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Reversal Learning Deficits in Young Monkeys Exposed to Lead[1]

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BUSHNELL, P. J. AND R. E. BOWMAN. *Reversal learning deficits in young monkeys exposed to lead.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 733–742, 1979.—The reversal learning capacity of young rhesus monkeys in visual discrimination tasks was examined during daily exposure to dietary lead acetate throughout the first year of life. While not affected in physical development, all lead-treated monkeys showed performance deficits on reversal learning tasks. These deficits were independent of lead-induced changes in motivation. Over a series of problems, the overall learning rate of monkeys with blood lead concentrations in the range of 70–90 μg/dl was retarded, which resulted partly from a pronounced difficulty in attaining criterion on the first of a series of reversals within a given problem. This latter deficit resulted from an increase in errors, balks, and total trials to criterion on the first reversal. Monkeys exposed to blood lead concentrations of 40–60 μg/dl required significantly more trials to finish all problems, but did not show the first-reversal deficit. Theoretical implications of these data were discussed.

Lead acetate Chronic lead exposure Visual discrimination Reversal learning sets
Cognitive development Rhesus monkey

HIGH doses of lead are known to produce pronounced learning impairment in the human [29,44]. However, the possible role and threshold levels of chronic, low-level lead exposure in the production of learning decrements is less well substantiated. The evidence for detrimental psychobiological effects of subclinical exposure in humans is correlative and is based primarily on studies of children adeventitiously intoxicated with environmental lead. Such studies sometimes have noted a negative correlation between performance on intelligence tests and various indices of lead burden [6, 31, 35] and sometimes have not [19, 25, 28].

Experimental evidence linking learning deficits to low-level intoxication with inorganic lead has been based mainly on rodents, and has generally found them to be relatively resistant to such effects if exposed after weaning. Thus, no learning deficits were noted in lead-exposed adult rats trained in a water maze [5] or in a Hebb-Williams maze [41]. On the other hand, increased response variability has been seen in lead-exposed adult rats [40] and sheep [46], and decreased spontaneous alternation has been noted in lead-intoxicated adult rats [26], thereby indicating that the behavior of the adult rodent may not be totally resistant to low-level lead exposure.

By contrast, adult rats exposed perinatally to inorganic lead have usually shown detrimental psychobiological effects. Such animals were retarded in visual discrimination learning and reversal [15], maze learning [3, 4, 41, 49], two-way avoidance learning and reversal of an operant discrimination [42], learning a tactual discrimination reversal [34], and were deficient in operant response inhibition [34]. Sobotka *et al.* [42] postulated a deficit in the inhibition of inappropriate responses as a mechanism for such lead-induced learning deficits. This hypothesis would suggest that lead exposure during development might impair the final maturation of central inhibitory processes involved in learning.

The nonhuman primate has received attention recently as a model for human inorganic lead poisoning [7, 10, 36, 37, 48]. This paper reports the effects of chronic, low-level, inorganic lead intoxication on reversal learning in infant rhesus monkeys.

The rationale for including reversal learning sets in a behavioral toxicology battery was considerable. First, the formation of learning sets [20] requires sophisticated cognitive abilities [1,38]. Second, such tasks offer a high potential for differentiating intellectual from performance deficits [15]. For example, performance deficits (e.g., sensory or motor impairment) should be manifested generally at all stages (reversals) of this paradigm, whereas cognitive processes might differ across the stages of learning so that cognitive impairment could affect some reversals more than others. Third, variations on this simple learning set procedure may be used with rhesus monkeys from 90 days of age through maturity [21]. Fourth, the paradigm has been analyzed theoretically in terms of cognitive processes to explain the overtraining reversal effect or ORE [43]. Fifth, reversal learning sets can be

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obtained on a wide range of species, from the rat to the human, and the efficiency of reversal learning has been empirically related to phylogenetic status [1,38], thereby offering some basis for extrapolating toxicological deficits between species. Finally, reversal learning would seem to emphasize or focus inhibitory learning processes at certain stages of the training, especially at early reversals within the learning set. If there is a basis to the hypothesis that lead exposure during development interferes with the final maturation of central inhibitory competence, then reversal learning sets, and early reversals especially, could be particularly sensitive to the neurobehavioral toxicity of lead.

The definition of low-level lead exposure in the primate can be made on the basis of the appearance of early clinical signs of lead toxicity, such as appetite loss, weight loss or decreased weight gain, effects on certain enzymes in the pathway for heme synthesis, and decreased hematocrit. In the human, the designation of undue lead absorption by the U.S. Center for Disease Control is based upon hematological criteria and the possibility of functional impairment of CNS processes. In children 1 to 5 years of age, a blood lead concentration about 30 μg/dl is presently cause for clinical concern, and exposure above 80 μg/dl is considered cause for therapeutic treatment [9].

Based on these designations, the experimental monkeys in the present study were fed daily doses of lead acetate sufficient to elevate their blood lead levels into the possibly psychotoxic range between 30 μg/dl and about 100 μg/dl, at which latter point the earliest clinical symptoms (such as reduced appetite and hematocrit) become evident. These levels were maintained throughout the first year of life, during which time many of the neurobehavioral capacities of these animals normally develop [21,50]. Reversal learning was examined within this period to determine the possible behavioral toxicity of concurrent lead exposure. Assessment of possible residual effects of this lead exposure, obtained in later years of life after blood lead levels have normalized, is currently in progress.

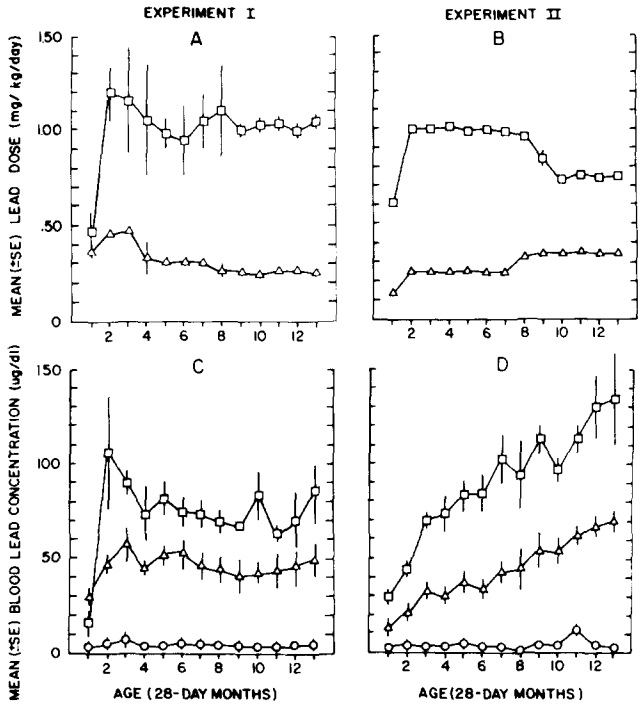

FIG. 1. Mean (± SE) lead dose and mean (± SE) observed blood lead concentration (PbB) values for all groups in Experiments 1 and 2 over 13, 28-day months of the treatment year. A. Experiment 1, lead doses: △, low-lead; □, high-lead. B. Experiment 2, lead doses: △, low-lead; □, high-lead. C. Experiment 1, PbB values: ○, control; △, low-lead; □, high-lead. D. Experiment 2, PbB values: ○, control; △, low-lead; □, high-lead. All SE values less than 0.01 for lead doses, and all SE values less than 1.0 for PbB values, are omitted for clarity.

EXPERIMENT 1

METHOD

Animals

Twelve infant rhesus monkeys were separated from their mothers within 72 hours following birth, were reared in individual cages with cloth diapers as surrogate mothers according to standard procedures [2], and were given daily 2-hr socialization sessions in groups of 4–6 animals throughout the first year of life. Three sex-balanced groups of 4 animals each were formed by random assignment at birth: *control* (no added dietary lead), *low-lead* (target blood lead concentration=50 ± 10 μg Pb per dl whole blood), and *high-lead* (target blood lead concentration=80 ± 10 μg/dl). Because of unexpectedly efficient absorption of lead by the neonatal monkey, one male monkey in each of the two experimental groups was lost due to lead overdosing in the first three months of the experiment.

Apparatus

All learning tests were carried out in a semiautomated Wisconsin General Test Apparatus, or WGTA [13]. A stationary formboard, food reward, and small wooden objects as stimuli were used.

Procedure

Dietary. Each animal was offered 100 cc of a milk formula (Similac with iron, Ross Laboratories, Columbus, Ohio) daily at 0800, and additional feedings of 100–250 cc each at 1200, 1600 and 2000 hr. Consumption of this formula averaged 390 cc/day/animal over the year. At 90 days of age, a standard laboratory chow (Purina Monkey Chow, 12% protein, Ralston Purina Co., St. Louis, Missouri) was added to the diet daily at 1500 hr. All animals had normal growth rates, and no lead-induced differences in body weight or physical development were observed.

Lead dosing. Lead acetate was administered to experimental animals in the 0800 milk feeding daily for one year beginning no later than 3 weeks after birth. Initial lead doses of 0.53 ± .02 (SE) and 1.15 ± .31 (SE) mg Pb/kg/day (0.0074 and 0.0161 mg/ml of Pb in milk for the low- and high-lead groups, respectively) were adjusted as necessary, but not more often than once per week, to maintain target blood lead concentrations. Group-mean lead doses (Fig. 1a) and blood lead concentrations (Fig. 1c) over the year are detailed elsewhere (Bushnell, Bowman and Allen, manuscript under review).

Behavioral. At 60 days of age, the monkeys were adapted in the WGTA to an appetite board containing 45 food wells

baited with candy, raisins, and marshmallows. Rates of food retrieval for each animal were determined at that time and subsequently at 20 and 40 weeks of age to monitor food motivation. When each animal took all the bait within a 20 min period, it was next adapted to the movement of the opaque and transparent screens of the WGTA and trained to displace practice objects from a two-foodwell tray to obtain the food rewards.

Reversal learning sets were obtained next as follows. In original learning (OL), each animal was trained on a two-stimulus discrimination to 90% correct on two consecutive 50-trial sessions (strict criterion). The reinforcement contingencies were then reversed, and the monkeys were retrained to a criterion of 9 correct responses in any ten-trial block (standard criterion), following which a second reversal was immediately begun, and so on through seven reversals. The correct stimulus was rewarded on every trial and the incorrect stimulus was never rewarded. Animals were trained in one 50-trial session per day, 3–5 days per week, between 0800 and 1500 hr. Within a session, each trial began with the removal of an opaque screen followed 2 sec later by removal of a transparent screen. The monkey was permitted to push only one stimulus (non-correction) to uncover the foodwell and obtain any reward present. The trial was terminated by interposition of the two screens. Intertrial intervals were 10 sec and any response latency exceeding the maximum trial duration of 30 sec was scored as a balk.

Three reversal problems were administered between 5 and 10 months of age. In Problem 1, two identical gray wooden blocks measuring 2.5×8×4 cm in width, depth and height were used, and the same position on the formboard was rewarded on each trial. For original learning, half the animals in each group were trained to the right side and half to the left. In Problem 2, one orange and one blue wooden block (each 5×5×3.4 cm) replaced the previous stimuli, and the monkeys were trained on a color discrimination, half to blue correct and half to orange correct in original learning. The positions of the stimuli associated with reward were randomized across trials. In Problem 3, the monkeys were trained on a size discrimination: two blue wooden blocks, measuring 5×5×3.5 cm and 3.5×3.5×3.5 cm, respectively, presented as in Problem 2, were used. For half the animals in each group the large object was correct in original learning; for the other half, the small object was correct.

Data analysis

The number of sessions to complete the entire three-problem series was analyzed nonparametrically (Kruskal-Wallis test [12]), due to significant heterogeneity of variance [11]. Frequencies of balks, errors, and total trials to criterion (including balks), all of which exhibited homogeneity of variance [11], were each subjected to an unweighted-means analysis of variance (ANOVA) with groups as a between-subject factor and problems and reversals, including OL, as repeated measures [32]. OL was included since effects of lead on this stage of learning could not be ruled out a priori. If main effects of lead treatment on any dependent measure were found to be significant, then experimental means were compared to the control mean by the procedure of Dunnett [16,17]. Significant interactions in overall ANOVAs were further analyzed by tests of simple main effects [24], which partitioned out effects due to lead on original learning and on each of the reversals separately, and by Dunnett's tests for group mean differences.

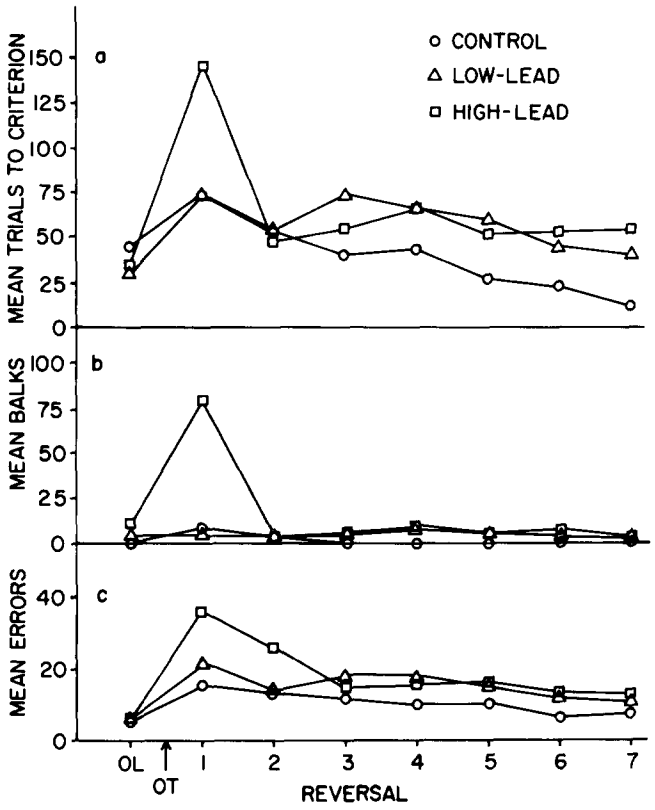

FIG. 2. Reversal learning sets obtained in the WGTA from each group of Experiment 1. Mean trials to criterion (a), mean balks per reversal (b), and mean total errors per reversal (c), averaged over three successive problems, are plotted as a function of reversals. OL=original learning; OT=overtraining.

Food retrieval rates on the appetite board tests were cal- 50-sec intervals for the first 300 sec of the 1200 sec test. culated from the number of food bits taken in successive Slopes of least-squares regression equations, relating elapsed time with the number of food bits taken, were computed for each animal and compared across treatments by a one-way unweighted-means ANOVA, or analyzed for covariation with performance variables on learning tasks.

RESULTS

At 20 and 40 weeks of age, the treatment groups did not differ in the rate of food retrieval in the appetite test, either before or after a 200 cc milk feeding. Nevertheless, the learning rates of both experimental groups were significantly retarded, as indicated by an increase in the number of sessions needed to complete the problem series (Kruskal-Wallis $H=6.71$, $p<0.013$: control mean ± SE=31.25 ± 0.95: low-lead, 53.00 ± 7.23: high-lead, 62.67 ± 9.53).

All groups required more trials to complete Problem 2 (color) than Problem 1 or 3 (mean trials per reversal: color, 100.3; spatial, 31.2; size, 55.0), $F(2,14)=10.03$, $p<0.005$. However, this variable did not interact with lead treatment, (lead by problems interaction $F[4,14]=0.63$: lead by problems by reversals interaction $F[28,98]=0.57$), so all further analyses considered data collapsed across problems. Across the three problems combined, all three high-lead animals

took more trials to learn Reversal 1 than did the other monkeys (Fig. 2a). The significant lead by reversals interaction, $F(14,49)=2.77$, $p<0.01$, was due to a significant effect of lead treatment at Reversal 1 only, $F(2,56)=8.38$, $p<0.001$. There was no lead effect on OL alone or on any other reversal tested separately. On Reversal 1, only the high-lead group differed from control. Across the reversal series, performance changed significantly for the control, $F(7,49)=4.37$, $p<0.005$, and high-lead, $F(7,49)=10.32$, $p<0.001$, groups. The lack of change in the low-lead group probably reflected both the absence of a peak in trials to criterion on Reversal 1 and their lack of improvement in performance after Reversal 2.

Third, the high-lead group (Fig. 2b) balked significantly more than the other two groups, $F(2,7)=7.01$, $p<0.025$, particularly at Reversal 1, as indicated by a significant lead by reversals interaction, $F(14,49)=24.86$, $p<0.001$, and a significant increase in balks by the high-lead group only at Reversal 1, $F(2,56)=112.80$, $p<0.001$. In addition, only the high-lead group showed a decrease in balk frequency after Reversal 1, $F(7,49)=75.75$, $p<0.001$.

Fourth, there was a dose-related increase in errors (Fig. 2c) across all reversals and problems combined, $F(2,7)=7.76$, $p<0.025$, but while the high-lead mean (18.01 ± 1.70) was significantly, $p<0.05$, greater than the control mean (10.31 ± 1.16), the low-lead mean (14.60 ± 1.49) was not. Across the reversal series, the high-lead group's errors were concentrated at Reversals 1 and 2, as indicated by a significant lead by reversals interaction, $F(14,49)=1.95$, $p<0.05$, with significant group differences at Reversal 1, $F(2,56)=13.43$, $p<0.001$, and at Reversal 2, $F(2,56)=5.79$, $p<0.01$, in which the high-lead group, but not the low-lead group, made more errors than the control group.

DISCUSSION

Exposure of infant monkeys to lead clearly interfered with their ability to form normal reversal learning sets. The low-lead group was significantly retarded in its learning rate over all problems and reversals, though its retardation on any given reversal or problem was insigificant by itself. Apparently, it was the accumulation of many nonsignificant increments in trials to criterion, reversal by reversal, which resulted in the significant overall retardation of learning rate. Retardation of the learning rate of the high-lead group resulted both from this accumulation of trials to criterion and from extreme difficulty with the first reversal in each problem, as evidenced by a significant increase in trials to criterion only on Reversal 1 (Fig. 2a). This latter effect was due in part to increased balking (Fig. 2b), yet these animals also emitted more errors than did the controls (Fig. 2c) on Reversals 1 and 2.

While transient within problems, this learning impairment recurred at each instance of a first reversal with a new pair of stimuli. The reasons for this recurrence are not clear; it is nevertheless noteworthy that impaired reversal learning was observed only on reversals following overtraining. Since overtraining has been shown to facilitate reversal learning in rats [45], it was possible that the high-lead monkeys did not benefit from overtraining as the controls and low-lead animals presumably did, and that the first-reversal deficit noted here reflected the performance of functionally nonovertrained animals.

The extensive balking in the high-lead group on Reversal 1 suggested the further possibility that the learning deficit was mediated by motivational impairment. However, no group differences in food retrieval rates on appetite tests could be demonstrated, nor did these rates correlate with balk frequencies in any problem. Balking by the high-lead animals on Reversal 1 reflected rather their tendency to give up after a series of unrewarded responses to the incorrect object; indeed, some of these animals required additional shaping at this time to reinstate the block-pushing response. It was as if their response to the originally-correct object had extinguished, but responding was not transferred to the other object, as normally occurs in this paradigm.

EXPERIMENT 2

Experiment 2 was designed to address two questions regarding the learning deficits observed in Experiment 1: (1) was the first-reversal deficit related to a motivational impairment? and (2) was overtraining involved in the expression of the deficit? Three reversal learning set tests were administered in Experiment 2. Test 1 consisted of a spatial reversal task in which contact with the animal's surrogate mother, rather than food, was used as the reinforcing agent, to determine whether the first-reversal deficit could be observed in the absence of food reinforcement. Test 2 was designed to assess the effect of overtraining, and Test 3 to provide additional information regarding the role of appetite and properties of the discriminative stimuli in the expression of the first-reversal deficit.

TEST 1

High doses of lead may suppress appetite by inducing stomach irritation, nausea, and malaise [8]. Mild forms of these symptoms might have reduced the appetite of the high-lead animals in Experiment 1 sufficiently to impair their performance on a demanding task such as reversal learning, but not on a simple task such as retrieving food bits from an uncovered tray. A reversal learning paradigm not involving food reward was thus devised to determine the specificity of the first-reversal deficit for food-motivated responses.

Infant rhesus monkeys exhibit strong affectional responses to cloth-covered surrogate mothers [22], and these surrogates demonstrate many of the properties of reinforcing stimuli. Indeed, in a free-choice test, nearly 100% of the surrogate contact of infant monkeys was directed toward a cloth-covered surrogate, as compared to a bare-wire surrogate, even when all of the animals' nourishment was derived from the wire surrogate [23]. To our knowledge, surrogate contact has not previously been used as a reinforcing agent in a discrimination learning paradigm. It seemed, however, to present an alternative to food reward in infant monkeys whose appetite for food might be disturbed by lead ingestion.

METHOD

Animals

Twelve infant rhesus monkeys, born the year following those in Experiment 1, were assigned to three treatment groups as defined in Experiment 1, with 2 males and 2 females per group. They were treated according to the procedures described for Experiment 1, with the following exceptions. Milk formula intake was restricted to 300 cc/day, divided into two daily feedings of 100 cc at 0800 hr (containing lead for experimental animals) and 200 cc at 2000 hr. Lead dosage and physiological parameters have been detail-

ed elsewhere (Bushnell *et al.*, manuscript under review). To avoid problems of overdosing, and because the lead doses administered at the end of Experiment 1 were maintaining blood lead levels in the target range, Experiment 2 was begun using these same dose levels. Thus, the high-lead group in Experiment 2 received lead at 1.06 ± .03 (SE) mg/kg/day and the low-lead group at 0.25 ± .01 (SE) mg/kg/day for the first 7 months, after which the high dose was adjusted downward and the low dose upward (Fig. 1b) to control the blood lead levels which are shown in Fig. 1d. Physical development was normal for all animals and no group differences in body weight were observed.

Apparatus

The Sackett Self-Selection Apparatus [39] was adapted for use as a two-choice maze by installing opaque doors such that a 152 cm path lead from a trapezoidal start box (with floor dimensions 81×33×33 cm and height 71 cm) through a central compartment (a hexagonal prism measuring 33 cm on a side and 71 cm in height) to one of two concealed side compartments (of the same dimensions as the start box), designated the left and right goal boxes. To facilitate the locomotion of the infant monkeys, the floor bars of the apparatus were covered with pressboard panels.

Procedure

Beginning at six weeks of age, each monkey and its diaper were placed in the maze with several familiar peers and their diapers. The animals were allowed to explore for about 30 min on three consecutive days. Next, each monkey was shaped individually over several sessions to find its diaper in the maze by separating the animal from its diaper and allowing it to run through the maze to the diaper over increasingly greater distances, until it would run from the start box to a diaper concealed in either goal box. A final 10-trial shaping session was then administered with diapers in both goal boxes. Each animal's side preference was defined as the side chosen on six or more trials in that session.

For discrimination training, each monkey was separated from its diaper, placed alone in the start box, and restrained by a transparent plastic door. Its diaper was then concealed in the goal box on its nonpreferred side only. After 5 sec, the door was raised by remote control and the infant's movements were monitored from overhead by closed-circuit television. A correct response was scored if the monkey's head first crossed the threshold of the goal box containing the diaper, an error was scored if its head first crossed the threshold of the empty goal box, and a balk was scored if its head crossed neither threshold within 180 sec after raising the start box door. The monkey was allowed to find its diaper, and to remain with it for 60 sec, on each of ten daily trials. After a balk, the animal was placed by hand on the diaper and left for 60 sec. The intertrial interval was about 15 sec. Response latencies were measured from the raising of the start box door to contact with the diaper. Learning criteria were: for original learning, two consecutive ten-trial sessions at 90% or better correct responding (modified strict criterion); for each of seven reversals, 9 correct responses in any ten-trial block (standard criterion).

Data Analysis

Response latencies, error frequencies, and total trials to the standard criterion were counted for each animal at OL and each reversal and analyzed in separate two-way ANOVAs with groups as a between-subject factor and reversals as a repeated measure. Balks were not analyzed due to their low frequency.

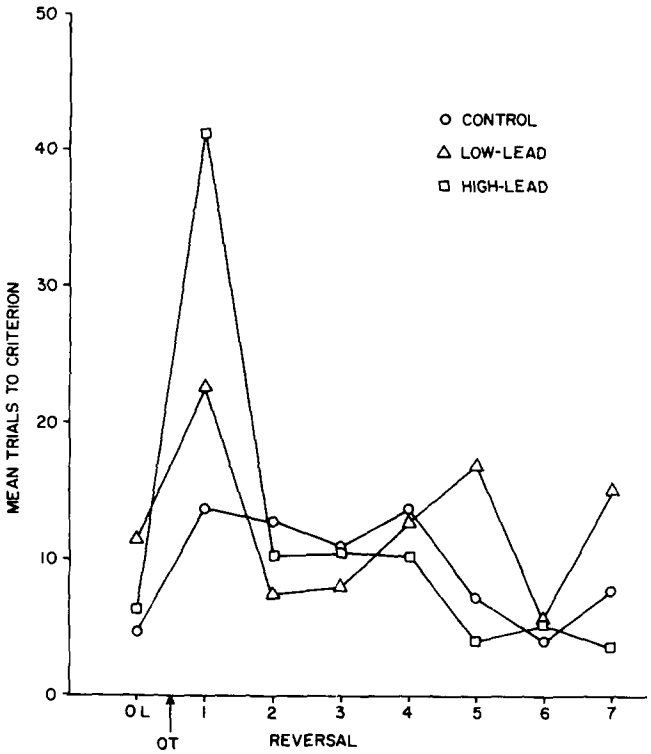

FIG. 3. Reversal learning sets obtained from the three groups of Experiment 2 in the two-choice maze with surrogate mother contact as reward for correct responses. Mean trials to criterion are plotted as a function of reversals for each lead group. Abbreviations as in Fig. 2.

RESULTS

Diaper contact proved to be a reliable motivator for learning in this apparatus, as the animals ran quickly and repeatedly from the start box to the diaper in the goal box. Acquisition of the spatial discrimination was rapid, and balking infrequent. Response latencies fell from an average of about 20 sec at the beginning of training to about 5 sec at asymptote. No differences in response latency as a function of lead treatment were observed.

As in Experiment 1, however, the high-lead animals required significantly more trials to reach criterion on Reversal 1 than did the control and low-lead animals (Fig. 3). The appearance of the first-reversal deficit in this test was confirmed in the ANOVA of trials to criterion by a significant lead by reversals interaction, $F(14,63)=1.99$, $p<0.05$, by elevated scores for the high-lead group compared to control at Reversal 1, $F(2,72)=9.95$, $p<0.001$, and no treatment effect on OL or any other reversal. In addition, only within the high-lead group were scores elevated significantly on Reversal 1, compared to OL and the other reversals, $F(7,63)=7.52$, $p<0.001$. Error frequencies paralleled counts of trials to criterion, and showed effects exactly comparable to those of the trials to criterion measure.

DISCUSSION

These results showed that the same discrete learning impairment was produced by lead treatment in a test situation in which both the reinforcing agent and the behavioral response differed from those used in Experiment 1. The phenomenon was therefore robust with regard to these parameters, yet specific to acquisition of the first of a series of reversals. These data also suggested that the deficit did not result from motivational impairment, since two independent motivational systems—food and contact comfort—were exercised separately in Experiment 1 and Experiment 2. Furthermore, physical debilitation could be ruled out as a factor since response latencies did not differ across treatment groups in the maze test, and since the groups did not differ in apparent health, body weight, or hematocrit.

TEST 2

In Test 2, a series of learning problems was administered in the WGTA in which the placement of overtraining trials in the reversal series was varied. If overtraining were a determinant of the lead-induced reversal deficit, it should appear following each overtrained reversal; if the deficit were specific to the first reversal, it should appear only on Reversal 1, regardless of the placement of overtraining.

METHOD

Animals

The Experiment 2 animals were about five months of age at the beginning of Test 2, which began about two weeks after the conclusion of Test 1, and which lasted a total of seven months.

Apparatus

The WGTA described for Experiment 1 was used.

Procedure

Test 2 consisted of a series of four five-reversal discrimination problems employing four pairs of multidimensional junk objects selected from the laboratory's learning-set stimulus file. For Problem A, no overtraining was given; for Problem B, overtraining (training to the strict criterion) was given after original learning and each reversal; for Problem C, overtraining was given after original learning only; and for Problem D, overtraining was given after Reversal 4 only, to observe its effect at the end of a reversal series. The order of administration of these four problems was counterbalanced within each treatment group, while each animal received the four pairs of stimuli in the same order.

Appetite tests were administered concurrently with learning tests, at 7 and 10 months of age. Shaping and reversal training procedures in the WGTA were the same as in Experiment 1, with the following exception.

To control balking, a forced-trials procedure was used: following four consecutive balks, the incorrect object was removed from the test tray for the next four trials, leaving only the rewarded object in place. Despite this hint, the monkeys typically reverted to the unrewarded object on the next few trials utilizing both objects.

Data Analysis

Procedures described in Experiment 1 were repeated, with the following exceptions. First, each dependent measure of reversal learning was analyzed both according to problem type (i.e., with regard to placement of overtraining) and according to problem order (i.e., the order in which the problems were given to each animal, regardless of the placement of overtraining). Second, the measure of total sessions to completion of the problem series was analyzed by a one-way ANOVA, since heterogeneity of variance was not evident.

RESULTS

As evidenced by trials to criterion, the performance of all animals improved markedly over the four successive problems, and this improvement, from 597 to 133 trials/problem, $F(3,27)=86.40$, $p<0.001$, obscured any effects that overtraining might have exerted. For example, a comparison of two reversals following overtraining (Problem C, Reversal 1 and Problem D, Reversal 5) with two reversals following training to the standard criterion (Problem C, Reversal 5, and Problem D, Reversal 1) showed no effects of lead, overtraining, or the interaction of the two, either on measures of trials to criterion (all p's>0.10) or of errors (all p's>0.05).

However, both experimental groups required more sessions overall to finish the problem series than did the control group, with mean ± SE sessions of 17.86 ± 1.16 (control); 23.54 ± 1.31 (low-lead); and 24.21 ± 1.71 (high-lead); $F(2,9)=6.12$, $p<0.025$. The retardation of the high-lead group was attributable in part to an increase in trials to criterion on Reversal 1 in the first two problems administered, regardless of the position of overtraining trials. Thus, on Reversal 1 of the first problem, the high-lead group required 189.00 ± 21.57 trials, compared to 132.25 ± 10.01 trials for the controls, $t(6)=2.39$, $p<0.05$, one tail, and on Reversal 1 of the second problem, the high-lead group required 79.75 ± 13.79 trials to the control's 31.50 ± 13.03 trials, $t(6)=2.54$, $p<0.025$, one tail. On Reversal 1 of both problems, the low-lead group performed at an intermediate level, which did not differ significantly from control. No effects of lead were observed in any reversal in the third or fourth problems.

Analysis of errors showed no significant main effects of lead treatment or interactions of lead treatment with problems or reversals in Test 2, with regard either to placement of overtraining or order of administration.

The high-lead group balked more frequently overall than the other two groups, $F(2,9)=4.78$, $p<0.05$, although the difference between the groups declined across the four problems in the series, $F(6,27)=3.08$, $p<0.025$. Analysis of this interaction showed that the high-lead group balked more frequently in the first problem (mean ± SE balk frequencies were: control, 57.75 ± 28.61; low-lead, 92.75 ± 27.61; high-lead, 220.00 ± 78.54; $F(2,36)=480.66$, $p<0.001$, and in the second problem, mean ± SE balk frequencies were: control, 4.50 ± 3.35; low-lead, 11.00 ± 3.74; high-lead, 35.50 ± 24.12; $F(2,36)=17.62$, $p<0.001$, but not in either the third or fourth problems. In addition, balk frequencies correlated significantly (Pearson's $r=-0.877$, $p<0.01$) with the rate of food retrieval in appetite tests conducted at 7 months of age, in which one low-lead animal and three high-lead animals with high balk frequencies retrieved food bits from the test tray at abnormally low rates.

DISCUSSION

Overtraining clearly exerted far less effect on reversal learning in these problems than did practice: reversal learning was

not affected significantly by overtraining at any time, while the average number of trials to criterion for all animals decreased by nearly 80% between the first and last problems in the series. However, learning rates over the entire problem series were retarded in both experimental groups with respect to control, in a manner comparable to the effect seen in Experiment 1. In addition, the high-lead group showed a reversal learning deficit on the first reversal of each of the first two problems administered, regardless of the presence or absence of overtraining on the original learning which preceded those reversals, indicating that this deficit was specific to the first reversal within these problems. Moreover, the fact that this first reversal deficit appeared only on the first two of the four problems suggested that amelioration of the deficit could follow from repeated exposure to a given problem type.

As in Experiment 1, balking again was evident in the high-lead animals, and it appeared in apparent association with the reversal deficit in the first two problems administered. This association, and the high negative correlation of balk frequency with rate of food retrieval on appetite tests, supported the possibility of motivational mediation of the reversal learning deficit.

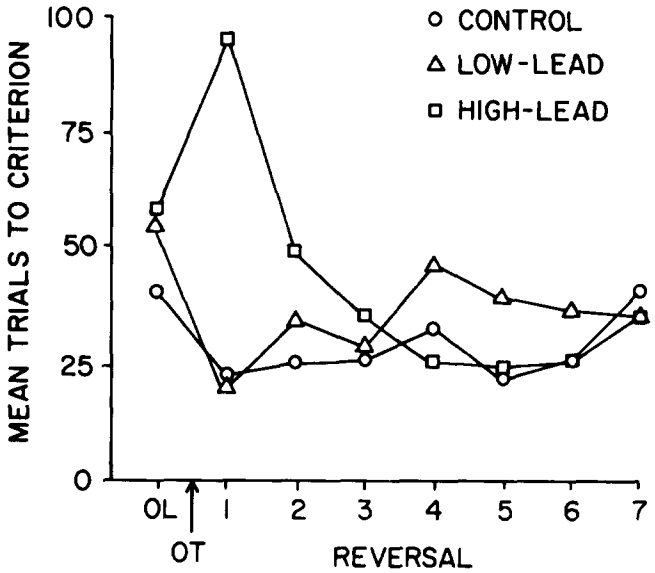

FIG. 4. Reversal learning sets obtained in the WGTA from each group of Experiment 2. This test (Test 3) required discrimination of colored planometric patterns. Mean trials to criterion are plotted as a function of reversals. Abbreviations as in Fig. 2.

TEST 3

Test 2 indicated the possibility that a motivational impairment interfered with the learning performance in the experimental animals. All animals were therefore retested under conditions in which appetite was controlled by manipulation of the food allotment for each animal in the home cage. By this time, lead dosing had already been terminated, as required by experimental protocol. However, administration of this test at this time was justified by the fact that the lead levels in the experimental groups remained elevated well above control. To reinstate the reversal deficit, which had disappeared by the end of the problem series in Test 2, the discriminanda were changed from three-dimensional objects to two-dimensional patterns for Test 3.

METHOD

Animals

The twelve animals began Test 3 at 15 months of age, two months after the slowest animal had completed Test 2. There was no significant difference across treatment groups in the mean duration of time between Tests 2 and 3. Test 3 required about one month to complete, at which time blood lead concentrations had fallen from peak levels at the termination of lead dosing (Fig. 1d) to 46.25 ± 6.74 μg/dl for the high-lead group and 18.75 ± 2.87 μg/dl for the low-lead group, and remained at about 5 μg/dl for the controls.

Apparatus

The WGTA described previously was used.

Procedure

Prior to Test 3, each animal was offered 4 monkey chow biscuits each day in its home cage after behavioral testing was finished. To equate appetite during the next day's testing, this number was increased or decreased as necessary for each animal to induce it to consume 30 reinforcers from the appetite test tray with a latency of between 100 and 300 sec. In some cases, it was necessary to feed animals ½ to 1 biscuit an hour or more prior to the appetite test to slow them down sufficiently, while in other cases, food rations were reduced to as little as 2.5 biscuits/day. One control, two low-lead, and two high-lead monkeys required deprivation of this magnitude. Periodic monitoring of food retrieval rates during Test 3, throughout which the food deprivation procedures were maintained, ensured that all animals were equally motivated for food, as measured by these appetite tests.

Test 3 consisted of a single seven-reversal discrimination problem using a filled blue 6.5 cm diameter circle pattern centered on a 7.5 cm square cardboard plaque and a similar red circle pattern on an identical plaque. Overtraining, i.e., training to the strict criterion, was provided after original learning only; otherwise, the procedures described for Test 2, above, were followed.

Data Analysis

Food-retrieval data from the appetite tests were analyzed as in Experiment 1. Error and balk frequencies and trials to criterion were counted as in Test 2 and analyzed as in Test 1.

RESULTS

Appetite tests administered concurrently with Test 3 showed no effects of lead treatment. In addition, balk frequencies did not differ significantly among the three treatment groups anywhere in the problem. Mean ± SE overall balk frequencies were: control, 10.50 ± 5.52; low-lead, 7.00 ± 2.04; high-lead, 21.50 ± 4.11.

All groups required 40–60 trials to attain criterion on original learning, but while the performance of the control and low-lead groups improved by nearly 50% on Reversal 1, that of the high-lead group was slowed by more than 60% at this point (Fig. 4). Statistically, this first-reversal deficit was confirmed by a significant interaction between lead and re-

versals, $F(14,63)=2.17$, $p<0.025$, and two significant simple main effects within the interaction: an increase in trials to criterion compared to control for the high-lead group at Reversal 1, $F(2,72)=6.16$, $p<0.005$, and an increase in trials to criterion at Reversal 1, relative to the other reversals, for the high-lead group only, $F(7,63)=4.51$, $p<0.001$. Analysis of error frequencies showed effects exactly parallel to those for the trials to criterion measure.

DISCUSSION

The performance of all three groups on original learning showed the difficulty of discriminating pattern stimuli, as compared to the stereometric objects used in Test 2. However, this initial difficulty was easily overcome by the control and low-lead groups, whose performance reached asymptotic levels on Reversal 1. These data suggested that these animals had learned to learn a discrimination reversal in prior tests and that this learning had transferred positively to Test 3. In contrast, the performance of the high-lead animals was characteristic of animals without prior training, suggesting that lead exposure at the high-lead level had interferred with transfer of prior learning to the present reversal task.

The lack of effect of lead on food retrieval rates and on balking in this test indicated further that the manipulation of dietary intake was successful in equating food motivation across the three treatment groups. It appeared therefore that this first-reversal deficit was not mediated by motivational impairment.

GENERAL DISCUSSION

The present data demonstrate that rhesus monkeys carrying blood lead burdens above 30 μg/dl during the first year of life were retarded in learning to criterion a series of discrimination reversal problems. In addition, monkeys with blood lead levels above 70 μg/dl were severely retarded in attaining criterion on the first of a series of reversals within a given problem, but performed normally on original learning and were usually unaffected on reversals subsequent to Reversal 1. This first-reversal deficit was characterized also by an increase in errors and often by an increase in balking among high-lead animals.

A similar retardation of reversal learning in a nonspatial discrimination task has recently been observed by others in cynomologous monkeys dosed daily with lead at 500 μg/kg [37]. The effect on Reversal 1 appears therefore to represent a behavioral response of macaques to chronic, low-level lead exposure which is not unique to the particular conditions of lead dosing or behavioral testing used here.

The differences between Experiments 1 and 2 (Fig. 1) in protocols of lead dosing and in PbB levels across the year did not appear to exert any effect on the learning deficits observed. Thus, the high-lead groups of Experiments 1 and 2 both showed the first-reversal deficit, despite steady-state PbBs in Experiment 1 and rising PbBs in Experiment 2. The clinical sign of appetite loss (but not that of hematocrit reduction) was observed at the end of treatment in Experiment 2, when PbB levels exceeded 100 μg/dl, yet were independent of changes in learning performance. In addition, the low-dose groups of Experiments 1 and 2 both showed retarded overall performance in long problem series, resulting from the cumulation of small increases in trials to criterion at each stage of the learning paradigm. This effect in low-lead groups was also observed whether PbB levels remained relatively constant, as in Experiment 1, or rose continually, as in Experiment 2.

The first-reversal deficit shown by the high-lead groups did not appear on every problem administered, although practice on reversal learning per se did not prevent its appearance. Thus, in Experiment 1, three successive seven-reversal problems using position, color, and size respectively all elicited the effect on Reversal 1 (Fig. 2), while in Experiment 2 it appeared initially on spatial reversal in a maze task at 3 months of age (Fig. 3), and subsequently only on the first two of four successive five-reversal problems, each of which employed multidimensional stereometric objects as discriminanda in the WGTA (Test 2). Later the deficit reappeared on a seven-reversal problem employing colored patterns as stimuli (Fig. 4), but was not evident in either experiment with pattern-based problems administered subsequent to the problems reported here. Thus the deficit tended to recur when succeeding problems differed in the quality of the stimuli used as discriminanda, as in Experiment 1, but tended to disappear when similar stimuli were used, as in Test 2 of Experiment 2. The persistent recurrence of the deficit suggests that the high-lead monkeys failed to transfer the appropriate reversal learning strategy across major changes in the discriminative stimulus dimension, but succeeded in doing so when changes in the discriminative stimulus dimension were minor. This pattern suggests further that lead treated monkeys should not be seriously affected on a series of problems of the same type, but should have serious difficulty if challenged with continuously novel problems.

This performance deficit can be interpreted in terms of impairment of one or more of the following processes: perceptual or motor function, motivation, or some aspect of central associative or cognitive function. Performance differences could not be due to body weight differences, since body weight was not affected by the lead treatment.

Impairment of perceptual or motor function seems unlikely to have retarded learning only of the first reversal, since interference with the ability to discriminate the stimuli or to make the necessary motor movements should have degraded performance across all stages of the reversal series, as has been argued [15]. In addition, no monkey appeared to have difficulty pushing aside stimulus blocks or retrieving small bits of food, nor were any group differences observed in response latencies in the maze running task.

Driscoll and Stegner's [15] argument cannot be applied rigorously to an interpretation of motivational impairment as accounting for the first-reversal deficit, however, since reduced motivation might degrade performance on a difficult task (e.g., learning a first reversal) but not affect performance on an easier task (e.g., learning an original discrimination or a reversal subsequent to Reversal 1). Motivational impairment as a determinant of the first-reversal deficit may be ruled out however by the fact that reversal learning deficits occurred both in the presence and the absence of other measures of reduced motivation. Thus, slowed food retrieval, indicating reduced appetite for food reinforcement, occurred in experimental monkeys only during Test 2 of Experiment 2. Increased balking, also indicative of reduced motivation, was observed in Experiment 1 as well as in Test 2 of Experiment 2. Nonetheless, the first-reversal deficit was observed in the high-lead monkeys not only in these tasks but also in Test 3 of Experiment 2, in which both slowed food retrieval and elevated balk frequencies were absent. In fact, the magnitude of the first-reversal deficit in Test 2 of Exper-

iment 2, accompanied by both of these indications of reduced motivation, was the smallest of those observed in the present series of experiments.

Finally, a large first-reversal deficit was observed in high-lead monkeys in the maze task of Experiment 2 (Test 1), in which the reinforcer was simply reunion with the animal's diaper. Clearly, differences in appetite for food could not directly account for this effect. Indeed, if reduced appetite for food existed and were the result of gastrointestinal malaise associated with lead ingestion, then the discomfort should have increased the drive for contact comfort, resulting in increased motivation in the experimental animals in the maze. In fact, observation of social behavior in these monkeys [7] indicated greatly increased clinging responses in experimental animals of both Experiments 1 and 2, supporting the contention of increased motivation for contact comfort under these conditions of lead exposure.

In sum, the first-reversal deficit appeared both in the presence and the absence of reduced appetite for food, as measured by food retrieval rates and balk frequencies, and both in food-motivated and contact comfort-motivated tasks. The deficit appeared therefore to be independent of the motivational factors measured here.

Cognitive interpretations of learning deficits in lead-exposed animals have been couched in terms of interference with attentional processes [49] and of impaired response inhibition [42]. The present data offer no insight into which of these two interpretations may be preferable, or indeed whether they might represent two different mechanisms at all. A deficit in response inhibition in an operant paradigm has been demonstrated in rats exposed postnatally to lead [34], but no adequate analysis of the possible cognitive impairment resulting from lead exposure has yet been offered in the literature.

Nevertheless, lead-induced interference with reversal learning, as an observable behavioral phenomenon, whether mediated by losses in attentional or response inhibitory mechanisms, points circumstantially to hippocampal damage as a potential mediating factor. Certainly, hippocampal damage has been related to deficits in reversal learning in rats [14] and in monkeys [27]. It is pertinent that lead has been reported to accumulate 7-fold or more in the normal rat hippocampus, compared to other brain regions [18], that increased lead concentrations have been noted in hippocampi of lead-poisoned children [33], and that lead exposure has been shown to decrease the concentration of zinc in the brains of 25-day-old rats [30]. It is possible that hippocampal function is impaired by accumulation of lead in the place of zinc in the mossy fiber system of the hippocampus [47] and that this interference is observable at a behavioral level as a reversal learning deficit.

Lead is also known to produce other forms of CNS damage (e.g., edema and lesions in the microvasculature), which cannot be ruled out as factors in any lead-induced behavioral change at this time. However, an hypothesis relating behavioral changes to interference with hippocampal function enjoys the advantage of relative consistency with available neuropathological and behavioral data regarding lead exposure and hippocampal function.

On the basis of the present data, it would be fair to speculate that the first-reversal deficit observed here might reflect slowed maturation of learning, or a learning process peculiar to the incompletely developed monkey. However, data currently being collected in this laboratory indicate that the deficit can be observed at least three years beyond final lead dosing. It therefore appears likely that this deficit represents a relatively permanent characteristic of the chronically lead-poisoned monkey.

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