

Behavioral Consequences of *in Utero* Exposure to Mercury Vapor: Alterations in Lever-Press Durations and Learning in Squirrel Monkeys

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Received November 9, 1995; accepted March 26, 1996

Behavioral Consequences of *in Utero* Exposure to Mercury Vapor: Alterations in Lever-Press Durations and Learning in Squirrel Monkeys. NEWLAND, M. C., WARFVINGE, K., AND BERLIN, M. (1996). *Toxicol. Appl. Pharmacol.* 139, 374–386.

Exposure to mercury vapor *in utero* results in the accumulation of mercury in the cerebellum, hippocampus, and other regions of the nervous system associated with motor function and learning, but little is known about the functional consequences of prenatal exposure. The offspring of pregnant squirrel monkeys exposed to 0.5 or 1.0 mg/m³ of mercury vapor during the last $\frac{2}{3}$ or more of gestation were studied. Median maternal blood levels ranged from 0.025 to 0.18 $\mu\text{g/g}$ and exposures were estimated to range from 20 to 62 $\mu\text{g/day}$, with cumulative doses of 1304 to 4305 μg . Unexposed monkeys born at about the same time served as controls. The monkeys' lever pressing was maintained under various Concurrent Random-Interval Random-Interval schedules of reinforcement. Time allocation on each lever was examined during behavioral transitions and in steady state. No difference in sensitivity to reinforcer ratios was identified in steady state, but there was much more variability in the steady-state performance of exposed monkeys, as indicated by the standard deviation of the regression, than in controls. Logistic regression was used to examine the transition to new schedule parameters. Exposed monkeys were found to produce smaller or slower transitions than controls. The magnitude and stability of lever-press durations for controls and exposed monkeys were indistinguishable early in the experiment, but at the end the exposed monkeys had longer lever-press durations and the session-to-session variability was much greater. One monkey's exposure began during the third week of gestation (earlier than any of the others) and the behavior of this monkey was so erratic that some of the analyses could not be accomplished. Long-term effects of prenatal mercury vapor exposure included instability in lever-press durations and steady-state performance under concurrent schedules of reinforcement as well as aberrant transitions. The levels used were close to those reported in occupational settings under conditions of poor hygiene, but were at least 10- to 50-fold greater than those more commonly reported. © 1996 Academic Press, Inc.

Mercury vapor exposure in the adult human results in a constellation of neuromotor effects, including tremor, ataxia, weakness, and a behavioral disturbance called erethism, which is characterized by withdrawal, depression, sensory and sleep disturbances, and emotional lability (Chang *et al.*, 1995; Echeverria *et al.*, 1995; Fawer *et al.*, 1983; Langolf *et al.*, 1978; Liang *et al.*, 1993; Weiss, 1983; Wood *et al.*, 1973). Studies in adult mammals and birds also point to a broad pattern of effects, including rate reductions of behavior maintained under fixed-interval schedules of reinforcement, disruption of nondiscriminated avoidance, and other effects on schedule-controlled operant behavior (Weiss, 1983). The more striking signs of inorganic mercury vapor exposure are reversible after exposure has terminated, but subtle emotional and motor effects may persist for years (Kishi *et al.*, 1993), perhaps due to the very slow elimination of inorganic mercury from the nervous system (Aschner and Aschner, 1990; Vahter *et al.*, 1994).

Mercury vapor is lipid soluble and readily crosses physiological barriers such as placenta and the blood–brain barrier (Clarkson *et al.*, 1972), but mercury vapor exposure during pregnancy is strikingly different from methylmercury exposure. After methylmercury exposure, fetal concentrations of mercury are higher than maternal levels, its distribution in the fetal nervous system is more homogeneous than in the maternal nervous system, and damage is more diffuse (Burbacher *et al.*, 1990; Eccles and Annau, 1987). By contrast, the concentration of mercury in fetal circulation and organs is less than (Warfvinge *et al.*, 1992, 1994; Yoshida *et al.*, 1986, 1992) or sometimes equal to (Vimy *et al.*, 1990) maternal levels after mercury vapor exposure, although its distribution, too, is more diffuse in the developing than in the maternal nervous system.

Little is known about the consequences of exposure to mercury vapor during development (Larsson, 1992). The distribution of mercury in the adult nervous system after exposure to elemental or inorganic mercury differs from that seen after methylmercury exposure (Møller-Madsen, 1994). Mercury vapor exposure during gestation results in the appearance of mercury in the CNS of rodents, sheep, and non-

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human primates, but the pattern of its distribution in the offspring differs from that seen in adults (Danielsson *et al.*, 1990; Warfvinge, *et al.*, 1994; Vimy *et al.*, 1990). A laminar distribution of mercury deposits with elevated concentrations in pyramidal cell layers of the pre- and post-central gyrus and visual cortex appears in the maternal brain but in the offspring some mercury is present in nearly all cortical regions and lamina and it is especially concentrated in the subiculum hippocampus, Purkinje cells of the cerebellum, and glia-rich areas such as the corpus callosum and Bergman cells of the cerebellum. The laminar distribution detected in the maternal brains was not seen in the brains of the offspring (Warfvinge *et al.*, 1994). Elevations in cerebellum have also been reported in rodents (Eide and Wesenberg, 1993).

In utero exposure to mercury vapor may have consequences on the behavior of the offspring (Danielsson *et al.*, 1993; Fredriksson *et al.*, 1992), but a full picture of its effects and their relationship to exposure is not available. The literature on methylmercury raises a general concern about the vulnerability of the developing nervous system to this metal, but because of the differences between inorganic and organic mercury (Aschner and Aschner, 1990; Berlin, 1986), the developmental toxicity of mercury vapor must be studied directly. Information about the developmental consequences of prenatal mercury vapor exposure not only will contribute to general knowledge of the developmental neurotoxicity of mercury compounds but also will have health implications. Employment in certain industries such as chloralkali plants, thermometer and fluorescent bulb assembly operations, and dentistry can result in significant exposure to mercury vapor among pregnant women. In dentistry, where mercury constitutes as much as 50% of dental amalgam, there is exposure to employees who often work with amalgam daily, and to patients receiving such fillings. Dental assistants may be the largest group occupationally exposed to mercury vapor and often they are women of child-bearing age (Skerfving, 1991).

The present study was designed to investigate the behavioral neurotoxicity of *in utero* exposure to mercury vapor, complementing an investigation of the localization of mercury in the squirrel monkey nervous system after similar exposures (Warfvinge *et al.*, 1994). Pregnant squirrel monkeys were exposed to different levels of mercury vapor and their offspring were evaluated using a protocol, behavior under concurrent schedules of reinforcement during transition states, that was especially sensitive to *in utero* methylmercury and lead (Newland *et al.*, 1994). In this procedure, a subject has two response alternatives and one produces reinforcers more frequently than the other. When behavior is stable, the time spent on the richer alternative is determined by the relative number of reinforcers it produces (Davison and McCarthy, 1988; de Villiers, 1977). When the reinforcement rates on the two levers are reversed, behavior

becomes reallocated systematically until more time is eventually spent on the newly rich lever. Describing this transition quantitatively permits the direct examination of learning, which can be defined as behavior change. The course of behavior change is smooth and can be fitted with a logistic equation in such a way that fitting parameters reveal the magnitude, speed, and number of reinforcers required to negotiate the transition (Newland *et al.*, 1994).

To investigate the possibility that motor effects are also a component of the developmental neurotoxicity, the duration of the lever press was recorded and analyzed. This measure provides a rough indication of motor effects of drugs or toxicants that is separate (Fowler, 1987; Newland, 1994, 1995) from and sometimes orthogonal to (Walker *et al.*, 1981) the information contained in response rates.

METHODS

Subjects. The offspring of timed-pregnant squirrel monkeys exposed in 2-m³ Rochester-type chambers to 0.5 or 1.0 mg/m³ of mercury vapor for 4 or 7 hr/day, 5 days/week (barring apparatus problems) during gestation were studied. Table 1 contains exposure parameters for the different monkeys. Exposure began a few weeks after mating and after diagnostic abdominal palpations and measurement of fundus height were performed to estimate gestational age (Lögberg, 1993). Exposure details can be seen in Warfvinge *et al.* (1994). Unexposed monkeys born at about the same time served as controls.

All monkeys but one (subject F490) were male. There was no difference in birth weight or weight gain between exposed and control monkeys. All monkeys selected for investigation derived from uneventful pregnancies and displayed no unusual complications during development. Informal observations revealed no gross differences in the behavior of the monkeys up to the end of the experiments reported here. At the beginning of the experiment the body weights of exposed monkeys were indistinguishable from those of controls. The monkeys ranged in age from 0.8 to 4 years at the start of the experiment, but age was not significantly related to the number or variability in responses, visits, or duration (*F* tests, all *p*'s > 0.1) so age was not included as a factor in the analyses.

Behavioral methods. The procedures reported by Newland *et al.* (1994) were replicated, with the addition that lever-press durations were also monitored. Monkeys were seated in a Plexiglas chair with loosely fitted plates that provided neck and waist restraint while permitting ample movement. The seated monkey faced an aluminum panel holding two conventional rodent levers (Gerbrands) situated about shoulder height and equidistant from a pellet dispenser located in the center of the panel at about the level of the waist. The monkeys were mildly food deprived to about 95% of their free-feeding body weight by permitting free access to a measured quantity of food after the end of the session. Body weights were monitored daily, before each session, and supplemental feeding was provided if a monkey could not defend its body weight under this regimen. Because these monkeys were still growing, they were permitted free access to food for 1 week after 3 months of testing and their free-feeding body weights were subsequently redetermined.

The monkeys were trained using conventional shaping techniques to eat sucrose pellets from the pellet dispenser and then to press the left lever. After they pressed the left lever under a fixed-ratio 1 schedule of reinforcement for 100 reinforcers during a 30-min session, they were required to press the right lever 100 times during a 30-min session.

After the monkeys reliably pressed each lever, a Concurrent Random-Interval 30" Random-Interval 30" (Conc RI 30" RI 30") schedule was intro-

TABLE 1
Summary of Mercury Vapor Exposures

Monkey number (code ^a)	Median (range) of mercury in blood ($\mu\text{g/g}$) ^b	Exposure duration		External Hg dose			Calculated exposure	
		Gestational age (weeks)	Number of exposure days ^c	Concentration (mg/m^3)	Hours per day (typical)	Total hours of exposure	Total (μg)	Daily ($\mu\text{g/day}$)
F490 (1)	0.025 (0.02–0.03)	5–19	63	0.5	4	247	1304	21
M497 (2)	0.09	5–21	79	0.5	4	323	1585	20
M485 (3)	0.08	6–22	76	0.5	7	510	2900	38
M470 (4)	0.16 (0.14–0.17)	7–21	69	1	4	283	2901	42
M477 (5)	0.12 (0.12–0.13)	3–18	72	1	4	287	3020	42
M447 (6)	0.18 (0.16–0.18)	8–21	69	1	7	402	4305	62

^a The single digit number in parentheses is used to identify the subject in the figures.

^b Maternal blood levels were determined after 4, 8, 12, and 16 weeks of exposure when possible—accomplished once for 497 and 485, twice for 490, and three times for 470, 477, and 447. Blood levels before exposure were 0.01 for 447 and nondetectable for the others.

^c Exposure occurred 5 days/week, with exceptions only for apparatus problems.

duced. This is listed as condition 1 in Table 2 where it is shown as having an average reinforcer rate of 2/min. Before describing the concurrent schedule, the implementation of a single RI schedule will be described. Under a single RI 30" schedule, a reinforcer follows a lever press on the average of once every 30 sec, but the exact time is unpredictable. In the present arrangement, reinforcer delivery was determined by arranging for a timer to query a uniform probability distribution every second and delivering a reinforcer with a probability of 1/30. Under the *Concurrent RI 30" RI 30"* schedule, a separate RI 30" schedule operated independently on each lever. That is, as the monkey responded on the left lever, a response-contingent pellet would be delivered on the average of once every 30 sec. Meanwhile the timer associated with the right lever continued to query its probability generator to determine if a reinforcer could be arranged for the next response on the right lever. If a reinforcer was scheduled to follow the next right-lever response, then the right timer stopped and the reinforcer was held until (1) the monkey pressed the right lever, and (2) a "changeover delay" of 2 sec elapsed. Under the changeover delay, a right-lever press after one or more left-lever presses (a "changeover") was not reinforced until 2 sec elapsed. An identical delay operated for switches from right to left. This changeover delay is a refractory period that is commonly used to prevent adventitious reinforcement of lever switching, which results in excessive switching between levers and insensitivity to the different reinforcement rates available. Table 2 shows the sequence of schedules used in this investigation. The transition from one schedule to another began with the first day

of the new schedule. The previous sessions on the older schedule served as the baseline and the transition was allowed to continue until a new steady state developed, which often required several sessions. The transition was charted by examining summary measures of behavior taken at the end of each 30-min session. Monkeys were not observed attempting to press both levers simultaneously with each arm, but if they did then the changeover delay would prevent that behavior from being reinforced.

Dependent measures. The dependent measures used to describe concurrent schedule performance were based upon the average duration of visits on the two levers. A timer began with the first response on the left lever (after one or more responses had occurred on the right lever) and stopped with the first response on the right lever. This time, called the left-visit duration, was accumulated over a session. Visits on the right lever were treated similarly. In steady state, the ratio of left-visit to right-visit durations was examined as a function of the programmed ratio of left-lever to right-lever reinforcer rates. To describe transitions, the proportion of time spent on the richer of the two levers was examined as a function of the cumulative reinforcers derived from responding on the newly rich lever. Overall response rates, derived by totaling left- and right-lever responses, were also examined as a general measure of response rate. The total number of visits in a session was examined as a measure of the rate at which the monkey changed levers.

Left lever-press durations were averaged across each session. A lever press was defined as the closure of a limit switch. A timer began when the switch

TABLE 2
Concurrent Schedule Parameters^a

Condition	Transition No.	Programmed reinforcer rate (reinforcers/min)			Proportion reinforcers on left	Reinforcer ratio (left/right)	Number of sessions
		Left lever	Right lever	Overall			
1		2	2	4	0.5	1	21
2	1	4	1	5	0.8	4	15
3	2	1	4	5	0.2	0.25	22
4	3	2.5	0.3	2.7	0.89	8	19
5	4	1	3	4	0.25	0.33	20

^a These parameters apply to all monkeys but 477, who required additional sessions at some parameters and fewer at others because response rates on one level plummeted to zero, and who occasionally required additional sessions to reestablish responding.

closed and stopped when the switch opened again. Accumulated time was divided by the total number of responses to obtain an average lever-press duration. The choice of the left lever for examination was arbitrary.

All stimuli were controlled, and lever presses were monitored, with 0.01-sec resolution using SKED 11 software (State-Systems, Inc.) running on a DEC PDP 11/73 computer.

Vision testing of monkey 477. Difficulties in establishing stable behavior in monkey 477 prompted an assessment of his visual function. First, small bits of food were placed in his visual field to determine if he would look at them and reach for them. Then monkey 477 and two control monkeys of the same age as 477 were dark adapted for at least 40 min before full-field electroretinograms were obtained, using Burian-Allen electrodes. The electroretinography examinations were performed under general anesthesia.

RESULTS

Summary measures. No exposure-related differences in the acquisition of lever pressing were noted. Table 3 shows the number of responses and changeovers per session averaged over the last five sessions of the Conc RI 30" RI 30" (the first condition) and the last five sessions of the Conc RI 60" RI 120" schedule (the last condition). While there were no consistent relationships to exposure on either measure, the performance of M477 is noteworthy in the extraordinarily low overall rate of responding and of changeovers. These low rates were present throughout the course of the experiment and at times the monkey ceased responding. The behavior of this monkey had to be treated separately from the others.

Steady state. One lever was richer than the other, i.e., had a higher programmed rate of reinforcement, for all but the first condition. The degree to which behavior reflected this discrepancy in reinforcer rates was examined by plotting the ratio of left-visit duration to right-visit duration against the ratio of left-reinforcer rate to right-reinforcer rate. The top three panels of Fig. 1 show this relationship for a control monkey and two exposed monkeys. On logarithmically scaled axes such a plot typically produces a straight line that can be described with a slope and intercept as

$$\log \left(\frac{T_l}{T_r} \right) = a + b \log \left(\frac{R_l}{R_r} \right) . \tag{1}$$

T_l and T_r are session times spent on the left and right levers, respectively. R_l and R_r are programmed reinforcer rates on the left and right levers. The slope (b) describes the sensitivity of behavior to changes in the reinforcer ratio and the intercept (a) describes bias (here a positive number indicates a leftward bias). These two parameters were estimated once by regressing the time allocation (time on left lever/time on right lever) against programmed reinforcer ratios and then regressing response ratio (left responses/right responses) against programmed reinforcer ratios. Linear least-squares regression was used in each case. The independent and de-

TABLE 3
Age, Body Weight, and Summary Behavioral Measures for the Monkeys Studied

Monkey (code)	At start of experiment			First baseline			Last baseline			Slope ^e		Intercept ^e	
	Dose ^a	Age ^b	Body weight ^c	Responses ^d	Changeovers ^d	Responses ^d	Changeovers ^d	Responses ^d	Changeovers ^d	Time	Response	Time	Response
M458	0	3.7	620	2136	72	2056	110	0.51	0.16	0.27	0.02	0.92	0.16
M465	0	3.1	795	2182	65	1217	45	0.61	0.50	0.02	0.16	0.61	0.12
M478	0	2.8	845	528	21	502	77	0.96	0.60	0.33	0.12	0.96	0.12
M484	0	1.8	595	604	21	830	35	0.92	0.71	-0.27	-0.02	0.92	-0.02
M493	0	1.2	520	336	52	292	34	0.75	0.71	0.33	0.20	0.75	0.20
F490 (1)	1304	1.2	535	363	78	235	11	0.67	0.59	0.43	0.33	0.67	0.33
M497 (2)	1583	0.8	500	734	103	496	33	1.70	0.44	0.12	0.36	1.70	0.36
M485 (3)	2900	1.9	650	614	40	272	44	0.97	1.31	0.72	0.28	0.97	0.28
M470 (4)	2901	3.0	860	718	76	722	87	0.79	0.71	0.18	0.01	0.79	0.01
M477 (5)	3020	2.8	795	99	2	86	5	0.65	0.82	-1.15	-0.96	0.65	-0.96
M447 (6)	4305	4.0	800	1652	169	1360	79		0.44	0.20	-0.20		-0.20

^a Estimated cumulative mercury exposure in μg .

^b Age in years at first session.

^c Initial body weight in kg under free-feeding conditions.

^d Average responses and changeovers per session at the end of the first and last baselines.

^e Slopes and intercepts describing steady-state time and response allocations.

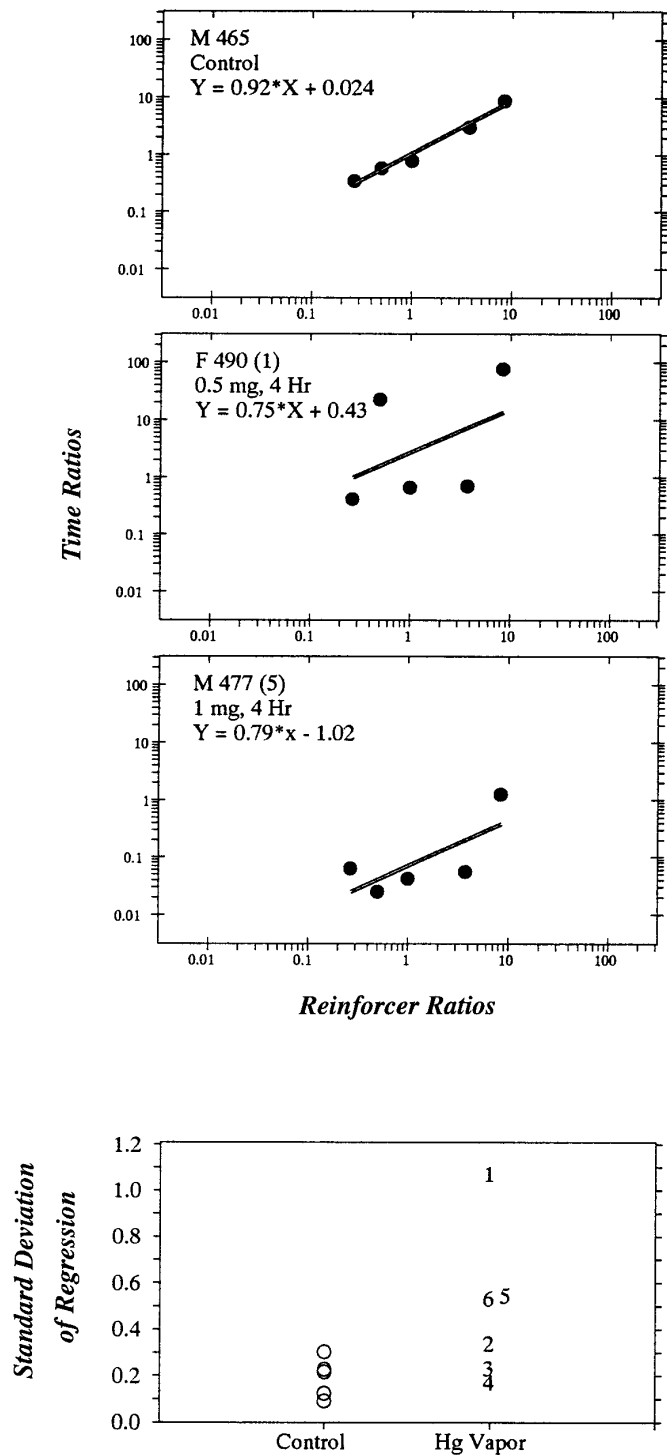


FIG. 1. The top three panels show steady-state performance at each of five different reinforcer ratios for selected subjects. The abscissas show the ratio of programmed rate of reinforcement on the left lever to that on the right lever. The ordinates show the ratio of left-visit duration to right-visit durations, averaged over the last five sessions on a condition. If the ratio of time allocation matched that of reinforcer rates then the resulting regression line would have a slope of 1.0 and an intercept of 0. The obtained regression lines are shown in each panel. Note the close alignment of the

pendent measures were log-transformed before the regression was accomplished. Table 3 shows the resulting slopes and intercepts for each subject. In general, the slopes obtained with time allocation were steeper, indicating greater sensitivity with this measure.

There was no difference between control and exposed subjects on slopes, intercepts, or variance accounted for by the regression (not shown) but there was a difference in the ability of Eq. (1) to describe the relationship between responding and reinforcer ratios. This difference was expressed as the standard deviation of the regression illustrated in Fig. 1. As seen in the control subject shown in the top panel of Fig. 1, the slope was positive, the individual points lay close to the regression line, and the linear regressions described the spread of points well. The slope was also positive for the exposed monkeys but the individual points lay further removed from the regression line, which provided a poorer description of the points. This spread was characterized by the standard deviation of the regression.

Note the third panel of Fig. 1 showing M477. The ratio describing time allocation was invariant across a 16-fold range of reinforcer ratios, but the rightmost point, above the reinforcer ratio of 8, had a y coordinate of about 1. Only when the left lever had a reinforcer rate that was eight times that of the right lever did that monkey's behavior begin to shift toward the left lever, and then, only about $\frac{1}{2}$ of the monkey's time was spent responding on the rich left lever. This point is sufficiently elevated above the other three to provide a positive slope of 0.79 and this fit accounted for 53% of the variability, even though inspection of the data indicates very poor control over behavior by the reinforcer ratios over a broad range of ratios for exposed monkeys.

The standard deviation of the regression, a measure of the degree to which individual points are scattered around the line of best fit, described the quality of the fit better than r^2 , the variance accounted for by the regression, which was very sensitive to points at the extreme ends of the data. The bottom panel of Fig. 1 shows the standard deviations of regression obtained from fitting Eq. (1). There was a general tendency for the fits from the exposed monkeys to be poorer, i.e., the standard deviation was larger, in exposed monkeys than controls ($p = 0.05$ from a t test on the log of the data), especially for monkeys 490, 477, and 447 (coded as 1, 5, and 6).

Transitions. When the concurrent schedule parameters were changed, the course of the resulting change in behavior was charted by following the proportion of session time

points to the regression in the top panel, representing an unexposed subject and the poor alignment for the exposed subjects in the next two panels. The bottom panel shows the standard deviation of the regression for all monkeys. The regression line provided a poorer fit to the data from the exposed monkeys.

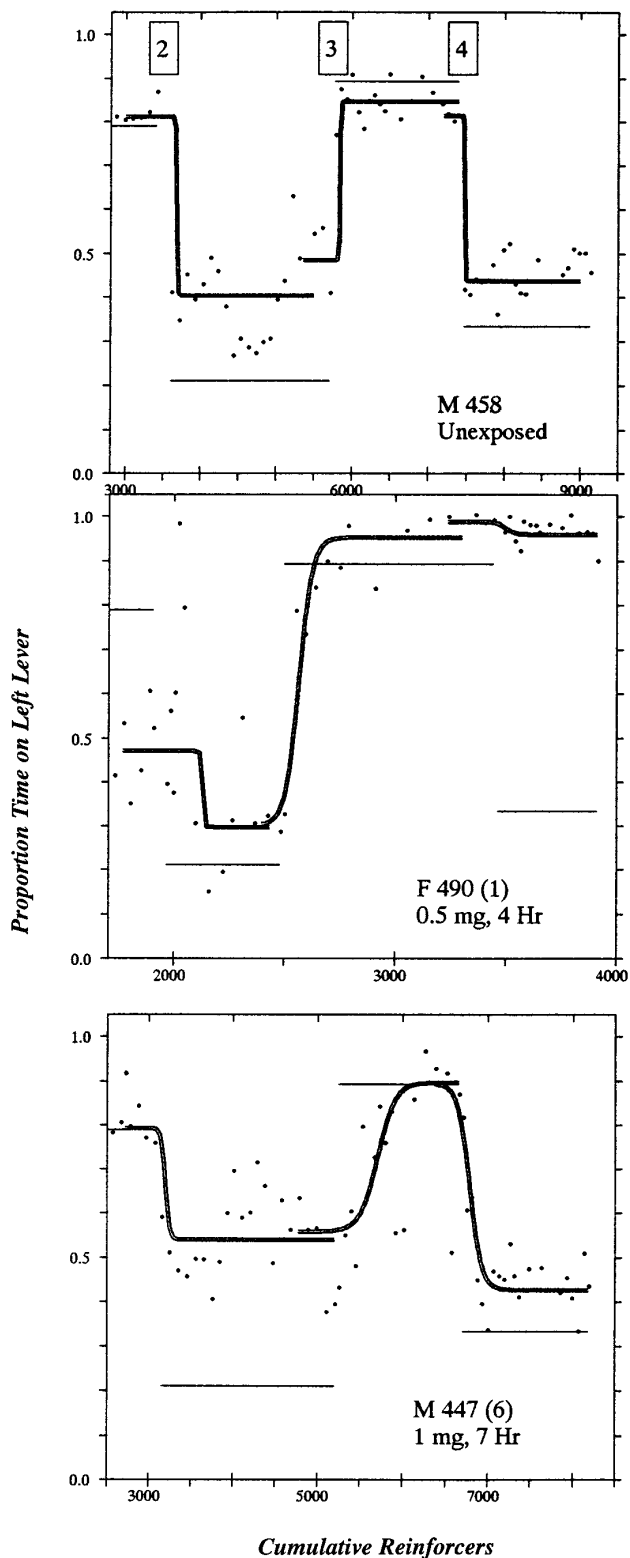


FIG. 2. Representative transitions for an unexposed (top) and two exposed (bottom two) monkeys. Transitions 2, 3, and 4 begin under the numbered boxes at the top. The transitions are plotted against cumulative reinforcers obtained since the beginning of the experiment. Each point represents an individual session. The ordinate is the proportion of time

spent on the newly rich lever across subsequent sessions. The time proportions were expressed as a function of cumulative reinforcers obtained for responding on that lever. Representative transitions are illustrated in Fig. 2. The behavioral transition from one concurrent schedule to another was analyzed by fitting a logistic equation:

$$Y = \frac{P}{1 + e^{(R_{\text{half}} - X)/t}} \quad (2)$$

In this equation, P represents the magnitude of the transition and is the difference between the baseline before the transition began and that at the end of the transition. The value R_{half} is the half-maximal reinforcers and represents the number of reinforcers required to complete $\frac{1}{2}$ of the transition. The value t describes the interval (in units of reinforcers) required to complete the transition once it begins and is numerically the number of reinforcers between $1/e$ and $2/e$ of the magnitude (approximately between $\frac{1}{3}$ and $\frac{2}{3}$ of the way through the transition). This is the same equation, but a slightly different form, as Eq. (2) used by Newland *et al.* (1994). The value t in Eq. (2) equals $1/k$ in that paper and the value P in Eq. (2) equals the value $P_{\infty} - P_0$ in that paper. Equation (2) was fit to transitions 2, 3, and 4 for each subject except M477. The first transition was excluded because Eq. (2) did not describe it well for some monkeys. The transitions, such as they were, for M477 were too erratic to fit.

Before fitting Eq. (2) to the data, the baseline time proportion was estimated from the last five sessions before the transition and that value was subtracted from all time proportions to normalize the dependent measure to the pretransition baseline. For each transition, the number of cumulative reinforcers at the beginning of the transition session was set to zero so that the number of reinforcers began accumulating when the reinforcer ratios were changed. These translations of the vertical and horizontal axes were accomplished to set the origin to (0,0), which simplifies the nonlinear least-squares regression used to fit Eq. (2). The regression was also performed with proportion responses on the newly rich lever as the dependent measure, but the fit of the equation was poorer for some subjects, due partly to a left-lever bias that emerged among some exposed monkeys during transition sessions. Only the results from regressions using proportion time are included here.

Figure 2 shows transitions 2, 3, and 4 for a control subject and two exposed monkeys. The solid lines show the results

spent on the left lever. The unconnected horizontal lines show the proportion of reinforcers programmed to derive from the left lever. The double line is the result of fitting a logistic equation (Eq. (2)) to the points, after normalizing as described in the text. Note that transitions 2 and 4 were relatively small for F490 and transitions 3 and 4 were slow for M447.

of the nonlinear least-squares estimate of the parameters in Eq. (2), after the data were returned to their original form (that is, after the two translations described above were reversed). Some transitions occurred within a single session (especially for the control subjects, including the one illustrated) and this resulted in estimates of t with high standard deviations in the estimates. When this happened the plausibility of the numbers was confirmed with visual estimates by estimating the number of reinforcers comprising the middle third of the transition.

Transitions for the control subjects closely resembled those of M458 illustrated in the top panel of Fig. 2. They were generally smooth, quick, and of a reasonable magnitude. Transitions for some of the exposed subjects differed in ways illustrated in the bottom two panels. The middle panel shows that transitions 2 and 4 for subject F490 were smaller in magnitude than the control transitions. In the case of transition 2 the previous baseline was well below the programmed reinforcer rate and in the case of transition 4 the subject's behavior showed little change after the transition began. The bottom panel shows that the transitions for M447 tended to be slow. This can be visualized as greater curvature in the best-fit line as compared with the control subject and described quantitatively as a larger value for the half-maximal reinforcers (R_{half}) and transition interval, t .

Figure 3 summarizes half-maximal reinforcers and transition magnitudes for transitions 2, 3, and 4 for all subjects except M477. The magnitude of the transitions for control subjects ranged from about 0.3 to about 0.6 and the half-maximal reinforcers clustered closely between about 40 and 80, with a single exception. Among the exposed monkeys, six transitions differed from the control pattern. Four transitions were especially small (less than 0.25) and two were especially slow, requiring more than 400 reinforcers to complete $\frac{1}{2}$ of it. In addition, the three transitions attempted with M477 were too erratic to fit. A similar analysis was performed on the transition interval. The values of t (not shown) ranged from about 4 to 125 reinforcers, with a median of 20, to complete the middle third of the S-shaped logistic curve. The value of t was correlated with R_{half} so it was not included in the following statistical assessment.

To determine the likelihood that such a pattern could occur by chance, the transitions were arranged into a 2 (control or Hg) \times 2 (normal or abnormal transition) table and evaluated with the Fisher's exact p value. One control transition was scored as abnormal and 14 were scored as normal. Eight transitions from the exposed monkeys were scored as abnormal (5 shown in Fig. 3 and 3 from M477) and 10 were scored as normal. This pattern was statistically different from chance (two-sided Fisher's exact test, $p = 0.021$). If M477, which is not represented in Fig. 3, is excluded, then the number of abnormal transitions in the exposed group decreases to 6 and the p value increases to 0.17.

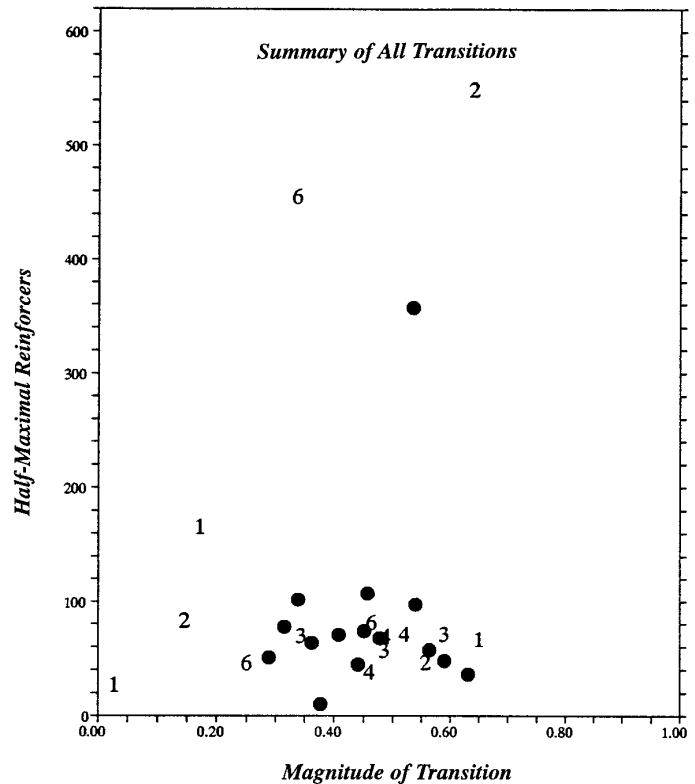


FIG. 3. Half-maximal reinforcers (ordinate) and magnitude of the transition (abscissa) for transitions from all monkeys except M477. One transition from control monkeys and five transitions from exposed monkeys were scored as aberrant. Of the five, two required a large number of reinforcers and three were small.

Lever-press durations. Lever-press durations were summarized by taking a mean and standard deviation over the last 10 sessions of conditions 1 and 5. The results are represented in Fig. 4. The median lever-press duration was about 0.3 to 0.4 sec for the two groups early in the experiment. At the end of the last condition, the median duration was 0.2 sec for controls but this value increased to about 1.0 sec for mercury-vapor-exposed monkeys. To determine whether there was a significant increase in lever-press duration, a difference score was computed for each monkey (late duration minus early duration) with a match-pair t test. The median increase for the exposed group was 0.51 sec while the controls showed a 0.02-sec decrease (not different from zero). The change in duration between the two groups was different ($p = 0.018$).

Figure 4 also shows that session-to-session variability in lever-press duration, as measured by the standard deviation in duration over 10 sessions, increased considerably for the exposed monkeys. The standard deviation increased by 0.19 sec in exposed monkeys but decreased by 0.09 sec in controls ($p = 0.03$ for a t test on the differences, after the data are made positive with a linear transformation and then trans-

formed logarithmically to stabilize the variability). It is clear from the bottom right panel of Fig. 4 that there was considerable scatter in variability for the exposed monkeys. With the exception of monkey F490 (coded 1), the only female, the variability in lever-press duration was related to cumulative exposure.

Visual function in 477. When food pellets were placed in this monkey's visual field he shifted his gaze toward them and reached for them with his fingers, as did other monkeys when evaluated this way. The visual examination revealed selective rod and cone response in all three monkeys, and no difference could be found between monkey 477 and the two control monkeys on these measures.

DISCUSSION

Young squirrel monkeys exposed *in utero* to mercury vapor showed alterations in three aspects of behavior maintained by a concurrent schedule of reinforcement: steady-state performance, transitions to new schedule parameters, and lever-press durations. In most of the exposed monkeys, the alterations were quantitatively, but not qualitatively, distinct from unexposed monkeys in that they acquired stable lever pressing and showed some degree of schedule-typical responding under the concurrent schedules of reinforcement. The performance of one monkey (M477) was qualitatively different from all others and will be discussed separately.

Steady-state performance. When behavior is maintained under a concurrent schedule of reinforcement, the allocation of time or responses to one alternative is directly related to the number of reinforcers available from that alternative as compared with other response alternatives. In the present case, if the richer schedule of reinforcement is programmed on the left lever then most behavior should occur on that lever. A large body of literature confirms the generality of this relationship across a variety of species, reinforcers, and responses both qualitatively and quantitatively by fits of Eq. (1) (Davison and McCarthy, 1988; de Villiers, 1977). This power function (taking the antilog of each side produces a power function) describes a measure of behavior, usually time or responses spent on one lever, as a ratio of that same measure on the other lever. Equation (1) normalizes the measure of behavior, and thereby reduces the influence of response or changeover rates, by expressing behavior as a ratio of time allocation on one lever to the other. In the present experiments, the quality of experimental control exerted over the behavior of the unexposed monkeys is evidenced by the close degree to which the performance of unexposed monkeys replicated performance under concurrent schedules seen in other contexts, and especially as measured by the ability of Eq. (1) to describe their steady-state behavior.

A positive relationship occurred between time allocation

and reinforcer ratios in exposed monkeys, as expressed by positive values of the slope, a , in Eq. (1). The scatter of points about the regression line was greater, however, for some of the exposed monkeys than for the controls, indicating that the degree to which reinforcement rate influenced behavioral allocation was poorer for these exposed monkeys, and this poor control was captured by the high value for the standard deviation of the regression. Thus, while there was a general trend for behavior to reflect reinforcer ratios over the 32-fold range of reinforcer ratios used here, many exceptions to strict monotonicity occurred with exposed monkeys. By contrast, a monotonic relationship appeared in unexposed monkeys: as the reinforcer ratio increased, so did the time ratio, and this monotonicity was maintained across nearly all data points. The summary figure describing the standard deviation of regression indicates that the weak relationship between time allocation and reinforcer ratios was characteristic of some exposed individuals.

The standard deviation of the regression has been used here as a measure of the quality of the fit of the regression equation. The variance accounted for by the regression, r^2 , is sometimes used as a measure of the quality of fit, but this measure was unsatisfactory in the present experiment. Visual inspection of the distribution of r^2 values revealed two possible reasons that this measure was unsatisfactory. First, the value of r^2 (and slope) is strongly influenced by data at the extremes if they are outliers (Parker *et al.*, 1988). The wide variation in reinforcer ratios used here resulted in shifts in time allocation at the extreme ratios in even the most insensitive monkey and the points at the extremes elevated r^2 . The second reason is that the values of r^2 for some of the exposed monkeys were extremely divergent from those of the other monkeys and no transform was found that could stabilize variability in r^2 sufficient for statistical analyses. The divergent points produced a large standard error for the exposed group, reducing statistical power. The statistical tests notwithstanding, it is evident in Fig. 1 that the variability in the regression was considerably greater for some of the subjects.

Transitions and learning. With the exception of a single transition for one monkey, the transitions were completed by the control monkeys rapidly and the magnitude of the transitions was of a reasonable size. The latter characteristic confirms the results seen in steady state, but with a different analytic route, since the magnitude of the transition represents the difference between the baseline (the previous steady state) and the end of the transition as estimated by nonlinear least squares while the steady-state analysis described the average performance of the last several sessions at each schedule value.

The transitions seen in unexposed monkeys corresponded qualitatively and quantitatively to those seen in somewhat older, unexposed monkeys in an earlier experiment (Newland *et al.*, 1994). The control monkeys in the present report

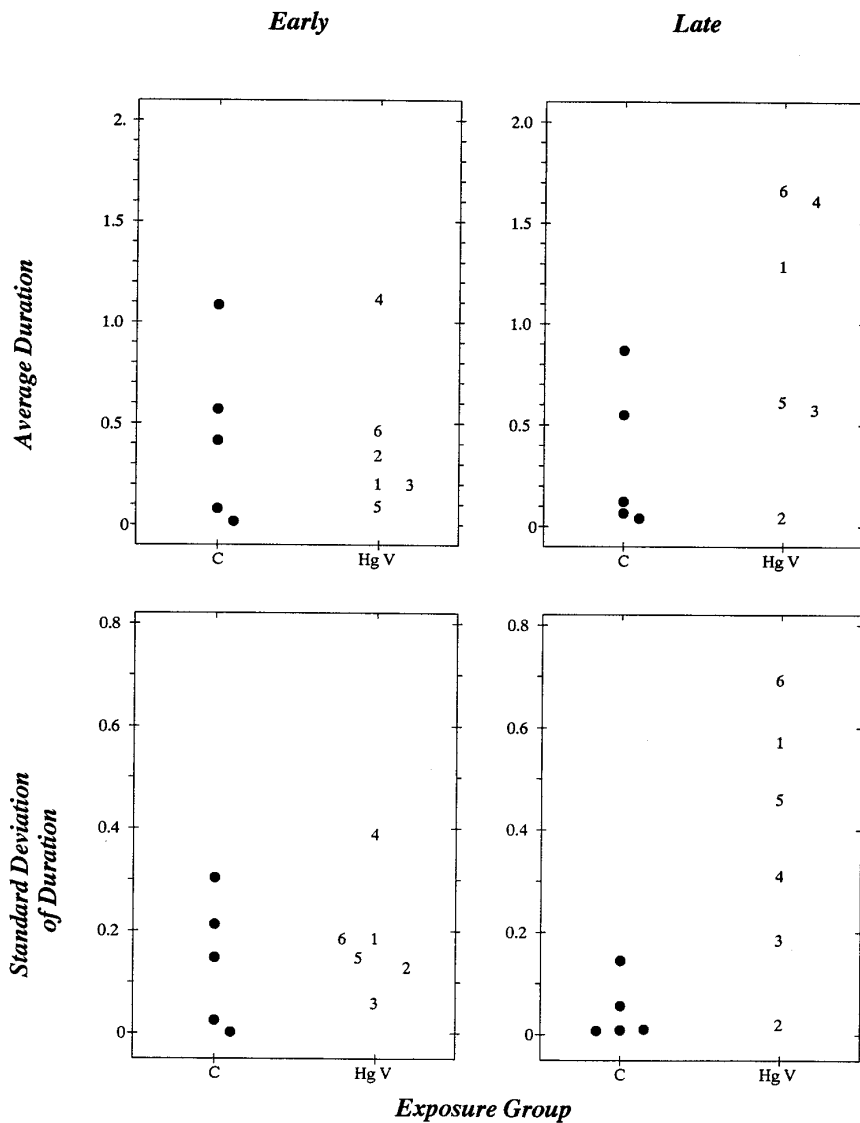


FIG. 4. Lever-press durations averaged over the last 10 sessions under a condition (top row) and their standard deviation (bottom row) taken from the end of condition 1 (left) and condition 5 (right). The length and stability of lever-press durations did not change for control but both measures increased for exposed monkeys.

completed transitions within a few sessions, and most transitions required 40 to 100 reinforcers to complete $\frac{1}{2}$ of it, as determined by the half-maximal reinforcers. Only one transition was described in the earlier report, but for that one the unexposed monkeys also completed the transitions within a few sessions and had half-maximal values between 40 and 90 reinforcers.

Sometimes the aberrant transitions were manifested as especially small transitions even in monkeys, such as F490, for which previous transitions had been of a reasonable magnitude. The presence of small and slow transitions represents a decreased sensitivity to changes in the reinforcement contingencies. This observation extends to those made in the

analysis of steady-state performance, which also indicated a deficit in the sensitivity of the exposed monkeys' behavior to the contingencies of reinforcement.

Whether altered steady-state performance, small-magnitude transitions, and slow transitions represent different disorders or different manifestations of the same disorder cannot be determined currently. The number of animals is too small and the application of concurrent schedule behavior in transition is too novel to support conclusions. It can be pointed out, however, that the pattern of disruption seen in transitions after prenatal lead showed a more consistent pattern and an overall dose-effect relationship than seen here (Newland *et al.*, 1994). After high-level exposure, the transi-

tions were too small to analyze and after low-level exposure transitions were of the same magnitude as controls, but they required more reinforcers to complete. Three monkeys described in Newland *et al.* (1994) were exposed to methylmercury, and the transitions for all of them were too small to analyze. In that case, a narrow range of mercury levels was used so it is not known whether lower levels of exposure would have produced more subtle effects. In the present experiment, both low-magnitude and slow transitions appeared but only a weak association with exposure could be discerned. The cumulative dose experienced by monkey M477 was the second highest and, perhaps more important, exposure began earlier for this monkey than for any other monkey and this monkey's behavior was the most aberrant.

Motor effects. The analysis of lever-press durations early in the experiment revealed no differences between exposed and unexposed monkeys, but late in the experiment there was a considerable difference. The average duration of all but one exposed monkey (M497, coded as 2) was longer at the end of the experiment than at the beginning, and for three monkeys they were as long as 1.5 sec. The average duration remained unchanged for the unexposed monkeys.

The longer lever-press durations in exposed monkeys were associated with instability in lever-press durations. The standard deviation of lever-press durations showed a striking increase in the exposed monkeys but, as expected, this measure only decreased in unexposed monkeys. The variability in lever-press duration of the exposed monkeys, which was indistinguishable from unexposed monkeys early in the experiment, increased as much as three- to nearly fourfold late in the experiment. The increased variability tracked calculated exposure very closely, with the only exception to this being the female monkey in which the effect was stronger than in the male exposed to similar levels. While conclusions about sex differences cannot be supported on the basis of these data, it is noteworthy that enhanced susceptibility of females to methylmercury's neurotoxicity, perhaps due to slower elimination, has been noted (Doi, 1991; Magos, 1987, but see Sager *et al.*, 1984).

Relationship to mercury in the central nervous system. Cohorts of these monkeys received similar exposure to mercury vapor and their brains were examined for the presence of mercury (Warfvinge *et al.*, 1994). The magnitude of mercury accumulation was less than that seen in the maternal brain, confirming other reports with guinea pigs (Yoshida *et al.*, 1986) and mice (Danielsson *et al.*, 1990). The concentration in many regions of fetal lambs approximated, but did not exceed, that seen in ewes (Vimy *et al.*, 1990). All these observations contrast with methylmercury, where the fetal brain shows greater mercury accumulation (Eccles and Anau, 1987).

The distribution of mercury in the CNS after mercury

vapor exposure showed regional differences, in contrast to the homogeneous distribution seen after prenatal methylmercury exposure. Some mercury was detected in nearly all regions examined (Warfvinge *et al.*, 1994) but in the neonate the concentration was especially great in the subiculum hippocampus and layers III and V of the pyriform cortex (Warfvinge *et al.*, 1994) and cerebellum of the rat (Eide and Weisenberg, 1993). It has also been noted in the molecular and Purkinje cell layers of the cerebellum, in deep cerebellar nuclei, and in cerebellar astrocytes (Warfvinge, unpublished data) after mercury vapor exposure.

The regions that accumulated mercury in the fetal brain are especially interesting in view of the behavioral effects noted. The hippocampus and pyriform cortex are associated with learning and memory (Kandel *et al.*, 1991) and plausibly could be associated with the learning deficits reported under the concurrent schedule. The cerebellum is a center for motor learning, and especially the repeated execution of learned motor acts, suggesting that the deficits in motor control evidenced by the highly variable lever-press durations could reflect cerebellar damage.

Experience from methylmercury exposure has shown that the squirrel monkey is the closest model of all studied nonhuman primates in terms of mercury kinetics. There is a good correlation between brain levels of methylmercury and toxic changes in the brain in most mammalian species. There is a wide variation, however, in the relationship between blood and brain levels of methylmercury among species and also among primates. This variation may be due to different binding to hemoglobin (Doi and Tagawa, 1983) and variation in hemoglobin structure between species and strains.

In the case of mercury vapor, elemental mercury is partially dissolved in blood (Magos, 1968) and much is bound to hemoglobin (Hursh, 1985) from which it can be released as vapor. Whether physical adsorption or a redox reaction is involved in hemoglobin binding is unclear. During ongoing exposure to mercury vapor, 90% of the mercury in blood is found in the erythrocytes. There is limited information in the literature on the relation between exposure levels and mercury concentration in blood for different species. Data from humans are rather similar to what we report here for the squirrel monkey (Berlin, 1986). Data relating brain level of mercury to toxic damage after exposure to mercury vapor are even more scarce. Toxic changes have not been seen at brain levels of mercury around 8 $\mu\text{g/g}$ in the squirrel monkey (Berlin, 1986). Cerebellar changes has been described in a clinical case with mercury concentration of around 5 $\mu\text{g/g}$. The lowest concentration of mercury resulting in cerebellar pathology has been reported for the Brown Norway rat, 0.71 $\mu\text{g/g}$, a rat which also developed autoimmune renal disease (Hua *et al.*, 1995).

Relationship to human exposures. The present experiment demonstrates that mercury vapor exposure during the

last $\frac{2}{3}$ or more of gestation has long-term effects on the offspring and it shows functional domains in which effects appear: motor function and learning. The erratic behavior of M477 suggests that exposure earlier in gestation might present a greater risk. The levels of exposure used in the present study, 0.5 or 1.0 mg/m³, have been reported in some industrial settings, such as chloralkali industries (Kishi *et al.*, 1993) or spills (Gelbier and Ingram, 1989; Warfvinge, 1995), especially if ventilation is poor, but are at least 10- to 50-fold greater than levels more commonly reported in chloralkali plants or other occupational settings (Roels *et al.*, 1990; Liang *et al.*, 1993; Skerfving, 1991). Dental amalgam might release as much as 0.3 to 0.4 $\mu\text{g}/\text{day}$ when fresh, but this decreases to less than 0.05 $\mu\text{g}/\text{day}$ after 30 days (Berdouses *et al.*, 1994), compared with estimated exposures of 21 to 62 $\mu\text{g}/\text{day}$ in the present studies. Exposures similar to those in the present study produced concentrations in the maternal brain of 0.8 to 2.58 $\mu\text{g}/\text{g}$ (Warfvinge *et al.*, 1994) while amalgam in the pregnant ewe produced concentrations of 2 to 20 ng/g (Hahn *et al.*, 1989).

The present study shows clearly the presence of effects but does not provide sufficient information about the shape that a dose-response relationship might assume, and it did not identify a no-effect level. A single safety factor would place the present exposure level into the realm of occupational exposures, but it is not clear how extrapolation to levels that might be experienced occupationally should be conducted, and whether safety factors should be applied.

Not all monkeys exposed to mercury vapor were affected in the same way. Individual differences in the susceptibility to neurotoxicants have been noted with neurotoxicants including methylmercury (Cox *et al.*, 1989; Rice, 1989; Rice and Gilbert, 1995), mercury medicinals (Warkany, 1966), lead (Cory-Slechta *et al.*, 1985; Rice and Gilbert, 1985), cadmium (Newland *et al.*, 1986), and toluene (Wood and Cox, 1995). When the behavior of individual subjects can be described quantitatively and meaningfully it becomes apparent that as the dose increases not only does the severity of effects, but also the number of subjects affected increases. Such individual susceptibility might have been undetectable had the results been presented only as group means.

The utility of the behavioral endpoints used. The analysis of behavior during transitions under concurrent schedules shows potential for being an effective way of quantifying learning, which can be defined as behavior change resulting from a change in the contingencies of reinforcement. Learning deficits rank among the most troubling of developmental neurotoxicities, but are also the among the most difficult to measure. Many applications of schedule-controlled operant behavior represent the end of learning, since in most applications steady-state performance is the target of analysis. This is also the case in human assessments of learning, which often comprise tests of achievement that are normalized

against a standard group. Such tests still tap the end of learning since they require effective use of a language, some knowledge of culture, and time in school. Both IQ and schedule-controlled behavior can be called measures of learning because the performance must be acquired and would never appear in a naive subject, but neither permits the assessment of the process of learning.

The course of learning has been difficult to study because, by its very definition, learning is a labile and temporary process that bridges one steady state to another. The orderly transitions reported with concurrent schedules stand in contrast to the apparent disorder that characterizes much of behavior change. The strength of concurrent schedules may lie in one fundamental difference between concurrent schedules and the application of single schedules more typically used in behavioral toxicology. The concurrent schedule can be conceptualized as a way of controlling not only the schedule in place, but also the context. In a single Random-Interval (RI) 60" schedule, for example, the context is uncontrolled and comprises everything else that the animal can do in the chamber, such as scratching, moving about, or exploring. With a *Concurrent* RI 60" RI *t*" schedule the context can be examined by changing the value of *t*. While it does not eliminate the alternate activities, it does provide a controllable alternative and, accordingly, additional experimental control.

Lever-press durations provide a relatively simple way of assessing the physical characteristics of the execution of a response. While they do not afford the detailed information available with more advanced techniques, they offer information that is separate and nonredundant from that available in measures of response rate (Newland, 1995; Fowler, 1987). For example, neuroleptics and benzodiazepines can be distinguished according to their different effects on lever-press durations, even though they had similar effects on response rate (Walker *et al.*, 1981). In squirrel monkeys exposed prenatally to lead (Newland *et al.*, 1996), or adult cebus monkeys exposed to manganese (Newland, 1994), the duration, but not the rate, of an effortful response was sensitive to body weight and toxicant exposure.

A characteristic of mercury vapor's effects is the elevated variability in the behavior of exposed monkeys, especially in durations of lever press. One source of variability might be found in the fine structure of behavior (Rice, 1988). For example, Zirix *et al.* (1993), Gott and Weiss (1972), Cory-Slechta *et al.* (1985), and Laties and Evans (1980) have reported disruptive changes in the variability of behavior associated with drug or toxicant exposure. The particular form that the disruption took differed across the studies, but all seemed to represent diminished control over behavior by its immediate consequences. Since lever-press durations, like other features of behavior, can be influenced by their consequences (Newland, 1995) a plausible hypothesis deriving

from the present results is that motor control over the execution of the lever press has been impaired by prenatal mercury vapor exposure.

ACKNOWLEDGMENTS

This work was supported by the Swedish Work Environment Fund, Medical Faculty of Lund University, the Swedish Medical Research Council (No. 24B-09243), and NIH Grant ES06466. Special thanks to Dr. Sten Andreasson for conducting the full-field electroretinograms.

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