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Behavioral Effects of Chronic Lead Exposure in the Adult Rat

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NATION, J. R., A. E. BOURGEOIS AND D. E. CLARK. *Behavioral effects of chronic lead exposure in the adult rat.* PHARMACOL BIOCHEM BEHAV **18**(6) 833–840, 1983.—Adult rats fed daily rations of laboratory chow laced with lead acetate, were tested for operant (schedule-controlled) responding and conditioned suppression. In Experiment 1, animals receiving 10 mg/kg lead showed significantly lower operant response rates (lever pressing) than controls. Conditioned suppression performance was not different between the two groups. During retraining that followed a 42 day no-training period, lead-treated subjects showed greater percent of prior baseline responding than controls. The groups were not different on a test for stimulus control or an appetitive resistance to extinction test. In Experiment 2, separate lead-treated groups were chronically exposed to either 10 mg/kg, 5 mg/kg, or 1 mg/kg lead daily. Behavioral tests showed that while the lowest lead level (1 mg/kg) occasioned higher rates of operant lever pressing relative to controls, the highest level (10 mg/kg) again produced lower rates. On a retraining task administered after an interpolated 90 day no-training period, the two highest exposure groups were significantly above controls regarding percent of baseline responding and there was evidence that the 5 mg/kg group was significantly superior to controls in terms of absolute response rate. No differences in conditioned suppression or resistance to extinction were observed in the second experiment.

Adults Lead Schedule-controlled responding Retraining

AN EXPANDING literature on the behavioral toxicology of recurrent lead exposure indicates a vulnerability even among animals exposed postweaning. Behavioral anomalies have been observed under varied operant training conditions to include fixed-interval [7], multiple fixed interval-fixed ratio [2] and differential reinforcement of high rates (DRH) schedules of reinforcement [16]. Further, rats exposed to lead after weaning have shown impaired performance on a schedule that differentially reinforces a minimum response duration [6].

The above demonstrations of aberrant performances under conditions of concomitant lead exposure underscore the potential toxic impact of lead for older animal populations and therein suggest that previous investigatory efforts in this area may have been underdimensioned. The assumption [3, 17, 22] that animals are largely refractory to the influence of lead beyond weaning has gained widespread acceptance over the past decade. But this assertion seems to rest more on the available biochemical and neurological data [22] than on the findings from perhaps more sensitive behavioral tests. The few postweaning treatment studies of behavioral phenomena on which the no-effects position is based [3] employed tasks such as the T-maze [4,18] and the Hebb-Williams maze [26]. Such tests are acknowledged to be insensitive procedures for assessing pharmacologic effects and as suggested elsewhere [8] there is reason to believe that they are equally inappropriate to index toxicologic effects. Certainly the aforementioned postweaning results showing lead-induced changes in schedule-controlled responding argue that behavioral effects are likely, given that the assessment protocol is sufficiently sensitive to permit their occurrence.

The purpose of this study was to examine the behavioral toxicity of chronic lead exposure in adult rats. By extending the age range of treatment subjects to include mature animals, a critical appraisal of the effects of lead on the functional status of a morphologically intact central nervous system (CNS) can be made. While this report is not directly concerned with the large literature on lead-induced behavioral abnormalities among humans (see Jason and Kellogg [17] for a recent review), maturational parallels would seemingly suggest a reasonable laboratory analog to adult human contamination. Occupational exposure is a major health problem in the industrial sector [10] and as has been noted [2], popular neonatal models of lead poisoning often fall short of the requisite criteria for extrapolating from animals to adult human populations.

The procedure used for behavioral testing in the two experiments reported here incorporated elements of both operant and classical conditioning. Operant training is ideally suited for chronic investigations of behavioral toxicology [11,19] inasmuch as schedules of intermittent reinforcement occasion stable performances. Departures from such steady-state patterns often serve to signal a change in the integrity of responding. While the operant format may be more familiar to behavioral toxicologists, classical (Pavlovian) conditioning, used extensively as a research tool in the Soviet Union [9], may be relatively more sensitive [23]. As

added features, retraining (schedule reintroduction following an interpolated period of no-training), behavioral contrast, and response persistence were examined.

EXPERIMENT 1

The experiment involved the serial presentation of different training conditions across five phases. The initial phase involved training animals to lever press for food on a multiple reinforcement schedule. This schedule is composed of two or more schedules correlated with different stimuli presented alternately requiring the same response [12]. During this first phase identical variable interval two minute (VI-2) schedules were used for each component of the multiple schedule. Identical schedule conditions were necessary for subsequent tests of stimulus control (see below). Variable interval schedules program reinforcement for the first response (lever press) after intervals of varying lengths elapse. Such schedules are defined by the average of the different intervals, thus the designation VI-2. A second phase involved the introduction of a probe (cue) paired with electric shock during operant training on each component of the multiple schedule. This classical conditioning procedure is known as conditioned suppression training. The typical reaction here is reduced operant responding when the cue is introduced [23]. Following discontinuation of suppression training, all subjects were shifted from a multiple VI-2 VI-2 to a multiple VI-6 VI-2 schedule of reward. Aimed at showing behavioral contrast [24] this stimulus control procedure assess the animal's ability to discriminate changes in reinforcement density on each component of the multiple schedule. The fourth phase involved a 42 day period of no-training. During the fifth phase, the multiple VI-2 VI-2 schedule was reinstated then suppression training followed. The final step during the fifth phase was a test of resistance to extinction (persistence).

METHOD

Subjects

The subjects were 10 male Sprague-Dawley rats approximately 100 days old at the beginning of the experiment. Animals were special ordered from the Holtzman Company (Madison, WI) to range between 200 and 210 g. On arrival, all subjects were placed on a 10 g food deprivation diet for the remainder of the experiment. Previous research in our laboratory had indicated that animals of the age and weights used for the present project did not show significant weight gains over a period of 16 weeks while on this diet. Nor was there any evidence of starvation-related health problems under such a diet. For the duration of the project, five randomly selected subjects received 10 g Purina lab chow daily without added lead while five subjects received a 10 g daily diet of lab chow sufficient to expose them to 10 mg lead (as lead acetate)/kg body weight. Thus, lead exposure occurred once a day (immediately following the daily training session) for a total of 162 days. Atomic absorption analyses (spectrophotometry) were performed on feed to insure that these dose levels were consistent throughout the study.

Throughout the experiment, animals were individually housed and had free access to water. Mean weights and standard deviations for each group of subjects at the end of weeks 1, 9, and 18 were: Week 1 Lead—mean=208 (SD=14), Control—mean=216 (SD=5); Week 9 Lead—mean=214 (SD=6), Control—mean=219 (SD=4); Week 18 Lean—mean=219 (SD=4), Control—mean=221 (SD=5).

Preparation of Feed

For lead-treated feed, pellets of Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) were ground in a small feed mill then transferred to a large stainless steel food mixer in 10 kg batches. One liter of distilled water was added to the mixer to facilitate repelleting, and the mixing process was continued until the mixture appeared homogeneous. As the mixing was continued, 100 ml of distilled water containing the appropriate quantity of lead acetate was added slowly. Mixing was continued 20–30 min to assure complete distribution of lead in the feed. The feed was then repelleted with a laboratory pelleter (Model CL Laboratory Pellet Mill, California Pellet Mill Co., San Francisco, CA) and stored at 0°C. Control feed was prepared in the same manner as lead-treated feed but with only water added.

Apparatus

Two identical small animal test chambers (LVE Model 1417) served as the apparatus. The chambers were housed in sound attenuated small universal cubicles (LVE Model 132-02) equipped with an air evacuation system. Each test chamber was 24.5 cm wide, 30.0 cm long, and 25.5 cm high with Plexiglas sides and top. The floor consisted of stainless steel grid rods located 2 cm apart. The front panel had a pellet trough centered on a stainless steel wall plate, approximately 4 cm above the grid floor. A Sonalert was centered at the top of the panel. When activated a 92 dB (SPL) tone (2900 Hz) operated as a cue. On the right side of the panel, 8.2 cm from the pellet trough, a single lever rested 6.5 cm below a tripartite light display. Bulbs were placed only in the red (far left) and green (far right) light locations. To insure that the force requirements were the same for each lever, counterweights were used to determine an equivalent static force (27 g) needed to define a response in each chamber. Continuous white noise was present to mask any extraneous sounds. Standard electromechanical scheduling (interval programming, food delivery, etc.) and recording (counters, cumulative records, etc.) equipment were located in an adjoining room. Chambers were thoroughly washed with a soap solution following each subjects' daily test.

A Grason-Stadler Model 700 constant current shock generator served to deliver shock to the grid flooring. A shock scambler was used on each occasion when administering current to subjects.

Training Procedure

Pretraining. Following 14 days of feeding on the respective control or lead diets, standard magazine training and shaping procedures were used during 30 min sessions for 2 days. By the end of the second day all subjects in the control and lead-treated groups had learned to press the lever to receive a 45-mg Noyes food pellet. On the following day all subjects operated on a continuous reinforcement (CRF) schedule for 15-min each. The final 2 days of pretraining required subjects in each group to perform on a multiple VI-15 "VI-15" schedule. During the first component, lasting 15 min, a green light came on cueing the subjects to lever press. Presses were reinforced on the average of every 15 sec. Similarly a red light cued the subject to lever press during the second 15 min component. All sessions were conducted on a

TABLE 1
SUMMARY OF SERIAL TRAINING PROCEDURES (EXPERIMENT 1)

Manipulation	Number of sessions (days)
Multiple VI-2; VI-2	40
Conditioned suppression	7
Multiple VI-2; VI-2	7
Multiple VI-6; VI-2	14
Multiple VI-2; VI-2	7
No-training	42
Retraining (Multiple VI-2; VI-2)	7
Retraining (Conditioned suppression)	3
Multiple VI-2; VI-2	7
Multiple EXT; EXT	9

daily basis. An overview of the various training procedures used in Experiment 1 is presented in Table 1.

Operant training—phase 1. Operant training began the day following pretraining and continued for 40 sessions. Throughout this phase, control and lead-exposed subjects operated under identical multiple variable interval (VI-2 VI-2) schedules where responding was reinforced every two minutes on the average. Control animals were tested in one chamber, lead-treated animals were tested in an identically programmed, time coordinated, parallel chamber. In all cases, subjects were exposed to schedule conditions under a red light for 15 min (component 1) and subsequently shifted to an identical schedule for 15 min under a green light (component 2 of the multiple schedule). The range on the VI schedules was from 30 sec to 3 min within a given component. Noyes food pellets (45 mg) served as reward. For each environmental cubicle and each component of the multiple schedule, five digital counters were programmed to count the number of lever presses made during successive 3-min intervals. The number of reinforcements was recorded on a cumulative recorder.

Conditioned suppression—phase 2. Conditioned suppression training began the day after phase 1 ended and lasted for 7 sessions (1 session/day). During this period a 92 dB tone came on at the beginning of the third 3-min interval of each component and remained on for the full length of that interval. Contemporaneously with tone offset, a scrambled electric shock of 1.3 mA was delivered to the grid flooring for 1.5 sec. Other than the introduction of the probe (cue) and correlated shock, procedures continued as described for phase 1.

Behavioral contrast—phase 3. This phase of the experiment began the day after the end of phase 2. For the first 7 sessions procedures were exactly as described for phase 1. During the following 14 sessions subjects in each group were shifted from the multiple VI-2 VI-2 to a multiple VI-6 VI-2 schedule. Other than the change in reinforcement density on the first component, all operations remained as they had been.

A 7 session re-stabilization period was included at the end of phase 3. During this period, subjects in each group were placed back on the multiple VI-2 VI-2.

No-training—phase 4. In this phase, lasting 42 days, subjects in each group remained in their respective home cages and did not perform on the operant task.

Retraining and persistence—phase 5. Following the no-training period the multiple VI-2 VI-2 schedule was reintroduced for 7 sessions. For 3 sessions following this initial test period, subjects in each group were again exposed to the suppression contingencies of phase 2.

After the suppression tests, subjects in each group were placed back on the multiple VI-2 VI-2 with no tone or shock for 7 sessions. Subsequently, all subjects were shifted to a multiple EXT EXT schedule where reward was not available in either component. This extinction test continued for 9 sessions.

RESULTS

Overt symptoms of lead toxicity such as ataxia, tremors, seizures, and anorexia were not observed in any subject.

Operant Training—Phase 1

Significance levels were set at 0.05 for separate Mann-Whitney comparisons of groups performing under each component of the multiple schedule.

Sessions 1–10. Analyses performed on the response rates in each component, averaged over the first 10 sessions, failed to show significant differences between control and lead-treated subjects.

Sessions 31–40. Individual response rates averaged over the final 10 sessions of phase 1 are depicted for each component in Fig. 1. Subjects exposed to lead lever pressed at 38 per cent of control levels. Statistical analyses performed on these data indicated that the group performance rates were significantly different. For both conditions, responding remained stable across the final 10 sessions of phase 1 and absolute as well as relative group performances were parallel across components.

Conditoned Suppression—Phase 2

The analyses performed on the conditioned suppression ratios of phase 2 failed to show differential performances for lead treated versus control subjects. While there was a trend for lead treated subjects to show less suppression initially and more suppression by the end of training, values did not reach acceptable levels for statistical significance. By the end of training, all subjects did show significant suppression of lever pressing while the tone was present.

Behavioral Contrast—Phase 3

Following the week of restabilization, group performance rates under the multiple VI-2 VI-2 were approximately at the same point as at the end of phase 1. Lead treated subjects were lower in each component relative to controls.

The shift to the multiple VI-6 VI-2 for the next 14 sessions did not produce differences. Neither control nor lead-treated subjects showed any tendency to exhibit behavioral contrast. That is, neither group tended to alter response patterns in the unchanged component (component 2). In fact, rates did not change significantly even in the changed component (component 1).

During the final 7 sessions, subjects in each group resumed the performance levels under the multiple VI-2 VI-2 that were apparent at the end of phase 1. On these last daily sessions, Mann-Whitney tests indicated that lead-treated

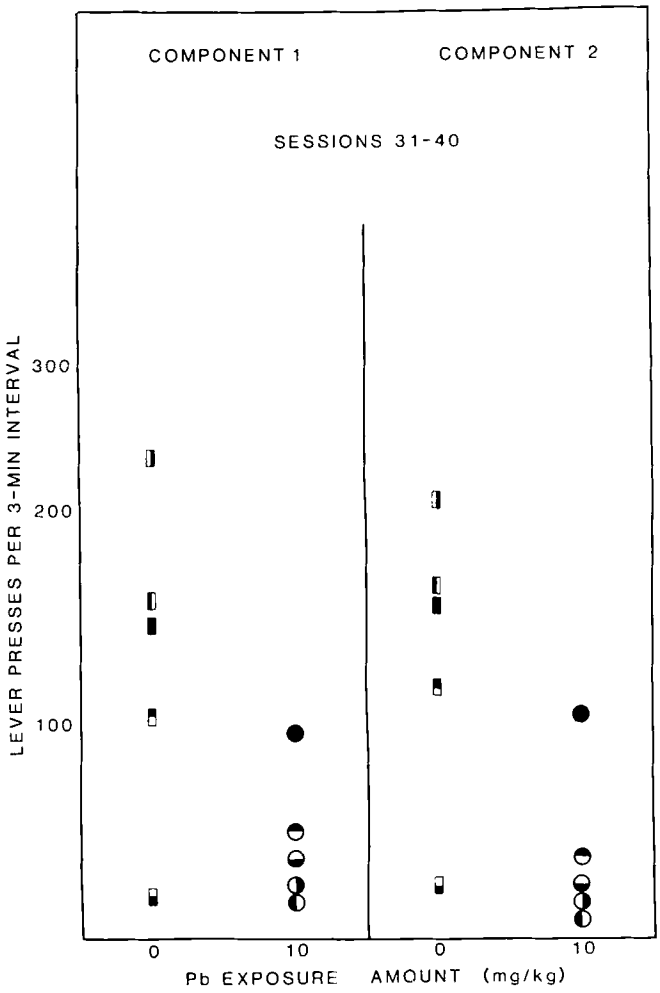

FIG. 1. Individual lever press rates for lead-treated (10 mg/kg lead acetate) and control rats for each component of the multiple schedule. Each data point represents the mean value collapsed across the final 10 sessions of phase 1 (Experiment 1).

subjects were again significantly below control subjects on each component ($ps<0.05$).

Retraining and Persistence—Phase 5

Operant retraining. Lever responding during the period of schedule reintroduction was assessed according to both absolute response rate and percent of baseline prior to the interpolated period of no-training. The latter measure was necessary due to the terminal rate differences that were present when training was discontinued at the end of phase 3. Percent of baseline was calculated for each subject by dividing the subject's daily session rate for each component by the corresponding mean of the last three sessions for the same component in phase 3.

Lead-treated subjects showed greater percent of baseline responding than controls. The reverse was the case on the measure of absolute response rate. The percent of baseline finding, while counter-intuitive, was pronounced and reliable across components.

Group profiles on each component are shown in Fig. 2. Rank tests (Mann-Whitney) performed on the percent of baseline scores for session 1 (top panels) indicated that lead-treated subjects were signficantly above controls on each of the two components ($ps<0.05$). It is worth noting that of the five subjects in the lead-treated condition, four responded at or above baseline phase 3 levels on session 1 of retraining, and this was true for both components of the schedule. While this was true of two of the five subjects in the control condition for component 1, it was not the case for any of the control subjects on component 2.

Rank tests performed on session 1 absolute response rates (bottom panels of Fig. 2) showed that control animals lever pressed at significantly higher rates on each of the two components ($ps<0.05$).

Conditioned suppression retraining. Analyses of suppression responding (interval 3) over the three sessions following the operant retraining test failed to reveal significant differences. This was the case for components 1 and 2. The lack of differences derived from almost complete suppression evidence by all subjects from the onset of the suppression training sessions.

Persistence. Analysis of terminal acquisition performances on the last session of the 7 session restabilization period following conditioned suppression training showed that lead-treated subjects were again significantly below controls on components 1 and 2 ($ps<0.05$). These differences necessitated a rate transformation on the extinction data [1].

Our analyses failed to find evidence of significant group differences in extinction rate.

EXPERIMENT 2

METHOD

Experiment 2 examined dose response functions attendant to exposure to differing amounts of lead (10 mg/kg, 5 mg/kg, 1 mg/kg) or a control diet. There were three subjects in each of the four groups for this second experiment. Mean weights and standard deviations by group at the end of weeks 1, 12, and 24 were: Week 1 Control—mean=203 (SD=1), Lead-1—mean=204 (SD=7), Lead-5—mean=203 (SD=8), Lead-10 mean=201 (SD=3); Week 12 Control—mean=208 (SD=5), Lead-1—mean=203 (SD=3), Lead-5—mean=207 (SD=7), Lead-10—mean=215 (SD=5); Week 24 Control—mean=223 (SD=15), Lead-1—mean=218 (SD=14), Lead-5—mean=225 (SD=16), Lead-10—mean=241 (SD=6). Subjects from the 10 mg exposure group were again paired against control subjects. Subjects from the 5 mg exposure group, run in the same chamber as the highest exposure group, were paired against subjects from the 1 mg exposure condition. The behavioral contrast manipulation was deleted and the interpolated no-training interval was extended to 90 days. Other than these changes, all procedures were as described for Experiment 1. An overview of the procedural manipulations of Experiment 2 are provided in Table 2.

Tissue Lead

To validate the exposure procedures used here, lead determinations of tissues were carried out 24 hr after the completion of the study. Subjects were rendered unconscious with CO_2 and subsequently sacrified via decapitation. Whole brain, liver, kidney, bone (left tibia), blood, and hair samples were collected for each subject and stored in a freezer (<0°C) until analyses. Tissue samples (0.2 to 1 g) were dissolved in

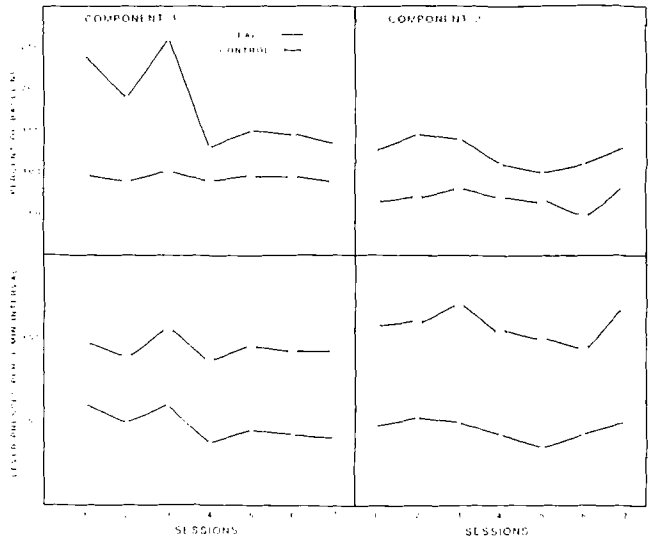

FIG. 2. Percent of baseline lever pressing (top panels) and absolute response rate (bottom panels) for lead-treated (10 mg/kg lead acetate) and control groups for each component of the multiple schedule used during the 7 session period of operant retraining (Experiment 1).

nitric acid, filtered and appropriately diluted. Lead residues (ppm) were determined by flameless atomic absorption spectrophotometry (Varian Techtron, Palo Alto, CA). National Bureau of Standard reference material 1577 (bovine liver) containing 0.34±0.08 ppm lead was tested to insure the accuracy of measurement.

RESULTS

There was no evidence of physical abnormalities among subjects.

Operant Training

An order statistic (median test) was used in all cases to determine group differences according to level of lead exposure. Significance levels were set at 0.05 for separate comparisons on components 1 and 2 of the multiple schedule.

Sessions 21–30. Individual lever press rates averaged over sessions 21–30 are shown for components 1 and 2 in Fig. 3 (top panels).

Statistical analyses of these data revealed that subjects in Group Lead-1 responded at a signficantly higher rate than control subjects on components 1 and 2. No other comparisons revealed signficant differences.

Sessions 31–40. Individual lever press rates averaged over sessions 31–40 are also shown in Fig. 3 (bottom panels) for each of the two components of the multiple schedule. Statistical analyses of these data indicated that Group Lead-10 subjects were significantly below control subjects on components 1 and 2. The only other control comparison to reach an acceptable level for significance was with Group Lead-1 on component 2. Overall, the importance of time course was evident from these data. It seems that enhanced operant responding associated with the smallest lead dose (1 mg/kg) onset relatively earlier than the performance deficits occasioned by the largest lead dose (10 mg/kg). And, it is clear from the figures and the statistical analyses that the

TABLE 2

SUMMARY OF SERIAL TRAINING PROCEDURES (EXPERIMENT 2)

Manipulation	Number of sessions (days)
Multiple VI-2; VI-2	40
Conditioned suppression	7
Multiple VI-2; VI-2	7
No-training	90
Retraining (Multiple VI-2; VI-2)	7
Retraining (Conditioned suppression)	3
Multiple VI-2; VI-2	7
Multiple EXT; EXT	9

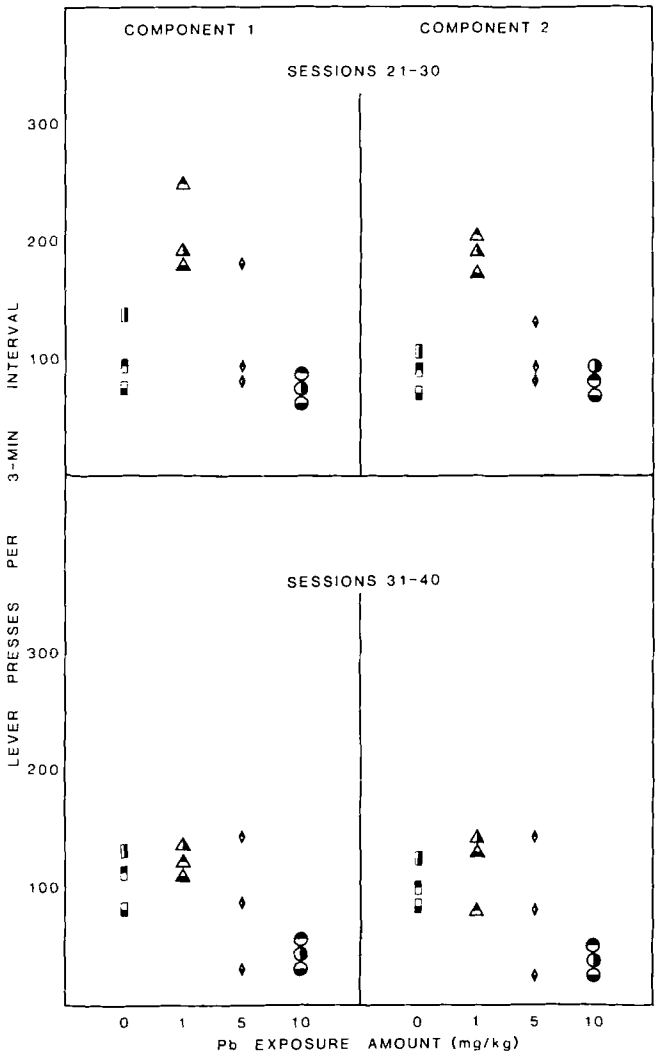

FIG. 3. Individual lever press rates for lead-treated (Lead-1=1 mg/kg lead acetate, Lead-5=5 mg/kg lead acetate, Lead-10=10 mg/kg lead acetate) and control rats for each component of the multiple schedule. Each data point on the top panels represents the mean value collapsed across sessions 21–30. Each data point on the bottom panels represents the mean value collapsed across sessions 31–40 (Experiment 2).

lead-induced increases in lever responding attenuated over sessions.

Conditioned Suppression

There was no evidence that any of the groups were different in terms of conditioned suppression. Inconsistent with the findings from Experiment 1, none of the groups in this second experiment showed pronounced suppression by the end of training.

Retraining and Persistence

Operant retraining. Terminal baseline differences prior to the interpolated 90 day no-training period again necessitated a baseline transformation like that employed for the analysis of the operant retraining data from Experiment 1. Separate analyses, therefore, were performed on absolute rate and percent of baseline (defined according to the formula response rate during retraining/terminal training response rate, with rate being both subject and component specific).

Percent of baseline responding, by component, is depicted in Fig. 4 (top panels) for each of the lead-treated groups and the control group. Differences on this measure (session 1) were assessed using the median test with a 0.05 significance level. The findings showed that groups Lead-10 and Lead-5 responded higher than control subjects on component 1. On component 2 Group Lead-5 evidenced greater percent of baseline responding than the control group on session 1.

Regarding the rate measure (bottom panels of Fig. 4), Group Lead-5 was greater than the control group on session 1 but this difference was limited to component 2. None of the other groups were significantly different on session 1.

Conditioned suppression retraining. Differential performances on the suppression retraining task were not shown.

Persistence. The disparate terminal baseline rates following conditioned suppression training again required a rate transformation [1]. Statistical analyses performed on the extinction data replicated the findings of Experiment 1, i.e., extinction rate did not differ for lead-treated and control subjects.

Biochemical Analyses

The results of the atomic absorption spectrophotometry analyses are presented in Table 3. The findings confirmed the presence of lead residues among lead-treated animals. Further, a positive relation was observed between the level of lead residues in tissue and lead exposure level. This is seen most vividly in the results for kidney and bone where lead residues tend to double with successive increases in doses.

The test analysis performed on the reference bovine liver tissue confirmed the validity of the spectrophotometry procedures used here (lead (ppm)—mean=0.37, SD=0.18).

TABLE 3

LEAD RESIDUES (ppm) IN TISSUES*: EXPERIMENT 2

	Tissue					
Group	Brain	Liver	Kidney	Bone	Blood	Hair
Control						
mean	0.01†	0.143	0.01	0.01	0.030	0.01
SD	0.01	0.064	0.01	0.01	0.020	0.01
Lead-1						
mean	0.01	0.085	0.338	0.658	0.020	0.01
SD	0.01	0.079	0.470	0.265	0.020	0.01
Lead-5						
mean	0.01	0.478	1.053	3.255	0.050	0.01
SD	0.01	0.394	0.450	1.006	0.020	0.01
Lead-10						
mean	0.01	0.423	1.388	10.263	0.090	0.01
SD	0.01	0.500	0.106	11.167	0.030	0.01

*Lead residues determined by flameless atomic absorption spectrophotometry.

†Values listed as 0.01 indicate lead residues (ppm) below the lowest level detectable.

GENERAL DISCUSSION

The findings from Experiments 1 and 2 indicate that subtle behavioral disturbances do occur among adult rats chronically exposed to lead. Ten mg/kg lead resulted in reduced rates of operant lever pressing relative to controls (Experiments 1 and 2) while 1 mg/kg lead enhanced lever responding (Experiment 2). While conditioned suppression and stimulus control performance were unaffected by lead exposure, both experiments revealed evidence showing relatively greater initial lever responding on an operant retraining task for lead-treated (10 and 5 mg/kg) compared to control subjects. Extinction performance was not affected by lead treatment in either experiment.

The operant findings from this study converge with earlier data [2, 6, 7] to provide needed closure on the issue of the vulnerability of older animals to lead contamination. Not only were our adult animals affected by lead-exposure, they further showed behavioral effects resembling those of younger populations similarly exposed. For instance, rats 20 to 22 days of age were exposed to either 50, 300, or 1000 ppm lead acetate drinking solutions for 35 days prior to testing on a fixed-interval (FI)-30 sec food reinforcement schedule [7]. It was found that postweaning exposure to the lower two concentrations (50 and 300 ppm) increased lever press responding relative to control levels, and that exposure to 1000 ppm lowered rates significantly below control levels. As Cory-Slechta dramatically reveals in a recent review paper, similar patterns have been observed by most investigators of schedule controlled phenomena when response events and dose levels respectively are converted to a common metric [5]. Ostensibly, prolonged low-level lead exposure, regardless of maturational status, produces a biphasic pattern of operant change defined by facilitation with low doses and retardation with higher doses.

The operant retraining results showing greater initial lever pressing among lead-treated as contrasted with control subjects were unexpected. The fact that absolute as well as percent of baseline differences were observed, accents the robust nature of these effects. Contending accounts of this phenomenon would include arguments favoring greater stimulus control under conditions of lead exposure, or perhaps lead functions as a nonspecific CNS excitant and thus performs a mediational role. Such positions are testable and future examinations conducted along these lines may prove to be especially profitable.

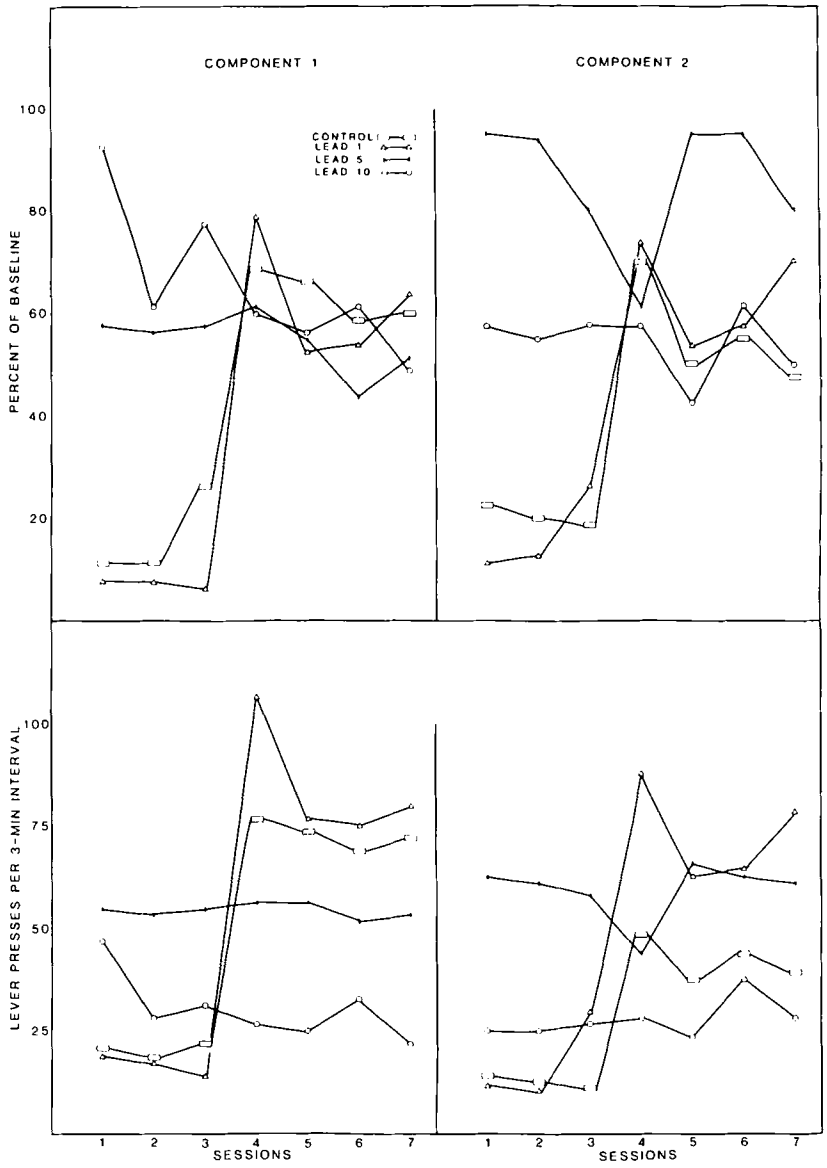

FIG. 4. Percent of baseline lever pressing (top panels) and absolute response rate (bottom panels) for lead-treated (Lead-1=1 mg/kg lead actate, Lead-5=5 mg/kg lead acetate, Lead-10=10 mg/kg lead acetate) and control rats for each component of the multiple schedule used during the 7 session period of operant retraining (Experiment 2).

Behavioral contrast is said to occur when animals operating under the contingencies of a multiple schedule adjust their response rates on an unchanged component of that schedule commensurate with but in a direction opposite to that of rate changes on a changed component of the same schedule [24]. The lack of behavioral contrast effects in Experiment 1, even among control subjects, points to the probable absence of stimulus control in the present study. In this regard, the current failure to observe lead-induced differrences with respect to contrast phenomena more likely should be viewed as a consequence of procedural shortcomings than as a matter of insensitive testing. Visual stimulus control is difficult to achieve with rats [13], and of course, such control would have been required for a critical appraisal of contrast effects. Despite the apparent lack of such control here, other stimulus control tests appropriately modified to meet the demands of the organism are to be encouraged.

While the reported tissue assays showed the expected increases in liver, kidney, and bone lead residues, lead burdens in brain and blood samples were virtually below the limits of detection. The lack of significant accumulations in the brain is perhaps understandable, for we analyzed whole brain residues and lead is known to produce focal neurochemical [14, 21, 25] and neurological [15] effects. Isolated concentrations would have been obscured by the gross

analysis. The blood lead level findings, which show amounts substantially below the modal values (0.4–0.7 ppm) reported in several other behavioral studies [5], are more difficult to reconcile. It is possible that the discrepancy may rest with blood lead periodicity following exposure via water or food. That is, with water exposure [2,7] access to the contaminant would be continual and thus the exposure regimen consistently applied. Conversely, with food (as used here) exposure would be acute on a daily basis. Consequently, blood lead amounts would increase rapidly permitting tissue residuals to accumulate, but as the system is purged blood lead amounts would be expected to return to near normal levels. In any case, caution should be exercised concerning statements about the incidence of behavioral toxicity that may exist apart from marked increases in blood lead levels.

Finally, behavioral studies that choose to use food as a reinforcer during operant training must be alert to the possibility of generalized conditioned flavor aversions. This is true for water as well as food exposure for even in the former case animals are given food in the home cage, the taste of which may be adventitiously associated with a toxicant-induced aversion. That aversion was not a confounding variable in the present study is supported by recent findings [20] which show flavor aversions developing to doses of 200 mg/kg lead acetate but not to doses of 100 mg/kg lead acetate. Obviously, our exposure values fell well short of the amounts necessary to produce such aversions.

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