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Drug Induced Activity in Lead-Exposed Mice[1,2]

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RAFALES, L. S., R. L. BORNSCHEIN, I. A. MICHAELSON, R. K. LOCH AND G. F. BARKER. *Drug induced activity in lead-exposed mice*. PHARMAC. BIOCHEM. BEHAV. 10(1) 95–104, 1979.—Three interrelated studies were conducted to examine the locomotor activity of lead-exposed mice. The effects of lead were examined as a function of the dose and duration of exposure. Exposure during the first three weeks occurred via the maternal milk supply. Exposure following weaning was achieved via the water supply. Mice received challenges with various pharmaceutical agents, including d-amphetamine, methylphenidate, apomorphine and phenobarbital. The spontaneous activity prior to injection and the drug-induced activity were monitored. Lead-exposed mice usually displayed spontaneous activity which was indistinguishable from that of the control animals. In only one set of observations did lead exposure result in a modest increase in spontaneous activity. The drug-induced activity varied in a complex manner as a function of the magnitude and duration of the lead exposure. Depressed body weight, which was concurrent with high lead exposure (0.5% $Pb(Ac)_2$) was also a significant parameter affecting both the spontaneous and drug-induced activity.

Lead exposure   Locomotor activity   Drug challenge

THE PREVALENCE of lead in the environment [3, 6, 22] and its unequivocal toxicity at high concentrations (cf [23], for review) has prompted concern that ambient exposure levels may pose a significant health hazard. Support for this hypothesis is found in reports of altered locomotor activity [4, 8, 12, 15–16, 18–21, 25] and atypical pharmacological responses [14, 18, 19, 21] in rodents which have received chronic, "low-levels" of lead during the early formative period of development. The validity of these reports rests on the assumption that control animals and animals receiving lead were reared and tested under identical conditions. However, this degree of experimental control has rarely been achieved because of a lack of attention toward three salient experimental parameters: body weight, litter size and sampling bias.

Several investigations in which lead altered locomotor activity employed lead exposure regimens which produced weight retardation [15, 16, 18–21]. However, growth retardation alone can alter the locomotor response of an animal [5]. Consequently, the results of these studies are open to an alternative interpretation: namely, that early growth retardation, and not lead per se, was responsible for the altered activity observed in lead-exposed animals.

At least two studies [12,25] utilized exposure regimens which selectively reduced the size of litters born to pregnant females ingesting lead to 20–28% of the size of litters born to control animals. Litter size was not adjusted prior to weaning, hence early neonatal social history and maternal care of these animals covaried with their exposure history. Since these two factors affect behavioral development (cf [13], for review), it is fallacious to infer that lead exposure was the sole factor responsible for altering locomotor patterns. Unequal litter size offers an alternative explanation.

Sampling bias is another factor often neglected by lead researchers. The use of an inordinately small number of litters within each treatment group [15,16] increases the probability that differences ascribed to exposure conditions were actually the result of normal genetic variation. Unfortunately, many activity studies reporting positive findings failed to indicate the number of litters represented within each treatment group [12, 18–21, 25]. This omission results in the possibility that the method of subject selection produced the behavioral differences attributed to lead exposure.

The purpose of the present investigation was to confirm earlier observations of hyperactivity and altered drug response in lead-exposed animals. Thus, litter size was controlled and under-representation of litters minimized in this lead study. Body weight was initially allowed to vary with lead exposure. Subsequent experiments either controlled for, or systematically manipulated, body weight to account for the contribution of this variable upon measures of activity.

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[2]Part of this research was reported at the Symposium on Effects of Prenatal and Postnatal Toxic Exposures on Behavioral Development, 85th Annual Convention of the American Psychological Association, San Francisco, California, 1977 (Experiment 1). An expanded version of Experiment 2 appears in *Life Sciences* 22(22): 1963–1970, June 12, 1978.
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GENERAL METHOD

Lead Analysis

A simple extraction procedure was used which was applicable to atomic absorption spectrophotometric determination of trace quantities of lead [26]. This method involved chelation of lead with ammonium pyrrodidine-dithiocarbamate (APDC) at pH 8.5 and extraction of the chelate into a small volume of methyl isobutyl ketone (MIBK) for aspiration into the burner of the instrument. Recoveries of known amounts of lead for this procedure have been reported at 97 to 105% for blood and 97 to 100% for urine [26].

Drugs and Injections

D-amphetamine sulfate and phenobarbital were dissolved in 0.9% saline. The vehicle for methylphenidate HCl (Ritalin®) was distilled water and for apomorphine HCl, 0.1% Na metabisulfite. All drugs were administered in a volume of 10 ml/kg body weight. Doses are indicated in the appropriate method sections and are expressed as the salt. The route of administration was intraperitoneal for all drugs except apomorphine which was injected subcutaneously.

Experimental Chamber

All testing was performed in a sound attenuated room having a light:dark cycle identical to the cycle of the main colony. Air was continuously filtered by an electrostatic air cleaner.

EXPERIMENT 1

METHOD

Animals

Sixteen timed pregnant CD-1 mice were obtained from Charles River Breeding Labs, Inc. (Wilmington, MA) on Day 14 of gestation and individually housed in polypropylene mouse cages (29×19×13 cm) with stainless wire tops (Econo-cages®, Fisher Scientific Co.). Bedding consisted of ground corn cobs (San-I-Cel®, Paxton Processing Co.). Animals were kept at a temperature of 22 ± 1°C in a room having a reversed 12 hr light:12 hr dark cycle; the dark phase commencing at 7:00 p.m. Food (Ralston Purina Rat Chow, 1–3 ppm Pb [1,8]) and tap water ($Pb<0.05$ ppm) were provided ad lib until treatment regimens were initiated at parturition.

At parturition, litters were each culled to eight pups (0 days old). An attempt was made to select out females over males as only the males were to be tested for activity. Litters were again culled to 5–7 pups at 23 days of age. The average male/female sex ratio following this second culling was 5/4. Animals which were extracted from each litter at 23 days of age were sacrificed and the brains removed and frozen for subsequent determinations of lead. At 35 days of age mice were separated on the basis of sex. Five days later animals were ear punched for identification. Mean pup weights were recorded at 7, 14, 21 and 28 days of age.

Exposure Protocol

At parturition ten of the 16 dams received water containing 0.5% lead acetate (2730 ppm Pb) in place of tap water. Suckling mouse pups were thus exposed to lead via its transfer from drinking water to the maternal milk supply [18–21]. The remaining six dams served as controls and continued to receive tap water. Drinking solutions were prepared from chemical grade lead acetate (Fisher Scientific Co.) dissolved in boiled distilled water. Maternal drinking tubes were inaccessible to the pups.

At Day 21, dams were removed from their litters. Pups from control dams were weaned to tap water (Group:H_2O) as were pups from five of the treated dams (Group:0.5% NEO). Pups from the five remaining treated dams were weaned to 0.5% lead acetate (Group:0.5% CON). Three exposures were thus obtained by this procedure: (1) Group:H_2O; a tap water exposure which served as a control, (2) Group:0.5% NEO; a neonatal lead exposure terminating on Day 21, and (3) Group:0.5% CON; a continuous lead exposure from birth.

Apparatus

Locomotor activity was measured in 10 clear polystyrene cages of the same dimensions as the home cage (29×19×13 cm). A red fluorescent light with a peak emission spectra of 630 nm (range:575–725 nm) was positioned above each set of five cages. Each cage rested upon a platform containing an array of 10 photocells with an average interphotocell distance of 7.6 mm. The photocells were connected to a solid state Wheatstone bridge circuit which in turn was connected to an analog-to-digital converter and solid state memory device. An animal's movement across the photocell network resulted in digitalized "counts" which were accumulated and printed out at 15-min intervals.

Procedure

Behavioral tests employed 10 males from each treatment group. They were selected from the larger population of exposed and control animals such that a minimum of four litters were represented in each treatment group; otherwise, selection was random. A set of 10 animals was tested over a block of 3 to 5 consecutive daily sessions before testing another set of mice. Assignment of the mice to the appropriate test session and cage was determined by a modified incomplete latin square (Youden Square) design. Differences in the sensitivity of testing devices, age at testing, and uncontrolled conditions which may have been unique to a particular session were thus distributed across treatment conditions.

Fifteen hours prior to the first session in a block, ten mice were removed from their group housing facilities and placed in 10 holding cages located in the test room. The holding cages, their bedding, and food and water (or lead acetate solution) were identical to the group cages, except that the animals were now singly housed. This procedure was instituted to minimize the excessive handling which accompanies identifying an animal in a group-housing situation.

Animals were weighed and transferred from their individual holding cages to the clear polystyrene activity test cages 60–90 min after the onset (7:00 a.m.) of the dark phase of the dark-light cycle. A session lasted 7 hr after which animals were returned to their holding cages until the next session on the following day. When drugs or vehicle were administered, the time of injection was fixed at 3 hr after the start of a session. At the completion of each block of 3 or 5 sessions, mice were returned to their group housing facilities.

The first block of activity tests occurred when animals were 141–161 days of age. *D-amphetamine:* Animals did not

receive injections during the first 3 sessions. During the fourth session the vehicle for d-amphetamine was administered. During the fifth session d-amphetamine (5 mg/kg IP) was administered. *Methylphenidate:* Animals were tested in a second block of 3 sessions when they were 190–198 days old. No injections were made during the first session, distilled water (the vehicle for methylphenidate) was injected during the second session, and methylphenidate (40 mg/kg IP) was injected during the third session. *Apomorphine:* Animals were tested in a third block of 3 sessions when they were 211–219 days old. No drug injections were made during Session 1, 0.1% sodium-meta-bisulfite (the vehicle for apomorphine) was injected during Session 2, and apomorphine (4 mg/kg SC) was injected during Session 3. Apomorphine challenges were again conducted when mice were approximately 350 days of age. For this fourth block of sessions, the dosage was raised to 6 mg/kg SC.

RESULTS

Fluid Consumption

The fluid intake of dams drinking 0.5% lead acetate (2730 ppm Pb; Groups 0.5% NEO and 0.5% CON) was 34% less than that of dams drinking tap water (Group:H_2O) during the lactation period. A one-way analysis of variance, ANOVA; $F(2,13)=34.08$, $p<0.001$, and post hoc Scheffe t-test, $t(2,11)=8.02$, $p<0.01$, indicated that this difference was statistically significant.

Body Weight

The reduced fluid intake of dams exposed to lead during the lactation period may partially account for an observed depression in the mean body weights of their offspring by Day 21. A two-way ANOVA (repeated measures at ages 7, 14, 21 and 28; $F(6,69)=27.58$; $p<0.001$, and post hoc Neuman-Keuls test, q(2 or 3, 30)=9.41, 11.07; $p<0.05$ indicated that, at this time, neonatal and continuous exposure groups had mean body weights which were significantly depressed from those of controls (75 and 78%, respectively) but did not differ from one another, q(2, 3, 30)=1.66; $p>0.05$. Seven days of additional lead exposure to mice in the continuous group resulted in a further depression in their body weight, 67.2% of controls; q(3,30)=24.74; $p<0.05$. However, the body weights of mice in the neonatal group were beginning to return to control values, 86.1% of controls; q(2,30)=10.49; $p<0.05$. A second ANOVA on the body weights of animals used for behavioral tests showed that the effect of lead exposure on weight was still evident at 154 days of age, $F(2,27)=5.66$, $p<0.01$. Mice in the 0.5% CON group had body weights which were 87.0% of H_2O controls, Scheffe t-test:$t(2,27)=3.07$, $p<0.05$. The body weights of mice in the 0.5% NEO group, by this time, had returned to control values, 98.6% of H_2O controls; $t(2,27)=0.33$, $p>0.10$.

Lead Determinations

The mean brain lead concentration for 5 animals (each from a different litter) from the 0.5% NEO group was 1.9 ± 1.4 ppm (mean ± SD) at 23 days of age. For 5 mice from the 0.5% CON group the mean lead concentration was 3.8 ± 0.8 ppm. The amount of lead in the brains of control animals was below the limit of detection for the flame atomic absorption procedure employed (0.05 μg/sample). To improve detection, brains from several 350 day old animals were pooled. The brain lead concentrations of these animals (2 or 3 pooled samples per group) were 0.05 ± 0.04, 0.05 ± 0.01 and 1.80 ± 0.20 ppm from the H_2O, 0.5% NEO, and 0.5% CON groups, respectively.

Activity

All activity data were square root transformed in order to normalize the distribution.

Spontaneous activity. Spontaneous activity over an initial 3 hr period for 5 consecutive sessions is plotted in Fig. 1. The data were analyzed using a 3-way ANOVA with repeated measures on the activity within each three hour segment and across sessions. Neither of the two durations of lead exposure had a significant effect on this activity $F(2,27)=0.13$, $p>0.10$. The analysis also failed to demonstrate any statistically significant interaction with the exposure conditions, $F(8,108)=1.35$, $p>0.10$; $F(22,297)=0.80$, $p>0.10$; $F(88,1188)=0.80$, $p>0.10$.

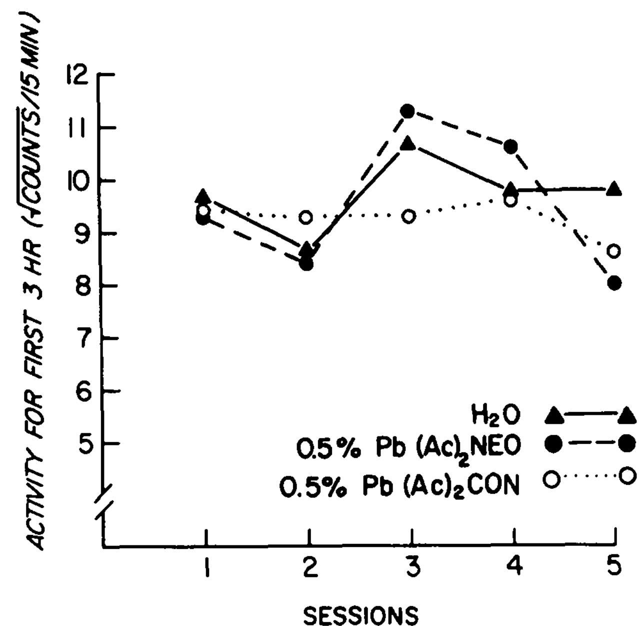

FIG. 1. Spontaneous activity recorded during a 3 hr period for 5 consecutive days.

Activity following drug challenges. Activity following each drug injection was analyzed using a two factor ANOVA with repeated measures over time.

D-amphetamine. D-amphetamine increased the mean activity of animals to a peak level within 30 min after injection (Fig. 2). There followed a gradual decline from this peak activity over the next 3.5 hr of testing, $F(15,405)=83.40$, $p<0.001$. Overall differences between the activity of exposed and control mice in response to d-amphetamine failed to attain statistical significance, $F(2,27)=2.84$, $p=0.076$. However, a statistically significant interaction between exposure history and time-after-injection, $F(30,405)=1.51$, $p<0.043$, meant that differences between treatment groups could be identified at individual time periods. The intervals in which treatment groups differed were identified using Neuman-Keuls post hoc comparisons [21]. Differences in the

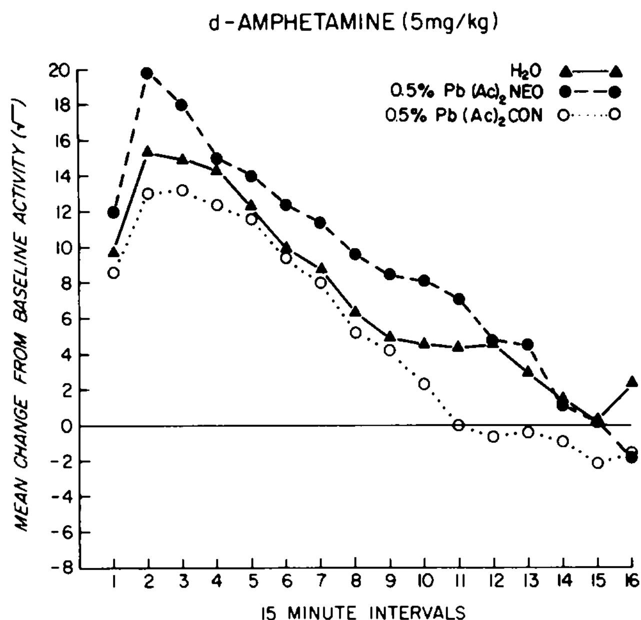

FIG. 2. Activity following injections of d-amphetamine (5 mg/kg, IP).

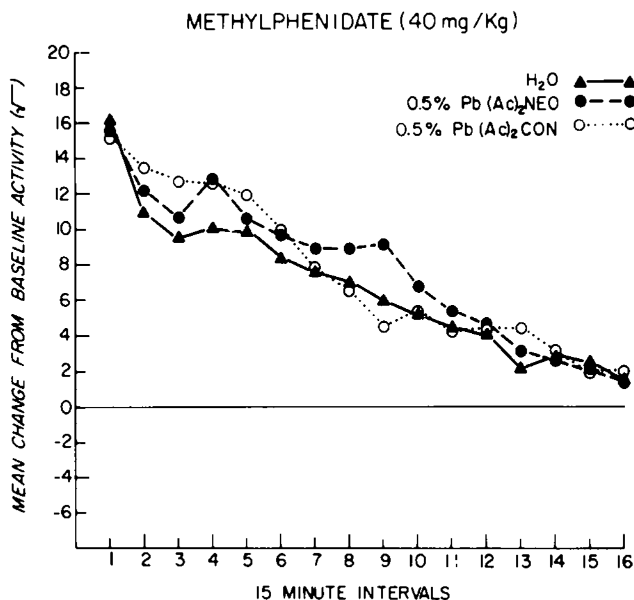

FIG. 3. Activity following injections of methylphenidate (40 mg/kg, IP).

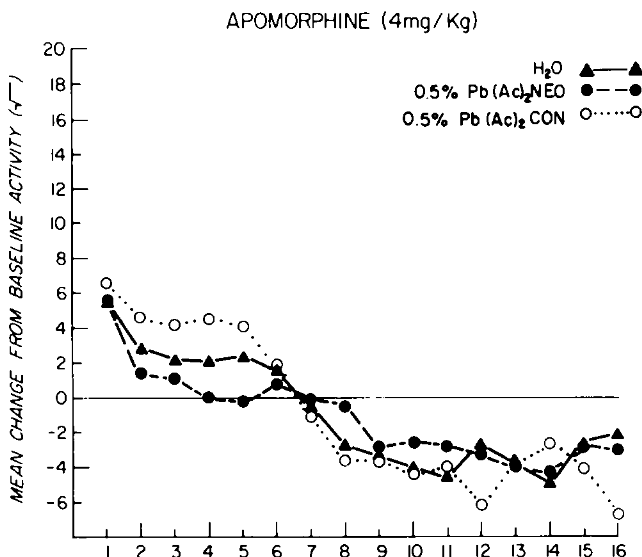

FIG. 4. Activity following injections of apomorphine (4 mg/kg, SC).

activity of treated and control mice were statistically significant at 9 of the 16 intervals. The tendency was for neonatally exposed mice to have larger increases in activity and for continuously lead-exposed animals to have smaller increases than controls. This trend is most clearly shown in the significant differences between lead exposure groups during intervals 8–13. These results were due to the specific effects of d-amphetamine, and not an artifact of handling, since saline injections did not produce differences in activity between treatment groups, $F(2,27)=1.46$, $p>0.10$; $F(30,405)=0.78$, $p>0.10$.

Methylphenidate

Activity following injections of methylphenidate peaked within the first 15 minutes and then declined over the 4 hours of testing, Fig. 3; $F(15,405)=42.36$, $p<0.001$. Unlike their differential response to amphetamine, lead-exposed and control animals did not differ in their response to methylphenidate, $F(2,27)=0.20$, $p>0.10$; $F(30,405)=0.78$, $p>0.10$.

Apomorphine

Apomorphine injections (4 mg/kg SC) caused a transient increase in activity lasting for approximately 75 min (Fig. 4). A significant time×treatment interaction, $F(30,405)=1.92$, $p<0.005$, indicated that this increase did not occur equally for all treatment conditions. Continuously lead-exposed mice were more active than controls, and neonatally-exposed animals were less active than controls. Activity between the continuous and neonatally-exposed mice was statistically significant during intervals 4 and 5, Neuman-Keuls post hoc comparisons: $q(3,120)=1.46$, $q(3,120)=1.39$; $p<0.05$, respectively. At a dose of 6 mg/kg SC neonatally lead-exposed mice again showed an attenuated response to apomorphine during the first 75 min, $F(30,405)=3.15$, $p<0.001$. Post hoc comparisons between the activity of H_2O controls and the 0.5% NEO group were statistically significant at 4 of the 5 intervals in this period, $q(3,120)=6.47, 7.54, 5.77$ and 4.94; $p<0.05$; interval 1:$q(3,120)=1.52, p>0.05$. No statistically significant differences occurred between the activity of the H_2O group and that of the 0.5% CON group.

EXPERIMENT 2

In Experiment 1 it was apparent that the nutritional status of the lead-exposed animals was compromised. This was evident from the reduced fluid consumption of dams drinking 0.5% lead acetate and from the attenuated weight gains of their offspring. Therefore, weight retardation, and not lead exposure per se, may have contributed to the behavioral

effects observed in Experiment 1. To test this hypothesis we conducted a second experiment which explored the relation between weight retardation and activity.

METHOD

Animals and Procedure for Weight Retardation

Mice were raised in either small litters of eight pups (controls) or large litters of 16 pups in order to produce growth rates approximating those which occurred in Experiment 1. Bedding and caging were identical to that in the first experiment. Six time-pregnant Charles River CD-1 mice (Wilmington, MA) served as the initiating pool. Newborn mice were mixed in a common pool and randomly assigned irrespective of sex so that each of two lactating dams suckled 16 pups and each of two lactating dams suckled 8 pups. Excess pups were maintained by the remaining dams and served as replacements for those which died prior to weaning. Replacements were not employed in measurements of weight, activity or drug response. There were no deaths among the small litters and 10 deaths in the large litters. Food (Ralston Purina Mouse Chow) and tap water were provided ad lib to the lactating dam. Daily consumption was monitored. Mice were weighed at six day intervals throughout the course of the experiment. All pups were weaned at 21 days of age.

Apparatus

Locomotor activity was measured in 8 translucent polypropylene mouse cages (Econo-cages®, Fisher Scientific Co.) with dimensions: 29×19×13 cm. The sensing device was a touch (drinkometer) circuit attached to 8 stainless steel plates which lined the cage floor. Passage of an animal across two plates resulted in the conduction of <1 μa of current through the animal's body. "Counts" produced by this method were stored in a solid state memory device identical to that used in Experiment 1.

Procedure

Mice were tested for activity when they were 35–37 days of age. Testing occurred during the light phase of an animal's 12 hr light:12 hr dark cycle. Activity was recorded for two hours following which each animal received a 10 mg/kg IP dose of d-amphetamine. Activity under the influence of the drug was monitored for an additional 2 hours.

RESULTS

Body Weight

Growth curves (Fig. 5) approximated those reported earlier in Experiment 1 and those reported in the literature under similar conditions of lead exposure [15–18]. A three factor ANOVA with repeated measures over time indicated that litter size significantly influenced body weight, $F(1,34)=117.26$; $p<0.001$; pups from larger size litters having depressed body weights compared to pups from litters of intermediate size. A significant age×litter-size interaction, $F(4,136)=34.26$; $p<0.001$, indicated that these differences were exacerbated over time.

Activity

All activity data were square-root transformed in order to

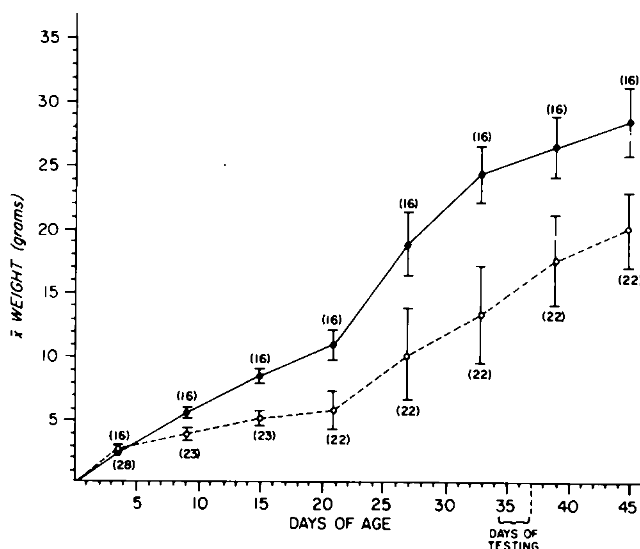

FIG. 5. Growth of CD-1 mice (mean body weight ± SD) raised in small (closed circles) and large (open circles) litters. Parentheses indicate total number of animals contributing to each data point.

normalize the distribution. Two separate ANOVAs were performed; one on the pre-drug data, and one on the post-drug data. Each analysis factored litter-size and sex as between subject parameters, and activity over time as a repeated measure. For the post-drug analysis, data were adjusted to each animal's activity one hour prior to injection.

Spontaneous Activity

The spontaneous activity of all animals (Table 1) decreased throughout the initial 2 hr period of testing, $F(7,238)=93.99$, $p<0.001$. However, the rate of decrease depended upon nutritional status; growth-retarded animals displayed a slower rate of decline over the two hour period than normal-sized animals, $F(7,238)=5.95$, $p<0.001$. This decreased rate of habituation was primarily due to those growth-retarded mice which were male, $F(7,238)=2.21$, $p<0.05$.

TABLE 1

PREDRUG ACTIVITY IN CD-1 MICE RAISED IN LARGE (16 PUP) AND SMALL (8 PUP) LITTERS

Time (Minutes)	Litter Size: Large (L) x̄	SD	Small (S) x̄	SD	Ratio L/S
15	20.09	2.68	22.26	2.74	0.90
30	15.82	2.59	18.62	2.47	0.85
45	14.08	3.62	16.24	3.40	0.87
60	13.46	5.20	14.11	3.46	0.95
75	11.11	5.75	10.83	6.51	1.03
90	8.87	6.82	7.04	6.31	1.26
105	7.83	7.93	2.74	4.41	2.86
120	7.14	7.04	1.38	2.76	5.17

Activity Following Amphetamine Challenge

The activity of all animals increased to a maximum within 30 minutes after injection and then declined over the next 90 min of testing, $F(7,238)=17.59$, $p<0.001$. Growth-retarded mice, as a group, increased their overall activity to a lesser extent than normal-sized mice, $F(1,34)=11.91$, $p<0.01$; growth-retarded *males* showing the smallest increases in activity, $F(1,34)=7.09$, $p<0.02$. The *rate* of decline in activity also differed between normal and growth-retarded mice, the latter having a more rapid decline than the former, $F(7,238)=2.71$, $p<0.001$.

Mice from both large (16 pup) and small (8 pup) litters were combined for an analysis of trend using polynomial regression. Two analyses were performed; one correlated body weight to the activity which occurred during the first hour post-injection and another correlated body weight to the activity which occurred during the second hour post-injection. Scattergrams of body weight and activity are plotted in Fig. 6. A statistically significant quadratic (polynomial of degree 2) relationship was found between body weight and changes in activity for both the first hour, $F(2,35)=14.87$, $p<0.01$; $R=0.68$, and for the second hour post-injection, $F(2,35)=11.00$, $p<0.01$; $R=0.62$.

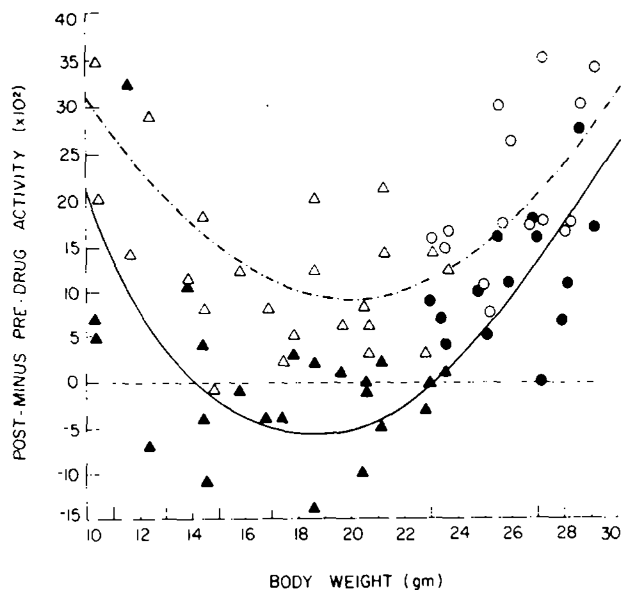

FIG. 6. Relative locomotor activity in mice one (open symbols) and two (closed symbols) hours post d-amphetamine (10 mg/kg) as a function of body weight (triangles represent mice from large litters; circles represent mice from small litters).

EXPERIMENT 3

In Experiment 1 weight loss varied systematically with an animal's lead exposure history. Experiment 2 demonstrated that weight retardation can be responsible for altering an animal's behavioral response both before and after drug treatment. Consequently, it is not possible from the first experiment to establish the causal agent, lead exposure or weight, responsible for the behavioral changes which were observed.

Accurate assessment of the behavioral effects of lead required that they be made in the absence of weight loss from lead exposure. Experiment 3 was designed to accomplish this objective.

METHOD

Animals

As in Experiments 1 and 2, time-pregnant CD-1 mice were procured from Charles River Breeding Labs, Inc. (Wilmington, MA) on Day 14 of gestation. Cage facilities and bedding were also identical to that of the earlier experiments. Dams were housed under a 12 hr light: 12 hr dark cycle; the light phase commencing at 7:00 a.m.

At parturition litters were culled to 10 pups, irrespective of sex and weight. Following weaning, littermates of the same sex were group housed for a major portion of the experiment. Exceptions are noted below under *Procedure*.

Exposure Protocol

Pregnant and lactating dams were maintained on an open diet prepared commercially by Ziegler Bros. This diet protocol was instituted to provide a stable dietary matrix from which lead could be absorbed in the gastrointestinal tract of the dam. This diet also compares more favorably with the National Research Council's recommended requirements [11] than do oversupplemented commercial mouse diets. Composition of the diet (Catalog No. 39–226) can be obtained from Ziegler Bros. (Gardner, PA, Box 95).

At parturition each of 30 dams and their litters were randomly assigned to one of 3 treatment groups. Each treatment group was composed of 10 litters. Dams were provided access to one of the following drinking solutions: deionized water, 0.02% (109 ppm Pb), or 0.10% (546 ppm Pb) lead acetate. At 21 days of age pups were weaned to the Ziegler diet and deionized water. At 42 days of age the semipurified diet and deionized water were removed and mice were given ad lib access to tap water and commercial food (Ralston Purina Mouse Chow) for the remainder of the experiment.

Apparatus

Activity was measured in the same devices used in Experiment 2.

Procedure

Testing at ages 22–23, 42–43 and 62–63 days of age was performed during the light phase of an animal's light:dark cycle. At ages 110–134 testing was initiated 60–90 min into the dark phase of an animal's light:dark cycle.

Testing at age 22–23 days of age. The activity of 10 mice from each exposure group was measured when they were 22–23 days of age. Each animal was tested once for a period of 4 hr and 15 min. Following the first 2 hr, animals were injected with 10 mg/kg IP of d-amphetamine and their activity monitored for another 2 hr and 15 min. Assignment to the eight test cages was determined by a Youden square design. Unlike Experiment 1, an intermediate holding facility was not used, rather, animals were transferred directly from their group housing to the test chamber.

Testing at 42–43 days of age. A similar procedure was followed in testing 42–43 day old littermates of the above animals, except that the interval during which the post-drug activity was monitored was increased by 45 min.

Testing at 62–63 days of age. The same testing protocol

was again followed for 62–63 day old littermates. However, instead of d-amphetamine, these animals received injections of phenobarbital (20 mg/kg). The observational period was also extended from 2 to 3 hr pre-injection and from 3 to 4 hr post-injection.

Testing at 110–134 days of age. Activity tests were again conducted when animals were 110–134 days of age. One-half of the animals had previously been tested (at either 42–43 or 62–63 days of age) and one-half were unfamiliar with the testing situation. Each animal was tested four times in the same test cage, receiving either 0 (vehicle), 2.5, 5.0 or 10.0 mg/kg, IP d-amphetamine at each session. Since only eight mice could be tested at a time, animals were distributed among the activity chambers over a period of six sessions. An equal number of experienced and nonexperienced animals were tested in each session. After three sessions, each test cage had received animals from each lead exposure group (balanced incomplete block design). The order in which these three groups (0.02%, 0.10% and H_2O) were tested was varied from one cage to another using a Youden square design. The remaining three sessions employed the same sequence with animals of opposite testing experience occupying the cages. The order in which the 3 doses of d-amphetamine and one dose of vehicle were administered was balanced using a Latin Square Design. Testing was intended to be conducted on an equal number of mice in each treatment condition. However, animal deaths and equipment malfunctions reduced the actual number of animals tested at all 4 doses of amphetamine to 9 (controls), 17 (0.02%) and 11 (0.10%). The minimum number of litters represented by each treatment group was 6.

RESULTS

Lead Determinations

Lead analysis was performed on samples of blood or brain pooled from 3–6 mice at 21 days of age. Two or three pooled samples were separately assayed for each treatment.

Blood lead levels for controls were 8.3 ± 3.8 μg/100 ml (mean ± SD). Pups which suckled from dams exposed to 0.02% $Pb(Ac)_2$ had blood lead values of 63.5 ± 12.0 μg/100 ml and pups which suckled from dams exposed to 0.10% $Pb(Ac)_2$ had blood lead values of 67.5 ± 9.2 μg/100 ml. The corresponding concentrations of lead in the brains of these animals were 0.13 ± 0.02, 0.24 ± 0.02 and 0.36 ± 0.02 ppm, respectively.

Body Weight

An ANOVA of mean pup weights with repeated measures at 1, 14, 21 and 28 days of age indicated neither any statistically significant differences in overall body weights among the 3 treatment groups, $F(2,20)=0.34$, $p>0.10$, nor a statistically significant interaction between body weight and age, $F(8,80)=0.14$, $p>0.10$.

A second, between groups, ANOVA on individual pup weights at 23, 42, 62 and 110 days of age in mice tested for activity did reveal statistically significant differences between treatment groups, $F(2,85)=4.08$, $p<0.02$. Post hoc Neuman-Keuls comparisons indicated that animals in the 0.02% exposure group were generally heavier than either controls, $q(1,60)=4.41$, $p<0.05$, or mice in the 0.10% exposure group, $q(2,60)=2.91$, $p<0.05$. The magnitude of this increase in weight was small, however, being only a 5.6% increase above the mean weight of control animals.

Spontaneous Activity

Separate analyses of variance were performed on the spontaneous (pre-drug) activity of 22–23, 42–43 and 62–63 day old animals. Overall activity at any of these ages was not significantly affected by lead treatments, $F(2,27)=0.24$, $p>0.10$; $F(2,13)=0.86$, $p>0.10$; $F(2,24)=0.23$, $p>0.10$, respectively, nor were there significant differences between treatment groups in spontaneous activity at particular intervals within a test session, $F(14,189)=0.95$, $p>0.10$; $F(22,143)=0.67$, $p>0.10$; $F(22,264)=0.71$, $p>0.10$, respectively. Thus, lead-exposed mice had spontaneous activity levels at ages 22–23, 42–43 and 62–63 days which were indistinguishable from those of control animals.

At 110–134 days of age mice were tested over four sessions where each session was separated from one another by five days. Analyses of variance were computed for the spontaneous activity occurring in each session. A statistically significant increase in activity occurred in lead-exposed mice, as compared to control mice, during the second session, $F(2,41)=4.24$, $p<0.05$. Similar trends (see Fig. 7) were evident during sessions three and four, but these were not statistically significant, $F(2,39)=2.24$; $p>0.10$; $F(2,38)=2.55$, $p>0.10$, respectively.

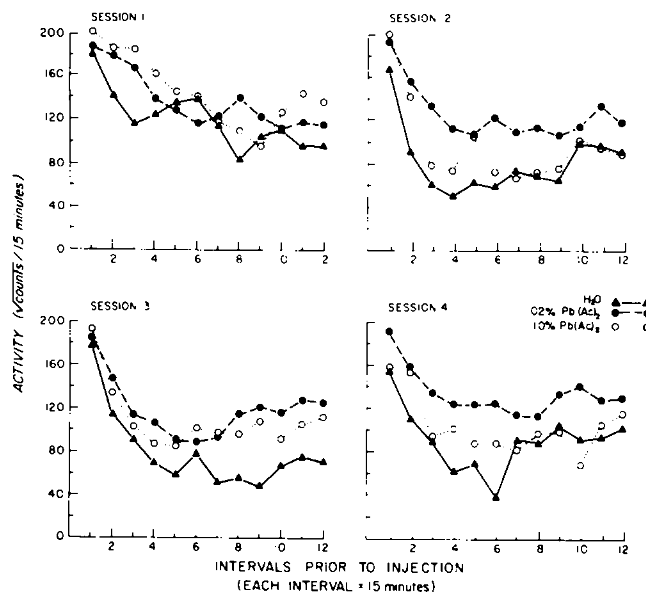

FIG. 7. Spontaneous (pre-drug) activity of 110–134 day old mice over four sessions.

Post-Drug Activity

Ages 22–23. A decreased response to d-amphetamine (10 mg/kg IP) noted in 22 and 23 day old mice failed to be statistically significant, $F(2,27)=0.44$, $p>0.10$.

Ages 42–43. An analysis of variance on the d-amphetamine-induced activity of 42–43 day old animals indicated that changes in activity produced by amphetamine were time dependent, $F(12,156)=34.79$, $p<0.001$, and that these interacted with treatment conditions, $F(24,156)=1.62$, $p<0.05$; see Fig. 8. The intervals in which treatment groups differed were identified using the Neuman-Keuls method [24] for post hoc comparisons. Activity in the 0.02% group

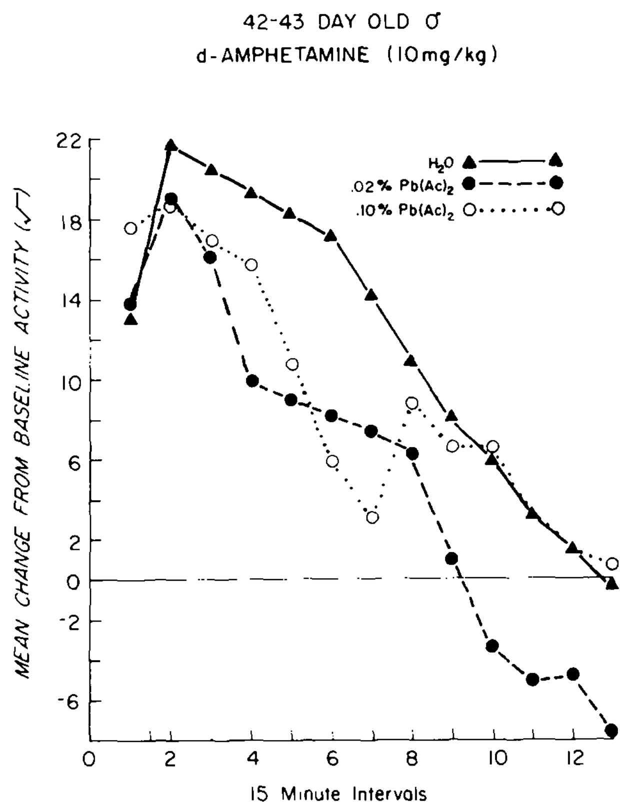

FIG. 8. Activity in 42–43 day old mice following d-amphetamine (10 mg/kg).

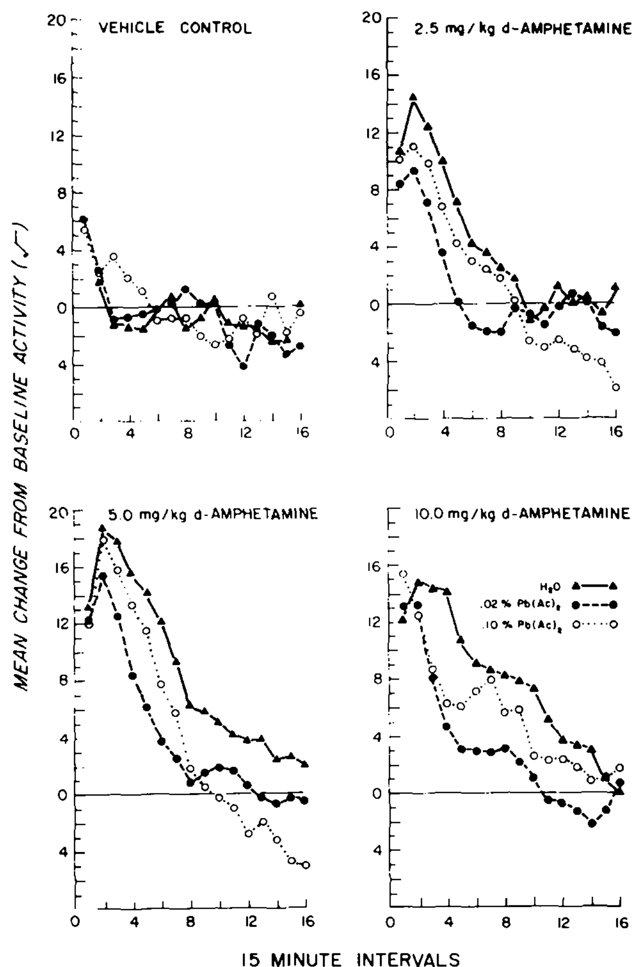

FIG. 9. Activity in 110–134 day old mice following injections of d-amphetamine (0, 2.5, 5.0 and 10.0 mg/kg).

differed significantly from controls during the 4th through 7th, q(2 or 3,120=4.21, 4.10, 4.07, 3.05; $p<0.05$, and 10th through 13th, q(2 or 3,120)=4.55, 3.91, 2.89, 3.31; $p<0.05$, postinjection intervals. Differences between the 0.02% and control groups in the remaining intervals failed to achieve magnitudes which were statistically significant. However, the trend was invariably toward a reduced amphetamine response. Post hoc comparisons between the activity of 0.10% and control animals revealed significant differences in activity only during intervals 5 through 7, q(2 or 3,120)=3.32, 5.08, 4.90; $p<0.05$.

Ages 62–63. Animals in all treatment groups responded similarly to a single challenge with phenobarbital, $F(2,24)=0.16$, $p>0.10$; decreasing their activity over the 4 hr period of observation, $F(15,360)=31.21$, $p<0.001$.

Ages 110–134. At 110–134 days of age, mice were tested for drug response with 4 doses of d-amphetamine: 0 (vehicle control), 2.5, 5.0 and 10.0 mg/kg IP (see Fig. 9). Analyses of variance, computed separately for each dose, indicated a significant treatment effect at the 5.0 mg/kg dose, $F(2,40)=3.76$, $p<0.05$, and a significant treatment by interval interaction at the 2.5, $F(30,600)=2.17$, $p<0.01$, and 5.0 mg/kg dose, $F(30,600)=1.64$, $p<0.02$. Differences in activity between 0.02% and control animals were compared using Neuman-Keuls tests. The activity of 0.02% animals was found to be significantly less than that of controls during intervals 2–8 and 3–8 at the 2.5 and 5.0 mg/kg doses, respectively. Control and 0.10% mice differed in activity following a 2.5 mg/kg dose of d-amphetamine only during interval 16. At a dose of 5.0 mg/kg, the activity of 0.10% animals was significantly less than controls during intervals 9–16. Activity between 0.02% and 0.10% animals did not generally differ in sufficient magnitude to be statistically significant from one another.

DISCUSSION

As one means of assessing the effects of lead on CNS development we have observed the activity of animals at various ages. In addition, we have challenged mice with pharmacological agents and observed the resultant changes in activity which accrue. We were generally unsuccessful in confirming earlier reports that hyperactivity occurs in lead-exposed mice [18–21]. Typically, we found that lead-exposed animals had levels of activity which were no different from those of control animals. Only in Experiment 3, in one of four sessions, was a slight increase in activity (52%) detected in mice exposed to lead (109 ppm to the dam). However, the magnitude of this effect, and the circumstances under which it could be elicited, were quite different from those previously described [18–21] where increases of 400% were noted.

Failure to observe hyperactivity following lead exposure

has also been reported for rats [10] in a study which exerted good experimental control over undernutrition, litter size and litter representation. Perhaps, as suggested in the introduction, these extraneous variables are more salient determinants of hyperactivity then is lead exposure per se. If so, then the use of lead exposed rodents as an animal model of childhood hyperactivity must be seriously questioned.

Lead did have pronounced effects on activity produced by drug challenges. We have viewed two types of alterations in activity following injections of the stimulant drug, d-amphetamine. These depended upon the dose and time course of the lead exposure. Mice exposed to lead neonatally (during the suckling period only) showed an attenuation in their response to d-amphetamine, increasing their level of activity to a lesser extent than controls. This effect predominated over much of the developmental history of animals, occurring at 22, 42 and 110 days of age. The largest effects occurred at older ages where blood lead concentrations were approximating those of controls. This attenuated response was not linearly related to external lead dose. Effects of the greatest magnitude were seen under conditions of lowest exposure (109 ppm Pb), while intermediate exposure (546 ppm Pb) produced little or no effects. At the highest lead exposure employed (2730 ppm Pb), we saw a slight augmentation in response to d-amphetamine. Further complicating an interpretation of these findings was the fact that high lead exposure, given over a more extended time course resulted in an attenuated response to d-amphetamine.

We were unable to confirm previous reports [18, 19, 21] that the stimulant drug methylphenidate depresses the activity of lead-exposed mice, nor did we observe an attenuated response to this drug. We were also unable to confirm previous reports [15,16] that phenobarbital increases the activity of lead-exposed mice [18,19].

Apomorphine, a dopaminergic receptor agonist, differentially altered the activity of lead-exposed and control animals. Continuously lead-exposed mice (the 0.5% CON group) showed a slight augmentation in response to a 4 mg/kg dose of this drug. This finding is generally consonant with a previous report [21], although the magnitude of effect previously observed was much greater. At a dose of 6 mg/kg this effect was not observed. In addition, neonatally-exposed mice (the 0.5% NEO group) demonstrated less activity than control animals at either dose of apomorphine.

Two disclaimers must be raised for any interpretation of the present results: (a) it was evident from the results of Experiment 2 that weight retardation, and not lead per se, may, at least partially, account for some of the behavioral effects observed at the larger doses and longer durations of lead exposure, and (b) some preliminary data [2] suggest that lead exposure does not have to alter the growth rate of suckling neonates to produce nutritional deficits. At two levels of lead exposure which do not alter the food or water consumption of lactating dams (0.02% and 0.20% $Pb(Ac)_2$ in H_2O) there is a depression in the zinc and copper concentrations of milk (33%, 28%, respectively) during the early stages of lactation. The essential role of these trace elements in brain development and function [7] suggests that such deficits may contribute to behavioral alterations later in life. These factors must be taken into consideration when interpreting the present findings.

Several litters, balanced across treatments, eliminated many of the biases evident in previous experimentation where little consideration was given to a sample's genetic composition. It is increasingly evident, however, that experimental designs need to incorporate an even larger number of litters and a larger number of offspring within a litter to obtain estimates of intra- and inter-litter variability. Through such estimates, susceptible subgroups can be discerned; a prerequisite to identifying parameters which interact with toxic exposure to produce behavioral and physiological alterations. Given these caveats, there is, nevertheless, one interpretation of the present results which remains inescapable: as demonstrated by response to centrally active drugs, exposure to relatively low levels of inorganic lead via the maternal milk supply can have subtle effects on the physiological and functional integrity of an organism long after the exposure has been terminated.

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