interaction between deprivation and the demands of the FR20 task may have been different between groups. However, it is worth reiterating that the groups did not differ in the length of the PRP which has been used as an index of motivational level (e.g., [6,13]).

Another factor that must be considered is the relevance of reduced birth weights of the lead pups as related to subsequent FR performance. The reduction observed in this study does not seem to stem from an undernutrition precipitated by a sharp decrease in food/water consumption on the part of the lead mother when presented with the treatment in one of these sources. Although such an explanation has been forwarded in other studies, in the present experiment, there were no differences between lead and control mothers in terms of weight, weight gain or water consumption during the course of the treatment.

TABLE 3

GROUP MEANS AND STANDARD DEVIATIONS FOR RATE TIMES AND POSTREINFORCEMENT PAUSE LENGTH (SEC)

Group	Rate Sessions 10	Rate Sessions 20	Postreinforcement Pause Sessions 10	Postreinforcement Pause Sessions 20
C/C	12.96 ± 4.31	8.73 ± 3.10	4.0 ± 2.41	3.18 ± 2.57
C/Pb	16.90 ± 6.08	11.42 ± 4.86	3.66 ± 2.23	3.42 ± 2.14
Pb/Pb	19.76 ± 5.41*	17.88 ± 4.03*	4.46 ± 2.78	3.87 ± 2.21
Pb/C	13.96 ± 6.25	10.25 ± 4.69	3.64 ± 2.81	3.57 ± 1.63

*$p<0.01$.

Whatever the process accounting for the reduced birth weights, it is questionable that this phenomenon can serve as a complete explanation for the FR differences in light of Group Pb/C's performance. These offspring also exhibited reduced birth weights; however, their FR performance was equivalent to the controls (Fig. 1). Furthermore, hypothesizing a continued nutritional deficit on the part of Group Pb/Pb is not reasonable in light of the absence of significant weight differences between groups at weaning. Although the interaction of factors underlying reduced birth weight and subsequent FR behavior cannot be dismissed, this relationship is not clear, and any stronger conclusions would presently be unwarranted.

It was surprising that the behavior of the developmental exposure-only group (Pb/C) was not altered in light of the numerous studies reporting disruption of behavior in offspring exposed developmentally. Rather the effect appeared to require the combined influence of developmental and direct exposure (Group Pb/Pb). In contrast to the popular belief espoused in the literature, the weaned rat may not be impervious to lead insult. However, the expression of this direct effect may require prior developmental exposure.

Interpretation of the results from this study is limited by the paucity of data on the effects of lead on schedule-controlled behavior. Avery et al. [1] examined the effects of a single i.p. injection of the organic compound, tetraethyl lead, on FR 5 behavior of adult rats. They found a significant decrease in rate from pretreatment levels in the highest dosage group which could not be attributed to anorexia. The several procedural differences between their study and the present one may account for the appearance of a depression on their much less demanding schedule. However, the assumption that the decreased rate observed in the Avery et al. study [1] is supportive of the results of the present study must be tempered, since no report is provided of the lead animal's behavior in the Skinner box.

The relationship of the peculiar behaviors observed in this study with that exhibited by lead offspring in other laboratories is not clear. As Reiter et al. [11] have commented, the lead offspring has been reported to show a gamut of behaviors ranging from hypoactivity to hyperactivity. However, it is premature to conclude that the behavior observed in the Skinner box is hyperactivity, a term that has been far too loosely applied to alterations in activity observed in the lead offspring. The inability of the Pb/Pb offspring to persist in responding could also be reflective of sensory, attentional, and motivational variables; all are a part of a myriad of factors that contribute to motor performance. Further experimentation with other schedule combinations should yield insight into the mechanisms underlying the behaviors observed in this study.

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