

0892-0362(95)02016-0

# In Utero Lead Exposure in Squirrel Monkeys: Motor Effects Seen with Schedule-Controlled Behavior

M. CHRISTOPHER NEWLAND,<sup>\*1</sup> SHENG YEZHOU,<sup>†</sup> BENGT LÖGDBERG<sup>†</sup> AND MATHS BERLIN<sup>†</sup>

<sup>\*</sup>*Department of Psychology, Auburn University, Auburn, AL 36849*

<sup>†</sup>*Institute of Environmental Health, Lund University, Sölvegatan 21, S-223 62, Lund, Sweden*

Received 7 November 1994; Accepted 18 August 1995

NEWLAND, M. C., Y. SHENG, B. LÖGDBERG AND M. BERLIN. *In utero lead exposure in squirrel monkeys: Motor effects seen with schedule-controlled behavior.* NEUROTOXICOL TERATOL 18(1) 33–40 1996. — Timed-pregnant squirrel monkeys were exposed orally to lead during the last 1/2 to 2/3 of gestation such that maternal lead levels ranged from 21 to 70  $\mu\text{g}/\text{dl}$  in blood. Offspring of these lead-exposed monkeys were compared to gender-matched, untreated controls (blood-lead levels from 4 to 9  $\mu\text{g}/\text{dl}$ ), born at about the same time. When the monkeys were 3 to 7 years old they were trained to pull a T-shaped bar against a 1 kg spring through a displacement of 1 cm. This performance was examined during acquisition of different fixed-ratio (1, 5, and 20) and fixed-interval (120", 300", and 600") schedules of reinforcement and during steady state under the fixed-ratio 5 and fixed-interval 600". Monkeys exposed prenatally to lead showed an increased number of responses failing to meet the requirement of pulling against 1 kg spring through a 1 cm displacement when behavior was maintained by a fixed-ratio schedule, which engenders a vigorous, high-rate pattern of responding. This increased number of incomplete responses first appeared in the acquisition of a fixed-ratio 5 and fixed-ratio 20 schedules of reinforcement, remained after the fixed-ratio 5 schedule was allowed to reach steady state, and did not appear under the fixed-interval schedule. Neither body weight nor response rate were affected by lead, but it was necessary to control for these variables using multiple regression to isolate lead's effect. The appearance of incomplete responses while the monkeys pulled vigorously against a 1 kg spring suggests that lead exposure during gestation produced subtle motor impairments years after exposure has ended. Deficits in the acquisition of behavior (learning) under Concurrent Random Interval schedules of reinforcement have also been reported with these monkeys. Together, these reports reveal prolonged deficits in learning and motor function resulting from in utero exposure to lead at maternal blood lead levels (21–70  $\mu\text{g}/\text{dl}$ ) that could result from exposure to ambient air in heavily polluted urban environments or in occupational settings meeting current World Health Organization standards.

In utero lead exposure      Squirrel monkeys      Scheduled-controlled behavior

FIXED-INTERVAL and fixed-ratio schedules of reinforcement have been used to examine the developmental toxicity of lead or other neurotoxicants, usually to characterize the organization of behavior around environmental contingencies after toxicant exposure (6–8,29,30,36) but sometimes to examine physical characteristics of responding that might reveal motor deficits (6,16,23,27). Fixed-ratio and fixed-interval schedules offer many strengths for such investigations, including important differences in the pattern and physical characteristics of behavior that they maintain and a long-standing literature describing drug and toxicant effects on behavior under these schedules that can be drawn upon to interpret behavioral effects of toxicant exposure (4,23,32).

Postnatal lead exposure produces monophasic rate reductions in behavior under fixed-ratio schedules at relatively high doses (7,8) an effect also seen with some behaviorally active drugs, such as psychomotor stimulants (32). Acquisition of fixed-ratio schedule performance in adulthood has revealed effects of chemical exposure during early development even when steady-state performance shows more modest effects. Delayed toxicity of neonatal cadmium exposure and prenatal ethanol exposure has been seen in altered acquisition of fixed-ratio responding (18,24).

Postnatal lead exposure produces biphasic effects on behavior maintained by fixed interval schedules; rate increases appear at low to moderate doses and decreases appear at

<sup>1</sup> To whom requests for reprints should be addressed.

higher doses, effects also resembling those seen with psychomotor stimulants. These effects have been reported in rats (1,6,9,13), sheep (5,35), and nonhuman primates (28,29,31) and have been interpreted as revealing deficits in the organization of behavior and perhaps reflecting the cognitive effects of lead.

In the present experiment, fixed-ratio and fixed-interval schedules of reinforcement are used with a response device designed to require effortful responding so that motor effects of prenatal lead exposure might become visible. Because fixed-ratio and fixed-interval schedules maintain very different patterns of responding they are capable of isolating deficits in the physical execution of a response. For example, the vigorous responding maintained by fixed-ratio schedules was especially sensitive to motor effects of manganese when the response entailed pulling a bar against a heavy spring, effects that appeared even when schedule-typical patterns of responding remained intact (27).

In the present study, the behavior of squirrel monkeys exposed prenatally to lead was examined under various fixed-ratio and fixed-interval schedules of reinforcement when the monkeys were 3 or more years of age. The response device, which required pulling through a displacement against a strong spring, was chosen to examine the possibility of deficits in effortful responding associated with prenatal lead exposure. The monkeys described in the present report have already been reported to display impairment in the acquisition and, at higher levels of exposure, maintenance of behavior under concurrent schedules of reinforcement (25).

#### METHOD

##### *Subjects*

Timed-pregnant squirrel monkeys were used for breeding. Twenty milligrams of iron was injected IM between gestational age of 9 to 18 weeks to prevent iron deficiencies, but no other drugs were given prior to the end of gestation. Pregnancies were timed to  $\pm 3.5$  days by limiting matings to 1 week every month, with diagnostic abdominal palpitations after a few weeks.

Twenty-four squirrel monkeys (*Samiri sciureus*, Roman Arch type) at least 3 years old served in the experiments. The subjects were born at the Institute for Hygiene, Lund University, Sweden, and housed according to Swedish standards for the care of primates. Lead-exposed and control monkeys were mixed and housed in small groups of three or four per cage. The home cages were  $5 \times 2 \times 2.3$  M, constructed of stainless steel, and had perches large enough for all monkeys to sit on. The room temperature was kept at  $23^\circ\text{C}$ , relative humidity at  $60 \pm 5\%$ . Lights were on from 0700 to 1900 h. Their diet consisted of pellets ad lib, amounting to about 80 to 100 g per monkey daily, supplemented with folic acid in the drinking water and 1/2 apple/day during the winter months.

The monkeys were tested in same-sex cohorts approximately matched for age (Table 1). While the monkeys were undergoing behavioral testing their body weights were maintained at about 90% of their free-feeding weight, which was redetermined semiannually by permitting unrestricted access to food. Each monkey's body weight was determined before the daily experimental session, and food (Nafag Affenfutten, Gossau, Switzerland) was provided to the group to maintain the individuals at a constant body weight. Supplemental feeding was provided individually as required, especially to low hierarchy monkeys, because they did not always obtain food

successfully when feeding was given to the group. Drinking water was provided ad lib.

##### *Exposure*

Lead acetate was administered orally in pieces of apple and in water. Blood-lead concentrations were analyzed using flame atomic absorption and were corrected for variations in hemoglobin by multiplying them by the mean hemoglobin value/simultaneous hemoglobin value (20,21). Doses were adjusted on an individual basis to provide consistent maternal blood concentration, which was monitored weekly. All doses represented a geometric mean across all weeks of exposure.

Exposures began during gestational week 8.5 for monkeys 310 and 325 (59 and 70  $\mu\text{g}/\text{dl}$  maternal blood lead) and at week 5 for all other lead-exposed monkeys, and exposure was maintained until parturition. Gestation is about 22 weeks. Exposures were timed to accord with the period during which mitosis and migration of cells to the outer cerebral cortex takes place (33). Unexposed monkeys born in the same year served as controls. Blood-lead levels in the unexposed monkeys averaged 6.5  $\mu\text{g}/\text{dl}$  (range, 4 to 9  $\mu\text{g}/\text{dl}$ ). When tested, neonatal blood lead levels closely matched maternal levels. The lead-exposed offspring were nursed by their mothers and separated from their mothers at 6 months of age. The monkeys were raised together in small groups with controls and exposed monkeys sometimes sharing cages.

Other cohort members were euthanized close to birth so brain levels of lead could be established and pathologic consequences of exposure could be determined. Detailed descriptions of exposure details, pathology, and tissue levels taken from cohorts of the monkeys described here can be found in Lögberg et al. (20,21).

##### *Experimental Apparatus*

During a behavioral session, a monkey was seated in a Plexiglas chair that provided only waist restraint. It faced a panel containing a light and a T-shaped bar (a modified "Lindsley Manipulandum," manufactured by Gerbrands, Inc.) mounted at about chest level and situated so that the monkey could reach it with its elbows slightly bent. To register a response, the monkey had to pull the bar horizontally through a distance of 1 cm against a spring that provided 1 kg resistance to movement. A food tray was centered under the bar and within easy reach for the monkey. This apparatus was housed in a sound-attenuating enclosure ventilated by a fan that provided exhaust and constant background noise to mask extraneous noises during testing. A Noyes 75 mg precision sucrose pellet was used as the reinforcer.

A transparent plastic window was installed on the top of the chamber, just over the manipulandum, permitting the behavior of a monkey to be monitored unobtrusively with a video camera. A PDP-11/73 computer running SKED11 was used to provide experimental control as well as to record and store data.

##### *Behavioral Procedures*

Monkeys were tested during 30-min sessions once a day, 5 days a week, excluding holidays or days required to maintain equipment. The monkeys were first trained to eat from the food dispenser, and then to pull the response bar through 1 cm against a light spring using conventional shaping by systematic approximations of the target response. When all monkeys completed at least 100 responses under a fixed-ratio 1 schedule

TABLE 1  
GENERAL DESCRIPTION OF THE SUBJECTS

| Cohort | Gender | Group   | Number | Range of Maternal Lead Levels ( $\mu\text{g}/\text{dl}$ )* | Range of Body Weights (gms) | Age Range in Years |
|--------|--------|---------|--------|--|-----------------------------|--------------------|
| 1      | Male   | Control | 5      |  | 1090-1180                   | 3-7                |
| 1      | Male   | Exposed | 3      | 44-79  | 950-1090                    | 3-7                |
| 2      | Female | Control | 3      |  | 795-830                     | 5-7                |
| 2      | Female | Exposed | 3      | 24-43  | 675-750                     | 5-7                |
| 3      | Female | Control | 5      |  | 620-710                     | 5-6                |
| 3      | Female | Exposed | 5      | 21-37  | 650-730                     | 5-6                |

\*Maternal leads for controls ranged from 4 to 9  $\mu\text{g}/\text{dl}$ .

(one response/reinforcer, where the reinforcer was a single pellet) during a 30-min session, a 1 kg spring was applied.

The Fixed Ratio parameter was changed systematically over the course of the next sessions to examine behavior change under the conditions shown in Table 2. After the monkeys completed the fixed-ratio schedules shown in the table, the lever-pulling response was maintained by fixed interval schedules. Under the fixed-interval 120" schedule, a single pellet was delivered for the first response to occur after 120" elapsed. Other fixed-interval schedules were defined similarly.

Table 2 shows the minimum number of sessions at each schedule value. More extensive testing was accomplished for some monkeys. Cohorts 1 and 2 completed 18 sessions under the fixed-ratio 5 schedule after all the schedule values shown in Table 2 were completed. Cohorts 2 and 3 completed an additional 18 sessions under the fixed-interval 600" schedule. These data were analyzed separately. Practical considerations of scheduling and the onset of estrous in some females prevented some animals from undergoing the more extensive testing. As female monkeys entered estrous during the winter months, the performance of many of the males and females were affected and their testing had to be suspended. Monkeys housed in rooms in which this was not occurring could be trained during these months. It is these considerations that prevented cohort 1 from undergoing extensive testing under the fixed-interval 600" schedule or cohort 3 from undergoing extensive testing under the fixed-ratio 5 schedule.

TABLE 2  
REINFORCEMENT SCHEDULES USED DURING ACQUISITION

| Schedule             | Minimum Number of Sessions |
|----------------------|----------------------------|
| Fixed ratio 1        | > 5                        |
| Fixed ratio 5        | 5                          |
| Extinction           | 1                          |
| Fixed ratio 1*       | 1                          |
| Fixed ratio 20       | 5                          |
| Fixed interval 120 s | 5                          |
| Fixed interval 300 s | 5                          |
| Fixed interval 600 s | 10                         |

\*Used to reinstate responding, data not analyzed.

### Performance Measures

The following dependent measures were examined:

1. Responses: total number of completed responses made during a session. A response entailed pulling the horizontal, T-shaped bar against the 1 kg spring through a displacement of 1 centimeter and allowing it to return to the home position.
2. Incomplete responses: total number of incomplete responses in a session. These responses failed to meet the criterion described in No. 1.
3. Response duration: the duration of a complete response, averaged through the session.

Data from the first session conducted at each schedule were used to examine sessions comprising schedule transitions. Steady-state performance under a fixed-interval 600" schedule was examined with cohorts 2 and 3. Steady-state performance under the fixed-ratio 5 schedule was examined with cohorts 1 and 2. The average of the last three sessions under a schedule was used to describe steady-state performance.

### Statistical Analysis

Each dependent measure was first related to dose using simple linear regression. Further analyses revealed correlations of response rate with body weight and of incomplete responses with response rate and with body weight. Therefore, subsequent analyses of response were conducted using multiple regression, with dose and body weight evaluated as independent measures. When examining incomplete responses, the number of completed responses was also included as an independent measure. For each analysis the data structure was examined for outliers and systematic variation in the residuals that could compromise the outcome. For some analyses transformations were accomplished to stabilize the variability. Weighting factors were also used if analysis of the residuals revealed unequal variability across different values of the independent measures. Statistical analysis was conducted with RS/1, version 4.3 from Bolt, Baranek, and Newman.

## RESULTS

### Fixed Ratio 5, Steady State

When the number of incomplete responses during steady state under the fixed-ratio 5 schedule was analyzed using simple linear regression, no relationship was found with dose, and only 5% of the variability in incomplete responses was

accounted for. When the number of incomplete responses was reanalyzed in a multiple regression, however, the variance accounted for increased to 69% (63% adjusted for the total model). Figure 1 illustrates the difference by showing both the raw data (top panel) and the full model (bottom panel).

The bottom panel shows the full model when the effect of response rate was controlled statistically. The adjustment for rate as a covariate illustrated in that graph was accomplished by calculating the model at each point (each point representing a monkey) using the slope terms for the independent variable as they apply to the subject represented, and then adding the residual associated with that point. Because the horizontal axis is dose, this analysis estimates the contribution to incomplete responses associated with prenatal lead exposure.

In the full model, the data were log-transformed (natural log) and one additional independent measure, total responses, was included. Body weight was also examined as an independent measure but the coefficient relating it to incomplete responses was not distinguishable from zero after the other terms were considered. The data and regressions were converted back to the original numbers by raising  $e$  (2.7183) to the appropriate power before making the figures. In addition, one outlier was removed from the analysis. This outlier, which came from a control monkey, was identified as exerting an inordinate influence over the outcome. The removed outlier is indicated in the bottom panel of Fig. 1 by an open circle. The equation describing the relationship between the natural log (ln) of incomplete responses and the different independent variables was:  $\ln(\text{incomplete responses}) = 1.85 + \text{dose} \times 0.019 (\pm 0.0073) + \text{responses} \times 0.0029 (\pm 0.00065)$ . Dose is in  $\mu\text{g}$  of lead/deciliter of blood, and body weight is in kilograms. The number in parentheses indicates the standard error in the estimate of the coefficient. The coefficients relating dose and total responses were both statistically different from zero ( $p = 0.019$  and  $0.001$ , respectively).

#### Fixed Ratio Acquisition

The number of incomplete responses during the first session of each fixed-ratio schedule was examined using linear regression first. The number of incomplete responses during the first session on fixed-ratio 5 and fixed-ratio 20 was significantly and positively related to exposure ( $p < 0.01$  for each analysis) but was also related to body weight and response rate. Therefore, the number of incomplete responses during the first session under a schedule was regressed against body weight, number of complete responses, and dose for performance under each schedule. The results are shown in Fig. 2.

The multiple regression conducted on incomplete responses during the fixed-ratio 5 schedule accounted for 49% of the variability (42% adjusted). The resulting equation was:  $\text{Incomplete responses} = 104 + 0.52 (\pm 0.27) \times \text{dose} + 0.18 (\pm 0.05) \times \text{responses} - 0.14 (\pm 0.042) \times (\text{body weight})$ . The  $p$ -values that the coefficients are not zero were 0.066, 0.0016, and 0.004, respectively.

A quadratic model was required to describe incomplete responses during acquisition under the fixed-ratio 20 schedule (note the downward trend at the higher doses in Fig. 2). The quadratic term ( $\text{dose}^2$ ) is included in the line shown in Fig. 2. The full model is:  $\ln(\text{incomplete responses}) = 2.67 (\pm 0.55) - 0.0016 (\pm 0.00075) \times (\text{body weight}) + 0.0044 (\pm 0.00072) \times \text{Responses} + 0.060 (\pm 0.013) \times \text{Dose} + 0.00086 (\pm 0.00021) \times \text{Dose}^2$ . Including the quadratic term did not change the magnitude of the regression coefficients for body weight and responses, but changed the linear coefficient from 0.012

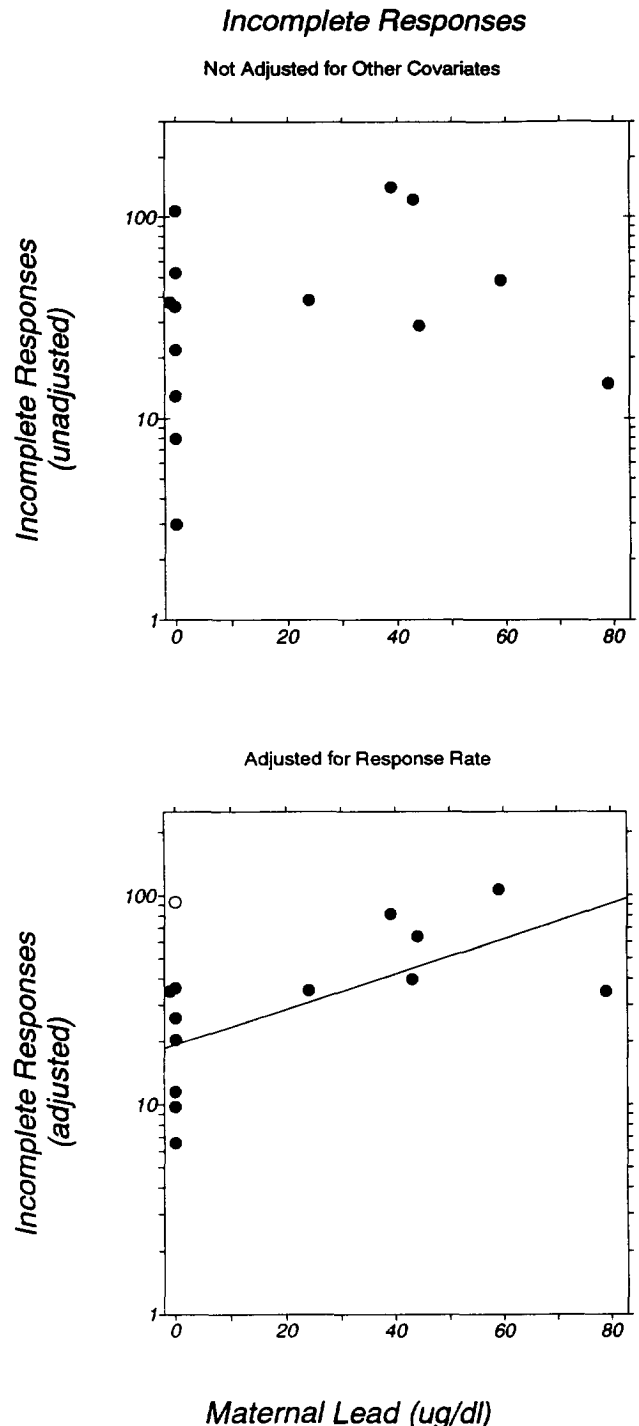


FIG. 1. The number of incomplete responses during steady-state performance under a fixed-ratio 5 schedule of reinforcement as a function of maternal blood-lead levels. An incomplete response is one that failed to meet a criterion of pulling a 1 kg spring through a displacement of 1 cm and allowing it to return to the home position before starting the next response. The top panel shows incomplete responses plotted against maternal lead exposure. The bottom panel shows incomplete responses adjusted for body weight and overall response rate plotted against maternal lead exposure. Note the logarithmic scale of the vertical axes. One outlier, indicated as an open circle, was excluded from statistical analysis.

*Incomplete Responses (Adjusted)  
During the First Session  
on a Schedule*

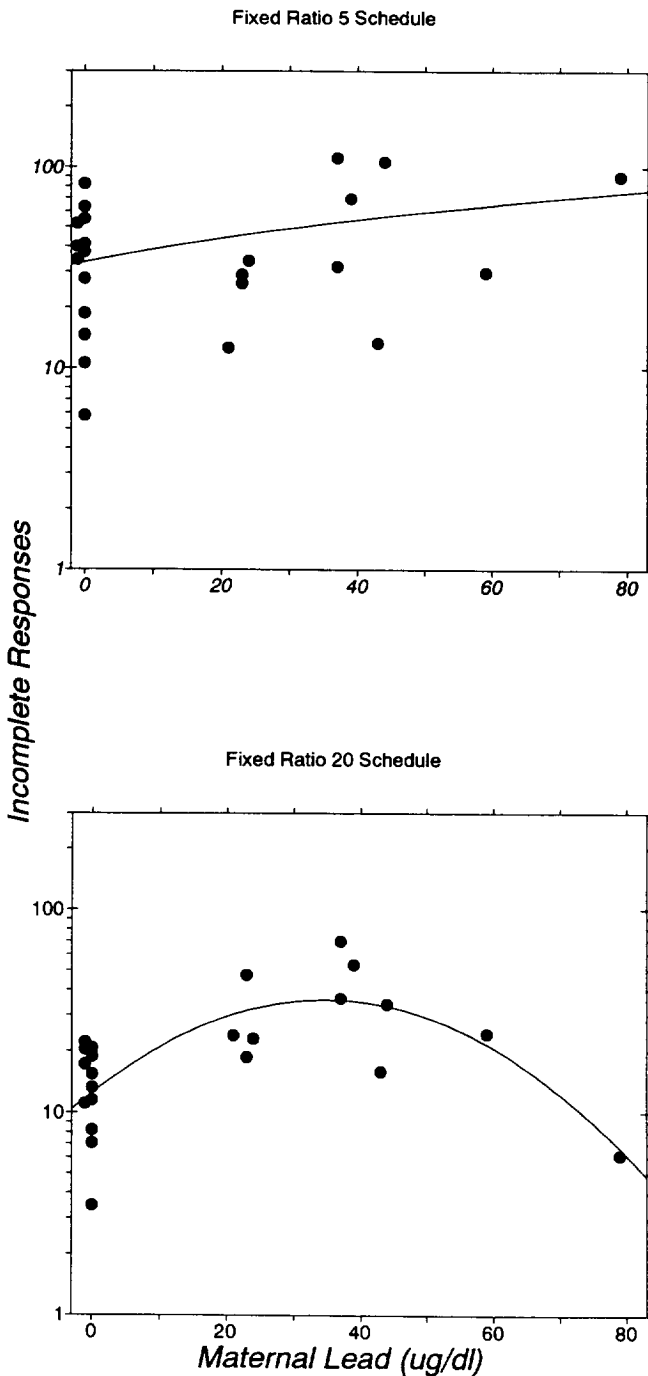


FIG. 2. The number of incomplete responses during acquisition of a fixed-ratio 5 (top) and a fixed-ratio 20 (bottom) schedule of reinforcement. In each case, the relationship between dose and incomplete responses was statistically significant, although the relationship is modest in the top panel. A quadratic component was included in the bottom panel due to statistical considerations, but it is not clear whether this is biologically significant.

$\pm 0.0055$  ( $p = 0.039$ ) to  $0.06 \pm 0.013$  ( $p = 0.07$ ), and increased the variance accounted for from 71% to 75% (67% to 70% adjusted). The  $p$ -value that the coefficients are not zero are 0.051 for body weight, 0.0001 for responses, 0.07 for dose, and 0.0007 for dose<sup>2</sup>.

An effect of lead on overall response rates was identified on the fixed-ratio 5 or fixed-ratio 20 schedules ( $p < 0.01$ ) only when simple linear regression was applied, but when body weight was included in the regression, no effect of lead was detected. This was true during acquisition and steady state and when data were stabilized by transformations and for weight-related differences in variability (all  $ps > 0.1$ ). No effects were identified during other phases of the experiment and no effects were seen on the duration of completed responses.

*Fixed Interval Performance*

No effect of lead on the number of completed or incomplete responses or on the duration of complete responses was found during the first session at each fixed-interval parameter or in steady state in the full regression model (all  $ps$  greater than 0.2). Effects had appeared in simple linear regressions of response rate against dose, but they disappeared when body weight was included as a covariate. The data were examined for the possibility of an inverted U-shaped dose-effect relationship, but no indication of one was visible. Detailed analysis of residuals and of the data structure supported this conclusion.

*Body Weight and Gender*

Because of the presence of a relationship between body weight and the behavioral measures, the possibility of an effect of dose on body weight was examined. This was accomplished using both simple linear regression and analysis of covariance, with gender as a covariate. A relationship between gender and body weight was identified ( $p < 0.0001$ ), with males being heavier than females, but no effect of dose appeared ( $p > 0.2$ ). Examination of Table 1 suggests that the lead-exposed males may have been lighter than the controls, but this tendency was not supported statistically.

DISCUSSION

The present study reproduced some of the testing procedures of Cory-Slechta using rats (6,7,11) and of Rice and colleagues (28,29,30) using cynomolgus monkeys and several others [see reviews by Cory-Slechta (6,8)] in its use of fixed-interval or fixed-ratio schedules of reinforcement. The present study differed from those in that lead exposure was exclusively prenatal and from many in that exposure had ended several years prior to testing, a condition also studied by Rice and colleagues (29,30). More important, the present study differed in the physical requirement required to complete the response. Rather than requiring a simple lever press with a small force through a nominal distance, the response in the present study entailed pulling a force of 1 kg, approximately the animals' body weight, through a displacement of greater than 1 cm. This physical requirement resembles one used to examine the neurotoxicity of manganese (27).

The requirement of pulling a high force through a displacement made it possible to detect deficits in the physical execution of the response. The number of incomplete responses, those that failed to meet the displacement requirement, was elevated in lead-exposed monkeys during initial sessions on

the fixed-ratio 5 and fixed-ratio 20 schedules, and this elevation persisted even with extensive experience on the fixed-ratio 5 schedule. During acquisition, body weight was an important covariate in the multiple regression analysis that identified a relationship to dose, but the significance of body weight waned during steady-state responding.

An increase in the number of incomplete responses appeared in behavior under the fixed-ratio, but not the fixed-interval schedule, reproducing the schedule specificity reported with manganese (27). It is likely that the schedule specificity seen in steady-state is related to the difference in response patterns maintained by fixed-ratio and fixed-interval schedules. The fixed-ratio schedule maintains a very high rate of responding, characterized by long vigorous bursts containing successive strings of short interresponse times, even when the response requires considerable effort (26). Such a behavior pattern appears to be especially vulnerable to disruption by compounds that interfere with motor systems (16,22,23,27). With manganese and in the present report with lead, disruption of the response's physical characteristics appeared even, while other characteristics of behavior were unaffected. Newland and Weiss (27) were able to allow manganese exposure to continue until more overt neurological signs developed, but such examination of higher doses or prolonged exposure was not possible in the present study.

The comparison with manganese is not intended to suggest that the neural mechanisms underlying the effects reported here are the same as those underlying manganese's neurotoxicity; it is more likely that they are quite different. The similarities do indicate, however, some similarities in behavioral mechanisms lying behind the expression of motor toxicity. Specifically, the physical difficulty entailed in pulling a force through a displacement, coupled with the high-rate response pattern that is an indirect effect of the fixed-ratio reinforcement contingency, seems especially capable of revealing certain motor deficits.

The detection of lead's effects on incomplete responses required statistical control over response rate and, during acquisition, body weight. Such statistical control has not typically been required with other behavioral measures, but because of the strength required and rapid alternation of flexion and extension that emerged, it should not be surprising that such performance is related to body weight, at least early in training. Gender was a significant covariate with incomplete responses (unreported analyses), but gender was also highly correlated with body weight. In the absence of other evidence for sexual dimorphism in the expression of prenatal lead toxicity, it would be premature to conclude that gender, and not body weight is the pertinent covariate.

While body weight was correlated with incomplete responding during acquisition, its influence waned during steady state. The reason for its declining influence is not clear, but two possibilities suggest themselves: more effective operation of the response device with extended practice, and increased strength. Behavior during acquisition is more labile and susceptible to influence by a large number of sources, so the natural variability and lability during acquisition may be partly responsible for the contribution of body weight during acquisition. As the monkeys learned to operate the response device more effectively, the role of body weight, or variables such as strength that could covary with weight, may have decreased. It is also possible that strength increased through training and, accordingly, the role of body weight declined.

It was also necessary to control for the overall rate of responding to isolate lead's effects. Although overall rate was

unrelated to lead exposure, it was related to the number of incomplete responses simply because the more responses the monkey executed, the more opportunities it had to produce an incomplete response. A simple ratio of incomplete responses/complete responses captured this relationship (unreported analyses), but accounted for less variability than when responses were included in the model as an independent variable.

The neural mechanisms underlying the effects reported here remain to be determined, except to say that it seems plausible that they involve some aspect of motor function. There is evidence of motor deficits associated with contemporaneous or prenatal lead exposure in children. Neonatal blood-lead levels, thought to estimate late trimester lead exposure, was associated with upper-limb speed and dexterity and bilateral coordination in children (15). Landrigan et al. (19) reported that lead-exposed children (PbB 40.5  $\mu\text{g}/\text{dl}$ ) showed an impairment in the motor skills measured by the finger-wrist tapping test which, like the current study, requires rapid flexion and extension, when compared with "control" children (PbB 20  $\mu\text{g}/\text{dl}$ ). Poor performance, especially visual-spatial and visual-motor integration skills tested at age 57 months has been related to the 24-month blood-lead level (2). Neonatal lead exposure has also been associated with deficits in motor control (3,15). The relationship between some of these effects and the current report must await further study to be clarified.

The monkeys exposed to lead showed no changes in rates or patterns of responding under the fixed-interval and fixed-ratio schedules. The effects of chronic lead exposure on performance maintained by fixed-interval schedules in other studies resemble those often seen after acute administration of psychomotor stimulants: low doses increase response rate, while higher doses decrease it and fixed-ratio response rates are only decreased (5,7,12,28,29,30). There had been some evidence of a modest rate increase in monkeys exposed to maternal lead levels of about 40  $\mu\text{g}/\text{dl}$  during the initial sessions on the fixed-interval schedule. This appeared as a modest effect when analyzed with simple linear regression, but the effect was not confirmed in the full model with body weight as a covariate.

The lack of rate changes in the present experiments do not necessarily mean that such effects would be absent under different conditions. No rate increase was seen after acute amphetamine exposure when the response required considerable effort (26), even though amphetamine commonly elevates response rates under fixed-interval schedules (32). The lack of an effect could be due to the physical effort required to respond. Rate increases under fixed-interval schedules normally seen with more conventional and less effortful responses after lead exposure or acute administration of a psychomotor stimulant may disappear when the physical effort required to respond increases. This could reflect relative insensitivity of effortful responding to some subtleties of fixed-interval schedule performance, or sensitivity of behavior early in the fixed interval to effort requirements. This means that the possibility that rate increases in fixed-interval schedules are not a component of the neurotoxicity associated with prenatal lead exposure cannot be ruled out.

The monkeys in the present study have already displayed disruption in behavior maintained under a two-lever (conventional, low-force levers) concurrent schedule of reinforcement whose schedule parameters changed from time to time (25). Under that schedule, one of the two levers was programmed to deliver reinforcers at 1 to 8 times the rate as the other. For

control monkeys and those exposed to less than about 40  $\mu\text{g}/\text{dl}$  of lead during gestation, the relative response rate on a lever approximately matched the relative reinforcer rate associated with that lever. Monkeys exposed to the highest doses showed little sensitivity to the relative reinforcement rates on the two levers, even with extended experience on the schedules. When the reinforcement rates on the two levers were changed, the performance of most monkeys changed, too, but the monkeys exposed to low levels of lead, whose performance was indistinguishable from controls during steady state, required about several times more reinforcers to achieve a new steady state as did controls.

The earlier report with these same monkeys (25) and the present one describe learning and motor deficits associated with prenatal lead exposure. The learning deficits were manifested as retarded behavioral transitions and appeared to be mediated by insensitivity to reinforcing consequences, or distractibility by alternative reinforcers. The motor effects were revealed as an elevated number of responses that failed to meet an effort requirement when vigorous responding was maintained. The motor deficit may reflect deficits in rapid

alterations of flexion and extension, fatigue, coordination, or other factors involved in effortful responding.

Exposure to lead at levels that might be tolerated occupationally or that could occur in some urban environments could result in maternal blood-lead levels similar to those used here. A time-weighted average of 25  $\mu\text{g}/\text{m}^3$  would result blood-lead in levels of greater than about 20  $\mu\text{g}/\text{dl}$  in 67% of workers and greater than 60  $\mu\text{g}/\text{dl}$  in about 0.5% of exposed workers (17,34). Residents in some urban areas, such as Mexico City, also have lead exposures up to 40  $\mu\text{g}/\text{dl}$ , within the range studied here (31). A close correspondence between blood-lead levels that produce adverse effects in human and nonhuman species has been noted (8,14). If this correspondence can be extended to prenatal exposure, then the present results would predict that lead exposure to pregnant women in some occupational or urban settings poses a long-term risk to the offspring.

#### ACKNOWLEDGEMENT

This work was supported by the Swedish Work/Environment fund.

#### REFERENCES

1. Angell, N. F.; Weiss B. Operant behavior of rats exposed to lead before or after weaning. *Toxicol. Appl. Pharmacol.* 63:62-71; 1982.
2. Bellinger, D.; Sloman, J.; Leviton, A.; Rabinowitz, M.; Needleman, H. L.; Waternaux, C. Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics* 87: 219-227; 1991.
3. Bonithon-Kopp, C.; Huel, G.; Moreau, T.; Wendling, G. Prenatal exposure to lead and cadmium and psychomotor development of the child at 6 years. *Neurobehav. Toxicol. Teratol.* 8:307-310; 1986.
4. Branch, M. N. Behavioral Pharmacology. In: Iversen, I. V.; Lattal, K. A., eds. *Techniques in the behavioral and neural sciences*, vol. 6: Experimental analysis of behavior. Part 2. Amsterdam: Elsevier; 1991:21-71.
5. Carson, T. L.; VanGelder, G. A.; Karas, G. C.; Buck, W. B. Slowed learning in lambs prenatally exposed to lead. *Arch. Environ. Health* 29:154-156; 1974.
6. Cory-Slechta, D. A. Behavioral toxicity of lead: Problems and perspectives. *Advances in behavioral pharmacology*, vol. 4. In: Thompson, T.; Dews, P. B.; Barrett, J. B., eds. New York: Academic Press; 1984:211-255.
7. Cory-Slechta, D. A. Prolonged lead exposure and fixed ratio performance. *Neurobehav. Toxicol. Teratol.* 8:237-244; 1986.
8. Cory-Slechta, D. A. Bridging experimental animal and human behavioral toxicology studies. In: Russell, R. W.; Flattau, P. E.; Pope, A. E., eds. *Behavioral measurement of Neurotoxicity*. Washington, DC: National Academy of Sciences Press; 1990.
9. Cory-Slechta, D. A. Exposure duration modifies the effects of low level lead on fixed-interval performance. *Neurotoxicology* 11: 427-442; 1990.
10. Cory-Slechta, D. A.; Bissen, S. T.; Young, A. M.; Thompson, T. Chronic postweaning lead exposure and response duration performance. *Toxicol. Appl. Pharmacol.* 60:78-84; 1984.
11. Cory-Slechta, D. A.; Thompson T. Behavioral toxicity of chronic postweaning lead exposure in the rat. *Toxicol. Appl. Pharmacol.* 47:151-159; 1979.
12. Cory-Slechta, D. A.; Weiss, B.; Cox, C. Delayed behavioral toxicity of lead with increasing exposure concentration. *Toxicol. Appl. Pharmacol.* 71:342-352; 1983.
13. Cory-Slechta, D. A.; Weiss, B.; Cox, C. Performance and exposure indices of rats exposed to low concentrations of lead. *Toxicol. Appl. Pharmacol.* 78:291-299; 1985.
14. Davis, J. M.; Otto, D. A.; Weis, D. E.; Grant, L. D. The comparative developmental neurotoxicity of lead in humans and animals. *Neurotoxicol. Teratol.* 12:215-230; 1990.
15. Dietrich, K. N.; Berger, O. G.; Succop, P. A.; Hammond, P. B.; Bornschein, R. L. The developmental consequences of low to moderate prenatal and postnatal lead exposure: Intellectual attainment in the Cincinnati Lead Study Cohort following school entry. *Neurotoxicol. Teratol.* 15:37-44; 1993.
16. Fowler, S. C. Force and duration of operant response as dependent variables in behavioral pharmacology. In: Thompson, T.; Dews, P. B.; Barrett, J. E., eds. *Advances in behavioral pharmacology: vol. 6. Neurobehavioral pharmacology*. Hillsdale, NJ: L. Erlbaum Associates; 1987.
17. Fronies, J. R.; Liu, W. V.; Hinds, W. C.; Wegman, D. H. Effects of aerosol size on the blood lead distribution of industrial workers. *Am. J. Ind. Med.* 9:707-712; 1986.
18. Gentry, G. D.; Middaugh, L. D. Prenatal ethanol weakens the efficacy of reinforcers for adult mice. *Teratology* 37:135-144; 1988.
19. Landrigan, P. J.; Baker, E. L.; Whitworth, R. H.; Feldman, R. G. Neuroepidemiologic evaluation of children with chronic increased lead absorption. In: Needleman, H. L., ed. *Low level lead exposure: The clinical implication of current research*. New York: Raven Press; 1980.
20. Lögdberg, B.; Berlin, M.; Schutz, A. Effects of lead exposure on pregnancy outcome and the fetal brain of squirrel monkeys. *Scand. J. Work Environ. Health* 13:135-145; 1987.
21. Lögdberg, B.; Brun, A.; Berlin, M.; Schutz, A. Congenital lead encephalopathy in monkeys. *Acta Neuropathol.* 77:120-127; 1988.
22. Newland, M. C. Operant behavior and the measurement of motor dysfunction. In: Weiss, B.; O'Donoghue, J., eds. *Toxicological interpretation of neurobehavioral data*. New York: Raven Press; 1994:272-298.
23. Newland, M. C. Motor function and the physical properties of the operant: Applications to screening and advanced applications. In: Chang, L. W.; Slikker, W., eds. *Neurotoxicology: Approaches and methods*. San Diego: Academic Press; 1995:265-300.
24. Newland, M. C.; Ng, W. W.; Baggs, R. B.; Gentry, G. D.; Weiss, B.; Miller, R. K. Operant behavior in transition reflects neonatal exposure to cadmium. *Teratology* 34:231-242; 1986.
25. Newland, M. C.; Sheng, Y.; Lögdberg, B.; Berlin, M. Prenatal exposure to lead or methylmercury impairs acquisition and maintenance of concurrent schedule performance in squirrel monkeys. *Toxicol. Appl. Pharmacol.* 126:6-15; 1994.
26. Newland, M. C.; Weiss, B. Drug effects on an effortful operant: Pentobarbital and amphetamine. *Pharmacol. Biochem. Behav.* 36:381-387; 1990.
27. Newland, M. C.; Weiss, B. Persistent effects of manganese on ef-

- portful responding and their relationship to manganese accumulation in globus pallidus. *Toxicol. Appl. Pharmacol.* 113:87-97; 1992.
28. Rice, D. C. Schedule-controlled behavior in infant and juvenile monkeys exposed to lead from birth. *Neurotoxicol. Teratol* 9:75-88; 1988.
  29. Rice, D. C. Lead exposure during different developmental periods produces different effects on FI performance in monkeys tested as juveniles and adults. *Neurotoxicology* 13:757-770; 1992.
  30. Rice, D. C.; Gilbert, S. G.; Willes, R. F. Neonatal low-level lead exposure in monkeys: Locomotor activity, schedule-controlled behavior, and the effects of amphetamine. *Toxicol. Appl. Pharmacol.* 51:503-513; 1979.
  31. Rothenberg, S. J.; Schnaas, L.; Cansino-Ortiz, S.; Herroni-Hernandez, E.; de la Torre, P.; Neri-Mendez, C.; Ortega, P.; Hidalgo-Loperena, H.; Svendsgaard, D. Neurobehavioral deficits after low-level lead exposure in neonates: The Mexico-City pilot study. *Neurotoxicol. Teratol.* 11:85-93; 1989.
  32. Seiden, L. S.; Dykstra, L. A. *Psychopharmacology: A biochemical and behavioral approach*. New York: Van Nostrand Reinhold; 1977.
  33. Sidman, R. L.; Rakic, P. Neuronal migration, with special reference to the developing human brain: A review. *Brain Res.* 62:1-35; 1973.
  34. Skerfving, S. Biological monitoring of exposure to inorganic lead. In: Clarkson, T. W.; Friberg, L.; Nordberg, G. F.; Sager, P. R., eds. *Biological monitoring of toxic metals*. New York: Plenum; 1988:169-197.
  35. VanGelder, G. A.; Carson, T.; Smith, R. M.; Buck, W. B. Behavioral toxicologic assessment of the neurologic effect of lead in sheep. *Clin. Toxicol.* 6:405-418; 1973.
  36. Zenick, H.; Rodriguez, W.; Ward, J.; Elkington, B. Deficits in fixed-interval performance following prenatal and postnatal lead exposure. *Dev. Psychobiol.* 12:509-514; 1979.