

## Gestational exposure to methylmercury retards choice in transition in aging rats

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Received 4 August 2003; received in revised form 9 December 2003; accepted 10 December 2003

### Abstract

Developmental exposure to methylmercury has behavioral effects that extend into adulthood and aging. In this study, methylmercury's prolonged effects on the acquisition of choice and sensitivity to changes in reinforcement rates were studied. Pregnant female rats were exposed to drinking water containing 0, 0.5, or 6.4 ppm Hg as methylmercury, resulting in about 40 and 500 µg/kg/day of mercury intake. Maternal exposure began at least 4 weeks before mating, and continued to postnatal day 16. Then all mercury exposure ended. The behavior of 1.7- and 2.3-year-old offspring was maintained under various concurrent schedules of reinforcement. Thus, one reinforcement schedule maintained left-lever responding and a separate one maintained right-lever responding. The animal could switch ("changeover") between the two levers at any time. For the first 30 min of a 3-h session, the left and right levers each produced reinforcement at the same rate and left:right response ratios were about 1:1. After 30 min, either the left lever became richer than the right; the right lever became richer than the left, or there was no change. Terminal reinforcer ratios (left:right) used were 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, and 1:9. Response rates on the two levers were tracked continuously through a session. This novel procedure for examining choice, and its acquisition, in a single session, was validated through many comparisons with the extant literature. Both response rates and changeover rates were influenced by the reinforcer ratios for the 1.7-year-olds. Changeover rates were not influenced by reinforcement rate for their 2.3-year-old littermates. For the 1.7-year-olds, there was no effect of methylmercury on changeover or response rates and there was no interaction between exposure and reinforcer ratio. In controls and most methylmercury-exposed rats, response ratios (the measure of choice) approximately matched reinforcer ratios by the end of the single session. This is commonly interpreted as reflecting sensitivity to reinforcement rates. Methylmercury exposure did not affect this measure systematically. The single-session transition from baseline (response ratios about 1:1) to terminal performance was retarded in many methylmercury-exposed rats relative to controls, especially in the older rats. The 2.3-year-old control rats required about 20 to 25 reinforcers to complete one half of the 9:1 and 4:1 transitions, respectively, and exposed rats required about twice as many. Thus, prenatal methylmercury exposure specifically retarded the acquisition of choice in older rats. Methylmercury did not interfere with the final expression of choice. Moreover, two rate measures, lever-press rates and changeover rates, were not systematically affected by methylmercury. The acquisition of choice appears to be very sensitive to subtle consequences of developmental methylmercury exposure. The specific tactics greatly reduced the time required to study behavior in transition from a month in previous reports to a single session here.

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**Keywords:** Methylmercury; Prenatal exposure; Operant behavior

### 1. Introduction

Evidence is accruing from human populations [39] and animal studies [49,61,63] that methylmercury's developmental neurotoxicity may become apparent when some challenge such as aging is imposed on the nervous system. Adult

victims of the Minamata disaster began to show declines in simple activities of daily living such as getting dressed or washing only as they aged. This morbidity was not associated with changes in mortality in the human populations [69] or in animal studies [47].

Age- and methylmercury-related deficits in auditory, somatosensory, and visual function have been described in nonhuman primates exposed developmentally to methylmercury [61,63]. Declines in the ability to sustain high-rate responding have also been noted in aging rodents exposed gestationally to methylmercury [48]. Evidence for the

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unmasking of methylmercury effects also derives from drug challenges, which revealed dose-related increased sensitivity to *d*-amphetamine and decreased sensitivity to pentobarbital in rats exposed prenatally to methylmercury [57].

While it is clear that methylmercury exposure has sensory and motor consequences, the issue of whether chronic, low-dose exposure to methylmercury produces cognitive effects remains murky. Studies of children exposed developmentally to methylmercury report effects on cognitive function in the Faro Islands [33,34] and in New Zealand [12,13], but not in the Seychelles Islands [14–16]. Animal studies using low exposure levels have been noteworthy in failing to identify effects of methylmercury on procedures used to characterize the neurotoxicity of compounds associated with deficits in memory or information processing speed [60], although effects suggestive of developmental delays in memory for faces, geometric stimuli, and object permanence have been identified in young monkeys [35–37].

Prenatal methylmercury exposure was reported to alter the acquisition of choice in three young squirrel monkeys [50]. In that study, a monkey faced a panel containing two levers. Pressing the left lever produced a reinforcer at one rate and pressing the right lever produced a reinforcer at a different rate. The monkey was free to switch levers at any time, and it did, but most behavior occurred on the lever that produced the most reinforcement. Relative response rates approximately matched the relative reinforcement rates, although methylmercury-exposed monkeys showed some position biases [50]. The reinforcement rates associated with the two levers were changed every few weeks and the resulting change in behavior was tracked across sessions. The effects of methylmercury (and lead) became clearest during these transition states. Exposed monkeys required more reinforcers to accomplish a transition than did controls. The effect was large and seen in all three methylmercury-exposed monkeys, but this is still a small sample and only a narrow range of doses was examined.

The present study was designed to replicate that procedure using a different species (rats), a larger sample size, and a broader range of exposure levels. The rats were exposed throughout gestation and allowed to age to up to 2 1/2 years old. They were littermates of those described in earlier reports [48,57]. Maternal exposure, which began weeks before mating, was designed to produce in the offspring mercury levels described as low and moderate [8]. The lower exposure level, 0.5 ppm in drinking water, resulted in approximately 40 µg/kg/day of intake. This approximated the highest level used in a study of methylmercury's effects on high-rate behavior [5]. The higher dose, 6.4 ppm in drinking water, approximated the lower level used in studies of the effects of gestational exposure on analog measures of response force and fine motor control [24,25].

The approach to investigating choice in transition was a modification of the procedure used in the earlier experiment [50] as well as in examinations of PCB 126 [65] and a PCB

mixture [64]. The modification permits the study of the acquisition of choice transitions in a single, 3-h session rather than in 3 weeks of 30-min sessions, making it possible to study a transition in a day rather than a month.

## 2. Methods

### 2.1. Subjects and exposure

Offspring of Long–Evans rats exposed throughout gestation to 0, 0.5, or 6.4 ppm of mercury as methylmercuric chloride in their drinking water and maintained on a chow diet were used in the experiments described here. The reported concentrations are those resulting from analyses of the rats' drinking water [47]. These concentrations produced daily exposure to approximately 0, 40–50, and 500–700 µg/kg/day of methylmercury, respectively. The higher number in each group reflects elevated exposure during gestation due to increased maternal water consumption. No overt maternal toxicity was noted, but there was some evidence, not statistically significant, of small litter sizes in the high-exposure group. No mercury-related effects were seen on growth or on the appearance of developmental landmarks. See Ref. [47] for details about exposure, biomarkers, and reproductive success.

Maternal exposure began 28 or 49 days before mating and continued to postnatal day 16 when pups could reach the drinking spout. The average concentrations of mercury in neonatal brains, as detected using atomic absorption spectrophotometry, were about 0.5 and 9.7 µg/kg (average of males and females) for the 0.5- and 6-ppm groups, respectively [47]. No mercury was detected in the rats exposed to 0 ppm of mercury. There was no difference between the 28- and 49-day groups so these groups are combined. By weaning, mercury concentration in the brain dropped approximately 10-fold, leading to the conclusion that there was no significant exposure via lactation. At weaning, offspring were selected and identified as subjects for Experiment 1 (adulthood), Experiment 2 (aging), other experiments, or as spares. After weaning, rats were maintained on a chow diet for life. Rats were free fed until they reached their targeted adult body weight. Adult males and females in Experiment 1 were maintained at 240 and 300 g, respectively. Older adult males and females were maintained at 250 and 320 g, respectively.

The rats used in Experiment 1 were about 1.7 years old ( $\pm 3$  weeks) at the beginning of behavioral training. The number of rats in the 0, 0.5, and 6.5 ppm exposure groups were 10 (5 male), 10 (5 male), and 11 (6 male) rats representing, respectively, 5, 5, and 6 litters. That is, there was a male and female littermate from each litter where possible. The rats in Experiment 2 were about 2.3 years old at the beginning of training. There were 8 (4 male), 7 (2 male), and 9 (5 male) rats representing, respectively, 4, 5, and 5 litters. The male and female rats were littermates where

possible and the rats in Experiment 2 were littermates of those in Experiment 1 where possible.

All rats were housed in individual cages in a room with a 12/12 h light–dark cycle (lights on at 7:00 a.m.). The experiments were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee. The housing facility and procedure rooms meet U.S. Public Health Service standards for housing laboratory animals.

## 2.2. Behavioral procedures

Except where noted in the following, Experiment 2 was a replicate of Experiment 1.

### 2.2.1. Apparatus

Behavioral sessions were conducted in conventional experimental operant chambers. Each chamber was enclosed in a sound-attenuating cubicle and equipped with two response paddles ( $2.5 \times 25. \times 0.16$  cm) situated 5 cm from the bottom of a grid floor and 14.5 cm apart. Reinforcers were delivered through a  $3.8 \times 3.8$ -cm opening centered between the two levers. White noise was generated from a speaker 7.2 cm above the food dispenser. Reinforcement contingencies and data collection were accomplished with 0.01-s resolution using a DEC PDP 11/73 running SKED 11 software (State Systems) located in an adjacent room. Sessions were conducted in the morning and early afternoon 5 days per week.

### 2.2.2. Training

Lever pressing was autoshaped by placing the rats in a chamber overnight in which food was delivered under a concurrent fixed-ratio 1 fixed-time schedule 60 s of food reinforcement. Every 60 s a food pellet was delivered, and this delivery was preceded by the illumination of a stimulus light over the lever for 5 s. Simultaneously, any lever press resulted in food reinforcement. After 10 presses of the left lever the free food was terminated and a fixed-ratio 1 schedule (every response produced a reinforcer) remained in place until 100 lever presses occurred on the left lever. Then, pressing the left lever no longer produced a reinforcer and a fixed-ratio 1 schedule was established on the other lever until 100 reinforced presses occurred on that lever, usually within one or two 30-min sessions. The reinforcer used in all experiments was a 45-mg food pellet.

Lever pressing was then reinforced under a concurrent random interval 60 s random interval 60 s (Conc RI 60 s RI 60 s) schedule of reinforcement. Under this schedule arrangement, a press on either lever produced a food pellet about once every 60 s, on average, but the exact interreinforcer interval was unpredictable. For example, as the animal responded on the left lever, the timer for the right lever continued to operate, potentially setting up a reinforcer to follow the next eligible right-lever press. Thus, under a Conc RI 60 s RI 60 s schedule an animal could receive two

reinforcers per minute from the two levers combined. This schedule was continued for approximately 30 one-hour sessions. Then the schedule changed to a Conc RI 180 s RI 180 s, and when behavior under this schedule was stable the session length was increased to 3 h.

Following common practice, a changeover delay of 2 s was imposed; no reinforcer was delivered following a changeover from one lever to the other until 2 s elapsed. If a reinforcer had set up on the right lever while the animal was responding on the left, then it would be delivered for the first right lever press after 2 s. A changeover delay reduces rapid switching between levers and enhances the sensitivity of behavior to differences in the reinforcement rate between the left and right levers.

### 2.2.3. Single-session transitions: 1.7-year-old rats

Three-hour sessions were used to generate single-session transitions. On initial exposure to the 3-h sessions a Conc RI 180 s RI 180 s schedule of food reinforcement was used throughout the entire session. Each lever produced about 0.33 reinforcers/min, and the rat would receive an average of 0.67 reinforcers/min from the two levers combined or 118 in a 3-h session. Cumulative records of responding through the 3-h session were collected and examined routinely to determine if response rates or switching rates declined toward the end of the sessions. Sessions were conducted 5 days/week. Behavior stabilized quickly under the Conc RI 180 s RI 180 s but the rats' behavior was continued under this schedule for about 21 sessions.

Then the transitions began. A typical 3-h session was divided into a 30-min baseline phase and a 150-min transition phase. During the baseline phase for the 1.7-year-old rats (Experiment 1) a Conc RI 180 s RI 180 s schedule of food reinforcement was presented. Thirty minutes into the session one of three things happened with equal probability: the left lever was assigned the higher density of reinforcement; the right lever was assigned the higher density of reinforcement; or the Conc RI 180 s RI 180 s schedule continued without interruption. Thus, for one third of the daily sessions (presented pseudorandomly) there was no transition. For the other sessions the schedule changed to one of three schedules, described in Table 1. No discriminative stimulus was presented at the beginning of the transition phase.

The different reinforcement densities used are presented in Table 1. The table also contains the number of sessions used in the analyses described here. The different pairs of reinforcement densities were imposed randomly with the constraint that no condition was repeated on 3 successive days. Lever pressing could always be reinforced on either lever, although for some implementations of the concurrent schedules one lever was nine times leaner than the other. Also note that the overall reinforcement rate, i.e., the sum of the two reinforcement rates, is the same under all schedules. Behavior under the 3:1 ratio differed little from that under the 4:1 ratio so the 3:1 sessions were not analyzed in detail.

Table 1  
Reinforcement parameters for the schedules used during transition portions

Schedule designation	Left:right reinforcer ratio	Reinforcement rate (reinforcers/min)			Number of sessions	
		Left	Right	Total	1.7 YO	2.3 YO
Conc RI 180 s	1	0.33	0.33	0.67 <sup>a</sup>	8	6
RI 180 s						
Conc RI 135 s	3:1	0.50	0.17	0.67	1	2
RI 270 s						
Conc RI 270 s	1:3	0.17	0.50	0.67	1	2
RI 135 s						
Conc RI 111 s	4:1	0.54	0.13	0.67	4	1
RI 461 s						
Conc RI 461 s	1:4	0.54	0.13	0.67	4	2
RI 111 s						
Conc RI 100 s	1:9	0.13	0.54	0.67	2	1
RI 860 s						
Conc RI 860 s	9:1	0.60	0.07	0.67	2	1
RI 100 s						

<sup>a</sup> Not equal to 0.33 + 0.33 because of rounding.

For each reinforcer ratio there was at least one presentation with the left side being rich, and one with the right side being rich.

#### 2.2.4. Single-session transitions: 2.3-year-old rats

Initial training proceeded much as described above for the 1.7-year-olds. For the experiments conducted with the 1.7-year-old rats, the left lever was programmed to produce 50% of the reinforcers during the baseline phase. Although the percentage usually fell between 40% and 60%, sometimes a more extreme value occurred by chance. This resulted in biased performance during the baseline and disrupted the subsequent transition. The problem was addressed in Experiment 2 by designing reinforcement sequences in such a way that 40% to 60% of the reinforcers were explicitly assigned to each lever during the last 10 min of the baseline phase. This approach reduced bias during the baseline phase. Technically, the baseline phase used in Experiment 2 could be described as a concurrent variable interval 180 s variable interval 180 s (Conc VI 180 s VI 180 s) schedule of food reinforcement. Random-interval schedules of food reinforcement were still used during the transition phases.

#### 2.2.5. Data analyses

Data were collected on a visit-by-visit basis. A “visit” to the left lever began with the first response on that lever and ended with the first response on the right lever. At the end of each visit, the number of responses, the number of reinforcers, and the time spent on that visit were all recorded.

**2.2.5.1. Response allocation during steady state.** The ratio of left-lever to right-lever responses was taken from the last 10 visits of a session. This was compared to the ratio of reinforcers programmed to derive from the left and right levers using regression analyses [4]. The log of the response

ratio was regressed against the log of the reinforcer ratio to fit Eq. (1) using linear least-squares regression.

$$\log\left(\frac{B_L}{B_R}\right) = \log a + b \log\left(\frac{R_L}{R_R}\right) \quad (1)$$

The intercept,  $\log a$ , is a measure of bias. A positive value indicates a bias to the left (the numerator), and a value of 0 represents no bias. The slope term  $b$  represents the sensitivity of behavior to the reinforcer ratios. To determine the effects of methylmercury exposure, the values of bias ( $\log a$ ) and reinforcer sensitivity ( $b$ ) were analyzed using an analysis of variance (ANOVA).

**2.2.5.2. Analysis of behavioral transitions.** The primary dependent variable was the logarithm of the ratio of left-lever to right-lever responses, a measure of response allocation or choice. The primary independent variable was the number of reinforcers delivered since the beginning of a transition. Thus, zero marks the moment that the reinforcer ratio changed 30 min into the session. Response allocation was related to cumulative reinforcers delivered during the transition by fitting the dependent measure to cumulative reinforcers using a logistic equation [Eq. (2)]. This relationship forms an S-shaped function that is described well by that equation [46]. An example will be presented in the description of Fig. 5.

$$B = \frac{B_{\max}}{1 + e^{k(R_{\text{half}} - R)}} \quad (2)$$

The parameters in Eq. (2) are as follows:

- $B$   $\log(B_L/B_R)$  as in Eq. (1).
- $R$  Number of reinforcers accumulated from the beginning of the transition.
- $B_{\max}$  Upper asymptote, representing response allocation at the end of the session.
- $k$  Rate of transition in units of 1/reinforcers and is equal to the number of reinforcers required to get from 1/e to 2/e (approximately the middle third) of the transition.
- $R_{\text{half}}$  Half-maximal reinforcers, or the number of reinforcers required to complete one half of the transition.

The parameters describing the transition,  $B_{\max}$ ,  $k$ , and  $R_{\text{half}}$  were compared using an ANOVA. In preliminary analyses, the reinforcer ratio at the beginning of the transition was estimated by incorporating a fourth, additive, term to Eq. (2). Further analyses (some described in Ref. [46]) persuaded us that a better approach was to subtract the response ratio seen in the last 10 min of the 30-min baseline from that seen during the 2.5-h transition, thus normalizing for any biases seen during baseline.

**2.2.5.3. Statistical analyses.** Steady-state performance and transitions were analyzed using linear [Eq. (1)] and nonlinear [Eq. (2)] least-squares regression, respectively. The param-

eters resulting from these analyses were then analyzed using ANOVA techniques. A one-way ANOVA was conducted for bias and intercept separately from Eq. (1) and then for the upper asymptote, half-maximal reinforcers, and transition rate deriving from Eq. (2).

For the 1.7-year-old group, repeated measures analyses (RMANOVA) were set up as a split-plot factorial design. Litter was treated as the “subject” nested within dose, making litter a random-effects variable. The analysis contained a single between-group factor, methylmercury exposure. There were two repeated measures factors, sex and transition size, making this a mixed-design ANOVA. The litter is the statistical unit, i.e., the “subject,” so sex must be treated as a repeated measure within this unit. Error terms used as the denominator in the  $F$  ratio were constructed as recommended by Kirk [40] for this type of analysis, which

he refers to as an  $SPF_{p,qr}$  design. The error term for the main effect of mercury was the Litter  $\times$  Dose interaction. The error term for main effects involving transition size was the Litter  $\times$  Transition interaction. The error term for sex was the Litter  $\times$  Sex interaction.

The analyses of the 2.3-year-olds were conducted similarly except that sex was not included as a variable since there was an insufficient number of males surviving long enough. When there was both a male and female from a litter, each contributed to the estimate of the effect from that litter by averaging, and  $N$  was still the number of litters represented. For graphical presentation, however, data from males and females were plotted separately in order to provide visual confirmation that sex appeared to be unimportant even if a statistical analysis of sex could not be conducted for these older animals.

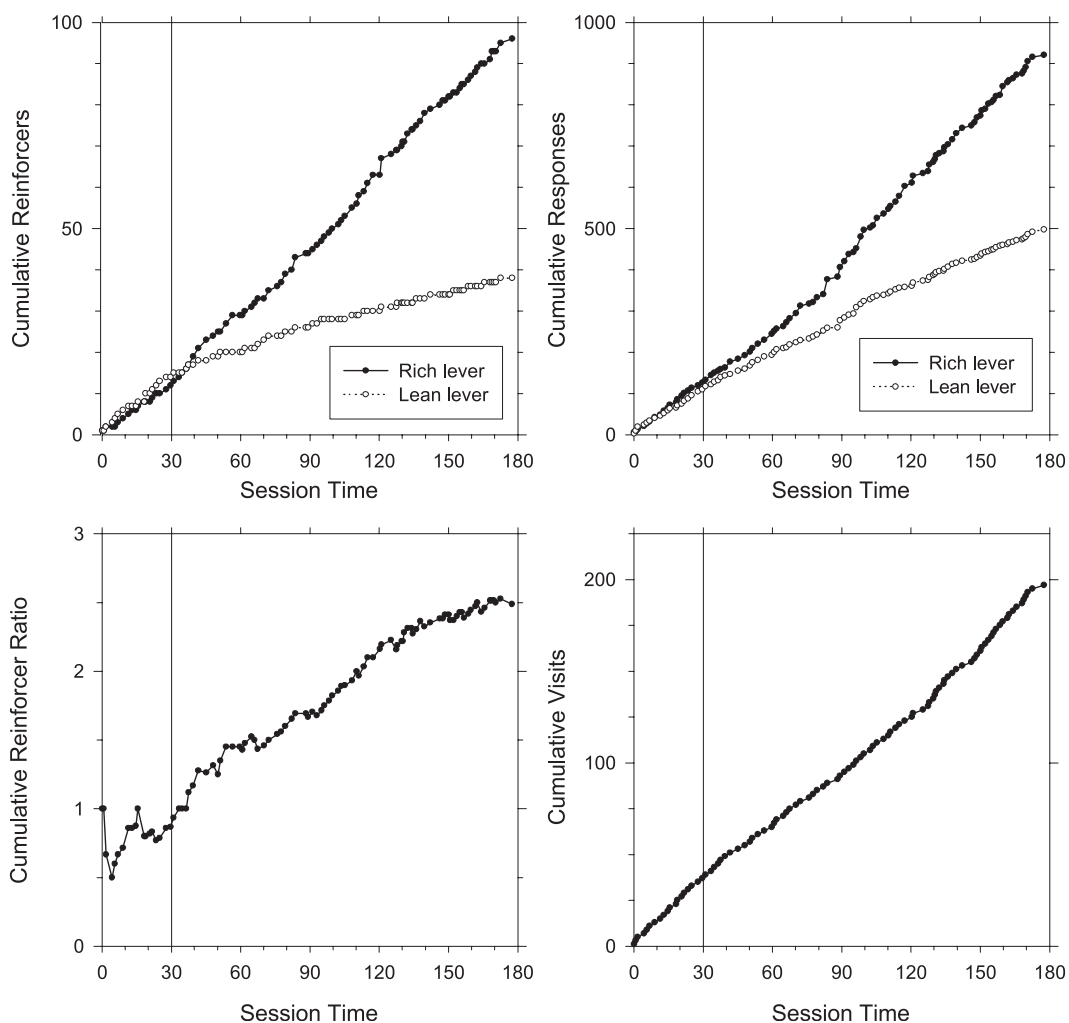


Fig. 1. Representative records showing cumulative responding on each of two levers for a transition to the left. The ratio of left:right reinforcement rates changed from 1:1 to 4:1 at 30 min into the session (vertical line). The top left panel shows cumulative reinforcers and the top right shows cumulative responses. There were slightly more reinforcers from the right lever at the beginning of the transition. This can be seen also in the bottom left panel showing the reinforcer ratio as it accumulates through the session. Response and reinforcement rates on the left (rich) lever accumulated at a higher rate than those on the right lever after the change of reinforcement rates. A new visit was registered every time the animal changed levers, and these are indicated in the bottom right panel. Note that response rates and changeover rates were steady throughout the 3-h session.

### 3. Results

#### 3.1. Autoshaping

All rats acquired lever pressing through autoshaping. No exposure-related differences were noted in the rate of acquisition.

#### 3.2. Overall responding

Daily inspection of response (lever press) rates and changeover rates showed that the rate of lever pressing and of changing levers occurred at a constant rate through the course of a session (Fig. 1).

Both response rates and changeover rates were influenced by the reinforcer ratios for the 1.7-year-olds, but not for their 2.3-year-old littermates (Fig. 2). For the 1.7-year-olds, there

was no effect of methylmercury on changeover or response rates and there was no interaction between exposure and reinforcer ratio ( $P_s > 0.1$ ). There was an effect of reinforcer ratio on changeover rate, or visits per minute, in the figure [ $F(4,50) = 14.4, P < 0.001$ ]. Post hoc comparisons confirmed what is visible in the top two panels of Fig. 2; when the left:right reinforcer ratio is one, the changeover rate is higher than it is in the other conditions, when one lever is richer than the other. There also was an effect of reinforcer ratio on overall response rate [ $F(4,50) = 19.1, P < 0.001$ ]. Post hoc comparisons showed that response rate was highest when the reinforcer ratio was equal on each lever (again, the 1:1 condition).

For the 2.3-year-olds, there was an effect of prenatal methylmercury on changeover rate [ $F(2,13) = 4.0, P = 0.04$ ] with the low-exposure group showing a lower response rate. There was also an effect of exposure on overall response rate

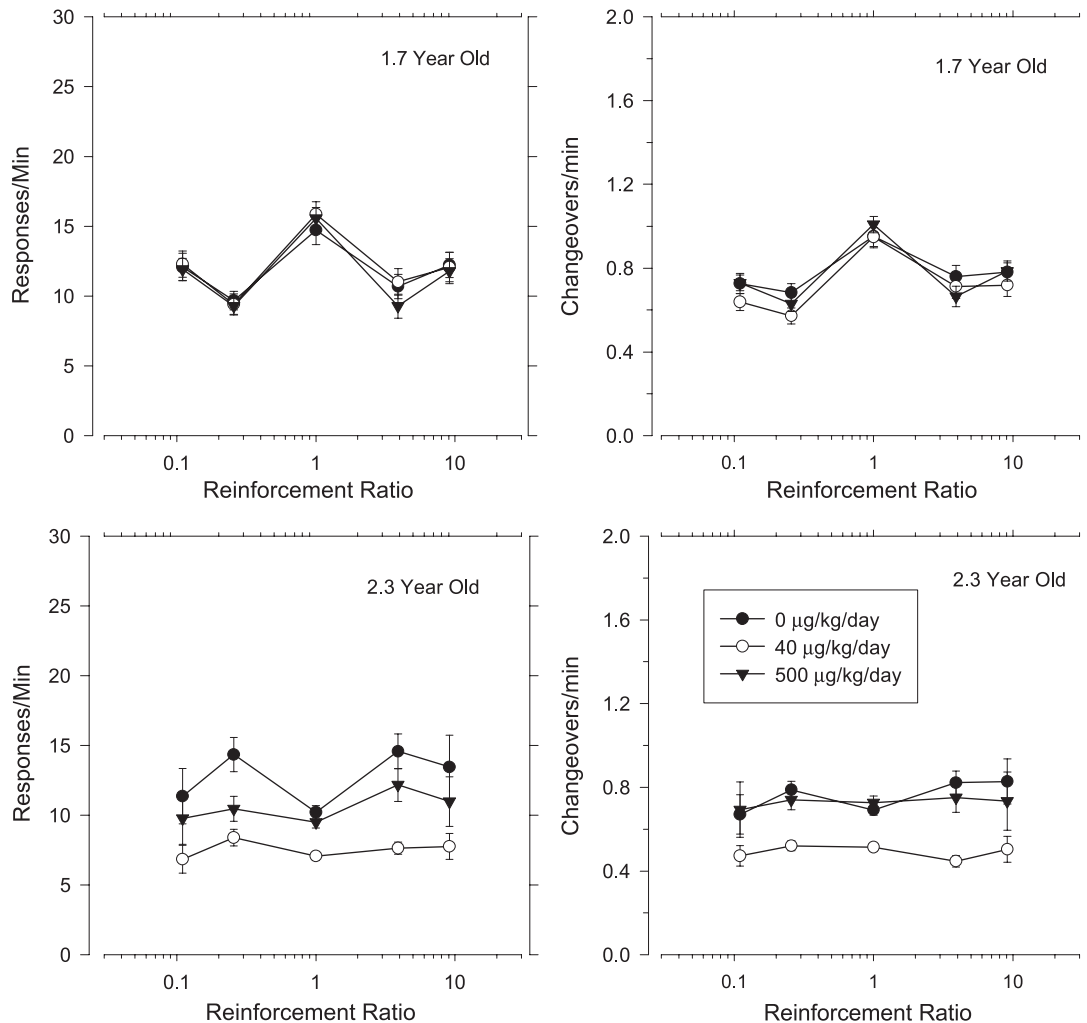


Fig. 2. Response rate (left) and changeover rate (right) for the three exposure groups expressed as a function of the ratio of left to right programmed reinforcers. Values for the 1.7-year-olds are shown in the top row and for their 2.3-year-old littermates in the bottom. For the 1.7-year-olds, response rates and changeover rates were highest during the condition in which each lever produced the same reinforcement ratio (ratio = 1) and there was no effect of prenatal methylmercury exposure on either measure. The relationship of response and changeover rate to reinforcer ratios did not appear in the 2.3-year-old littermates. Both measures were lower for the older rats exposed prenatally to the lower level of methylmercury.

[ $F(2,13)=4.1, P=.04$ ]. There was no effect of reinforcement ratio on changeover rate or on its interaction with dose ( $P_s>0.1$ ), but there was an effect on response (lever press) rate [ $F(4,52)=5.2, P=0.001$ ].

### 3.3. Examples of terminal response allocation

Fig. 3 shows representative analyses of terminal performance. The response ratios were calculated from the last 10 visits on each side. The reinforcer ratios were those programmed to derive from the left and right levers. The line in each panel shows the best fit line obtained by fitting Eq. (1) to the data after taking the log of response and reinforcer ratios, i.e., the regression was conducted on the linear function shown. If fitted to the raw data, the equation would take on a power-function form:  $Y=(10^{\log a})(X^b)$ . For example, the form for the equation in the lower left panel would be

$0.4(X^{0.53})$ . The intercept indicates a bias to the right lever (denominator) such that when each lever produces the same reinforcement rate, the ratio of left:right lever presses is 0.4. This condition was selected to illustrate biased performance. Very little bias was seen in the other three examples and in most cases.

### 3.4. Methylmercury and terminal responding

Fig. 4 shows slopes and intercepts describing terminal performance for the two age groups. One-way ANOVAs revealed no effect of prenatal methylmercury exposure on slope or intercept for the 1.7- or 2.3-year-old rats (all  $P_s>0.1$ ). Slopes ranged from about 0.2 to about 0.8 for all exposure groups and both ages. Intercepts averaged around 0, with some rats showing a left-lever bias (positive intercept) and some showing a right-lever bias (negative intercept). The

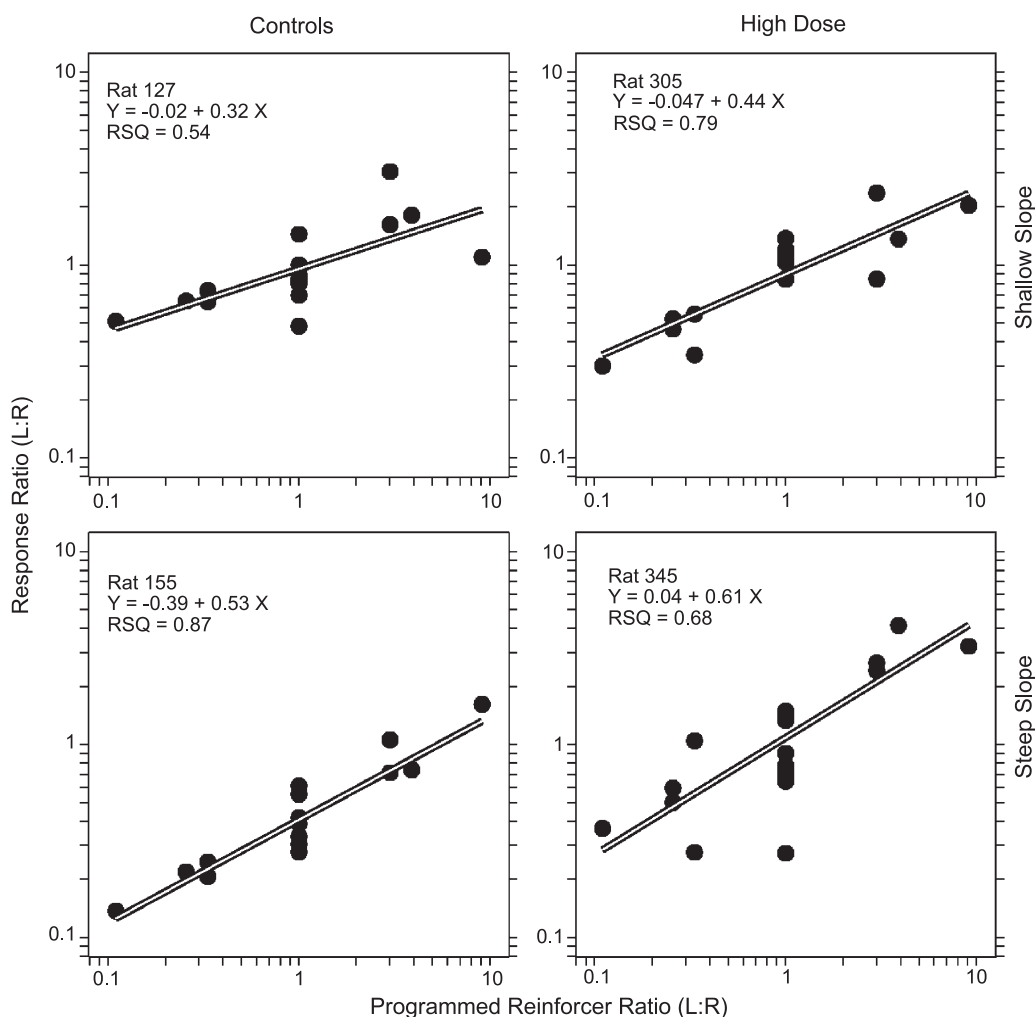


Fig. 3. Representative figures illustrating the relationship between asymptotic ratios of left- to right-lever responses (vertical axis) and the corresponding programmed reinforcement ratios (horizontal axis). Each panel represents an individual rat. Each data point represents a single session for that rat and, specifically, the last 10 visits from an individual session, i.e., the rightmost portion of a transition such as those shown in Fig. 5. The top row shows the shallower slopes from control (left) and high-dose groups (right). The bottom row represents the steeper slopes. The equation of the line of best fit and variance account for ( $R^2$ ) are indicated in each panel.

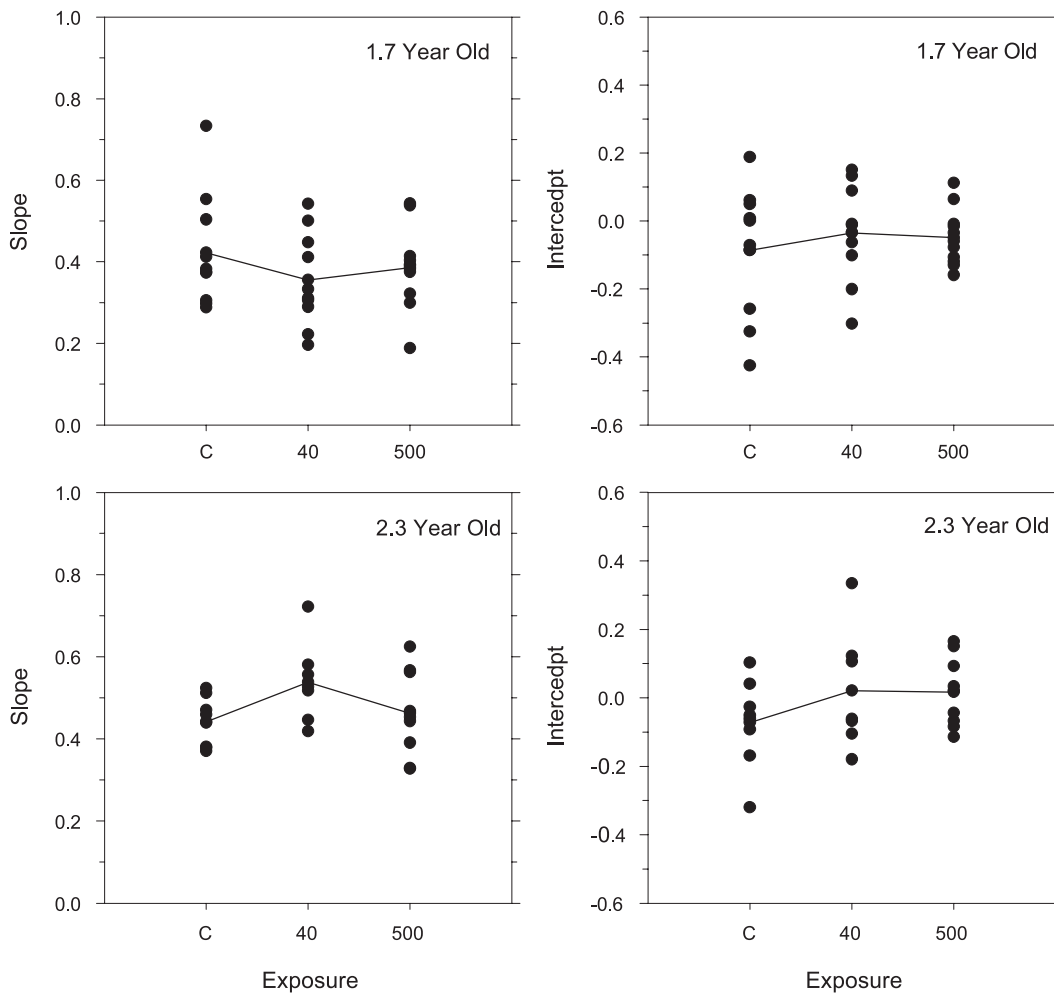


Fig. 4. Effects of methylmercury on slope (left) and intercept (right) for 1.7-year-olds (top row) and their 2.3-year-old littermates (bottom row) on asymptotic performance under concurrent schedules of reinforcement. Each symbol represents an individual subject. Lines connect group means. Prenatal exposure to methylmercury affected neither parameter of asymptotic performance.

large intercept from rat 155 ( $-0.39$ ) illustrated in Fig. 3 represents one of the more extreme biases seen in these experiments.

### 3.5. Examples of choice in transition

Fig. 5 illustrates how behavior change was quantified and prepared for curve fitting. Each transition was described as a change in the response ratio (shown as small points in the figure) as a function of cumulative reinforcers delivered. Analyses were conducted on a visit-by-visit basis. A LOW-ESS smoothing algorithm was applied to these data using a smoothing parameter of 9 (thin line on the figure). This means that a particular data point represents a weighted mean of that data point plus four to the left and four to the right, with weights becoming smaller for more distal points (special weighting was applied to the first and last four points, see Ref. [10]). The logistic function [Eq. (2)] was then fit to the smoothed curve using nonlinear least squares (thick line in the figure and equation in the inset). (The fit was actually

applied to the log of the reinforcer ratios, and the result converted back so the actual ratios could be plotted.) This produces three parameters whose interpretation can be understood by using the top left curve as an example. The numerator, 2.4, is the upper asymptote and represents the response ratio seen at the end of the session. The slope term, 0.14, represents the maximal slope of the transition curve, which is seen at the halfway point. This term characterizes the rate at which a transition takes place. A small value is seen if the transition progresses slowly. A little algebra shows that the inverse of this term (i.e.,  $1/0.14 = 7.1$ ) is the number of units on the  $x$ -axis (i.e., the number of reinforcers) required to get from  $1/e$  to  $2/e$  of the transition, where  $e$  is the natural log base and has a value of approximately 2.73. This is approximately the middle third of the transition. The value of 19 positions the logistic function horizontally and represents the point over the  $x$ -axis at which the transition is half finished. This is called the half-maximal reinforcers and is illustrated by a vertical line over 19 reinforcers. The control rat illustrated completed one half of the transition after 19 reinforcers,

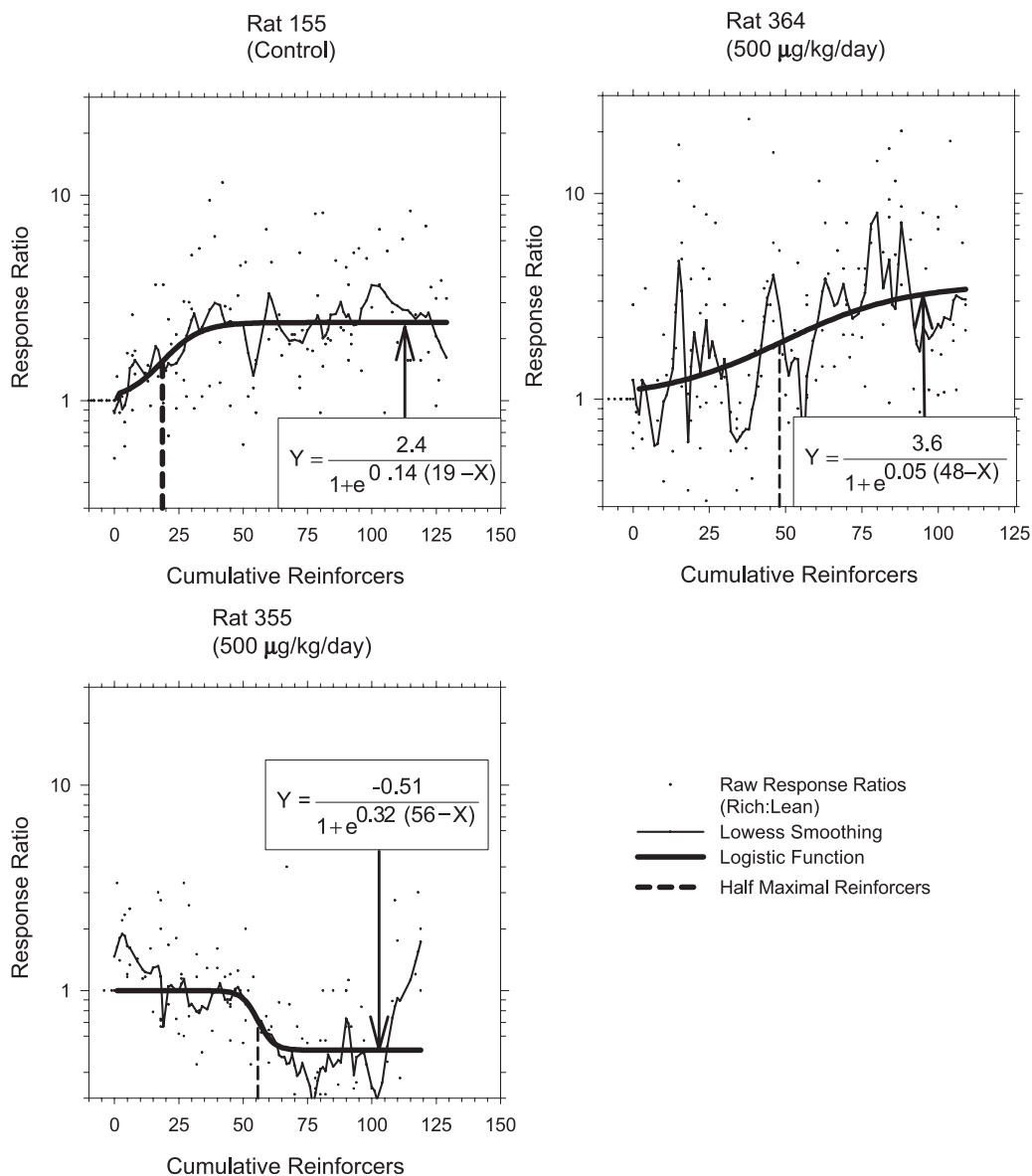


Fig. 5. Representative transitions depicted on a reinforcer-by-reinforcer basis and the equations describing their course. These were selected to illustrate different patterns of transitions seen in these experiments. The transition began 30 min into the 3-h session, at 0 on the x-axis. Behavior change is illustrated as a function of cumulative reinforcers (left + right) obtained under the final schedule, for which the rich:lean reinforcer ratio was 4:1. The vertical axis shows the ratio of responses on the rich to lean schedules. Note log scaling. Each point shows the reinforcer ratio for a pair of visits, one on the left and the other on the right. The thin wavy line shows the application of a LOWESS smoothing algorithm applied to the points. The thick line shows the result of nonlinear least-squares fitting of the logistic function [Eq. (2)] to the smoothed curve. See text for additional details and interpretation of the parameters. Note that for rat 355, responding tended to occur on the *lean* lever, a pattern seen in three exposed rats.

one exposed rat completed it after 48 reinforcers, and a third after 56 reinforcers. For one rat (355), behavior shifted to the *lean* lever, a pattern seen three times in exposed rats.

### 3.6. Effects of mercury on choice in transition

Fig. 6 shows the effects of methylmercury, age, and transition size on a key measure of behavior during transition sessions, the number of reinforcers required to complete one half of the transition, or half-maximal reinforcers. RMANOVAs were conducted separately for each age group. Transi-

tion size and, in the younger rats, sex were treated as repeated measures. Dose was a between-group measure and litter was the statistical unit.

There was no effect of sex on half-maximal reinforcement in the younger rats ( $P_s > 0.1$ ). Sex could not be incorporated into the analysis for the older rats because of small  $n$ s in some groups, but no effect is evident in the figure. There was no effect of transition size on this measure for the young or old rats ( $P_s > 0.1$ ). There was no effect of methylmercury on half-maximal reinforcers for the young rats [ $F(2,12) = 1.2$ ,  $P = 0.32$ ], although inspection of the figures indicates large

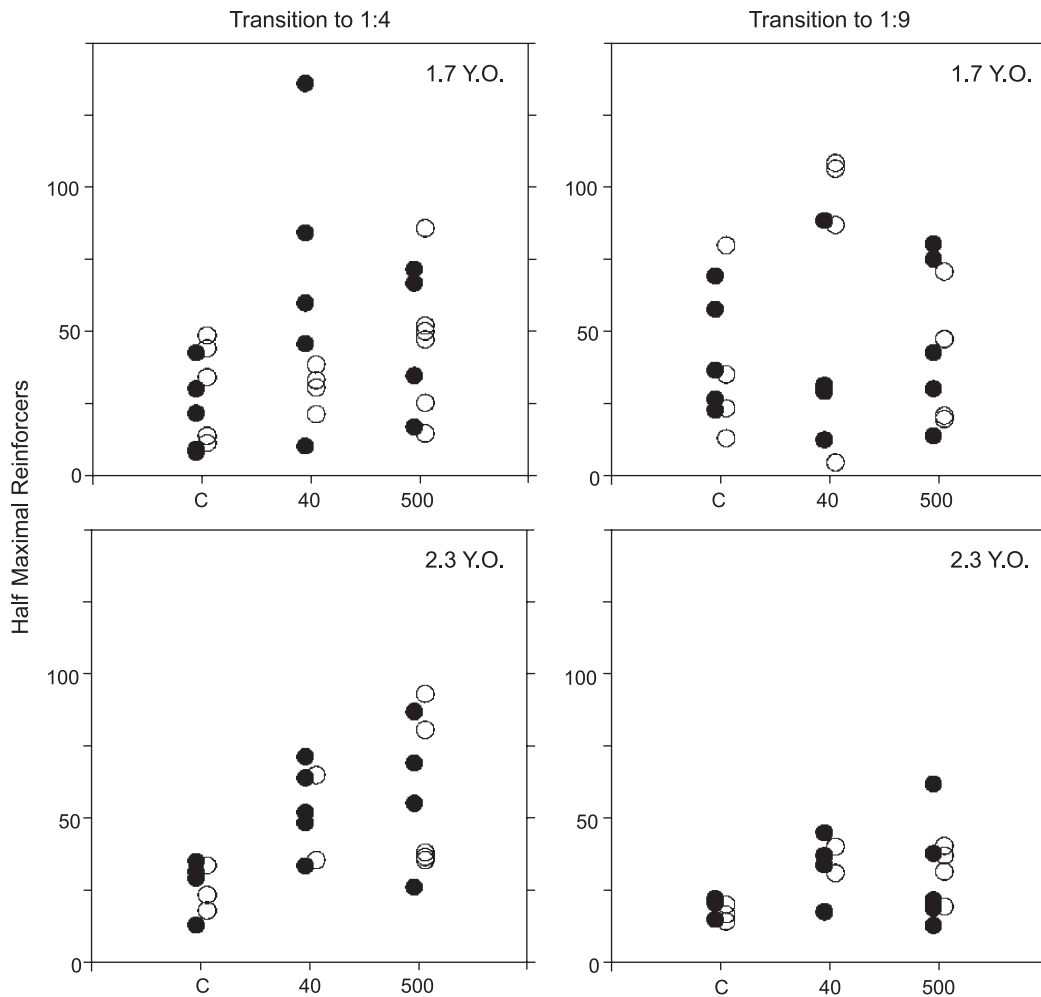


Fig. 6. Effects of prenatal methylmercury, age, transition magnitude, and sex on behavioral transitions as represented by half-maximal reinforcers. The 1.7-year-old rats are shown in the top rows and their 2.3-year-old littermates in the bottom row. Males (filled symbols) and females are separated, but there was no effect of sex on transitions. The number of reinforcers required to complete one half of the transitions was not affected by sex, transition size, or age. Methylmercury retarded both transitions in the 2.3-year-old rats. See text for details.

values for some methylmercury-exposed rats. Exploratory analyses revealed that these contributed inordinately to the variability, so the analysis was conducted a second time on the log of half-maximal reinforcers. There was a main effect of dose on log (half-maximal reinforcers) [ $F(2,13)=3.68$ ,  $P=0.054$ ], but no interaction appeared with the other measures. No other conclusions changed after this transform.

For the 2.3-year-olds, there was a main effect of dose [ $F(2,13)=5.24$ ,  $P=0.021$ ] and transition size [ $F(1,13)=13.69$ ,  $P=0.003$ ], but no interaction between these two variables [ $F(2,13)=2.0$ ,  $P=0.17$ ] on half-maximal reinforcers. For consistency with the analysis conducted with the 1.7-year-olds, the log(half-maximal reinforcers) was also examined, but this transformation did not change any conclusions.

There was no effect of methylmercury on the magnitude of the transition [ $B_{\max}$  in Eq. (2)], consistent with the analyses of the matching functions above (data not shown in figures). There was no main effect of methylmercury on the rate of the transition [ $k$  in Eq. (2)] for either age group (data not shown in

figures). For the 1.7-year-olds, there was an effect of transition size on the rate of the transition such that transitions to a 1:9 schedule tended to occur more slowly than those to the 1:4 transition [ $F(1,13)=4.39$ ,  $P=0.056$ ]. The interaction between dose and transition did not quite reach conventional levels of statistical significance [ $F(1,13)=2.97$ ,  $P=0.087$ ]. The main effect of transition was not replicated in the 2.3-year-olds [ $F(1,13)=0.006$ ,  $P=0.94$ ] but, once again, an interaction between dose and transition approached conventional levels of statistical significance [ $F(2,13)=3.32$ ,  $P=0.069$ ]. In both cases, transition rates tended to be slower for methylmercury-exposed rats. For the 1.7-year-olds this effect was more prominent for the 1:9 transition and for the 2.3-year-olds it was more prominent for the 1:4 transition.

#### 4. Discussion

The offspring of rats exposed during pregnancy to 40 and 500  $\mu\text{g}/\text{kg}/\text{day}$  of methylmercury showed retardation in the

acquisition of choice when they were 2.3 years of age. The effects were seen on choice, or the allocation of behavior between two response alternatives, as it was undergoing a transition. No effect appeared on asymptotic or terminal performance. The results of the experiments described here replicate, using rodents, many important features of an earlier report in which methylmercury was shown to disrupt transitions under concurrent schedules of reinforcement in three prenatally exposed squirrel monkeys [50]. There are some important differences, including an apparent interaction with age. From a methodological perspective, the present experiments introduce a technique for conducting behavioral transitions in single sessions, rather than over the course of about 3–4 weeks of testing, as in the earlier experiment. These issues are discussed below.

#### 4.1. Single-session transitions

The first question to be addressed in validating the procedure used for examining transitions in single sessions is whether behavior can be maintained steadily for 3 h. The answer to this question is yes, and both the overall rates of changing levers (changeovers) and of responding shown in Fig. 2 support this conclusion. We also examined many hundreds of daily records similar to those in Fig. 1 showing response rates and changeover rates to ensure that behavior could be maintained in these long sessions. Both lever pressing and changeovers occurred at a steady pace throughout the course of the entire session. This figure also shows that the approach taken to control reinforcement allocation during the baseline portion of the session also worked for the 2.3-year-old rats. Reinforcer biases, occasionally present in the transitions from 1.7-year-olds, were never seen after this adjustment was made. By the end of the baseline portion of a session, the proportion of reinforcers from the left lever was always between 0.4 and 0.6.

Most studies of behavior under concurrent schedules use pigeons and examine performance under steady-state conditions in which behavior is allowed to stabilize over the course of many sessions, often consuming many weeks of time. When rats have been used, they have not differed in important ways from pigeons in these types of experiments [17,22] and the results here do nothing to alter this conclusion. To validate the single-session transition, it should be demonstrated that both transitional and steady-state performance in our rats is similar to that seen in other experiments. Three other performance parameters are included for comparison: overall response rate, the relationship between changeover rate and reinforcer ratio, and the relationship between response ratio and reinforcer ratio.

In steady-state conditions, the changeover rate is highest when the two available schedules produce equal reinforcement rates, i.e., when the reinforcer ratio = 1 [43]. Here, changeover rates were highest, about 1/min, representing approximately 60-s visits under the 1:1 condition for the 1.7-year-olds. Interestingly, no relationship between reinforcer

ratio and changeover rate was discernable in the older rats. No comparative studies could be identified, so this may be a novel observation. The 2.3-year-old rats exposed to the lower dose of methylmercury had lower changeover rates and lower response rates than those in the other two exposure groups. In the absence of a systematic dose–effect relationship or a plausible way of interpreting it, this will remain simply an observation for now.

For the 1.7-year-olds, response rates were influenced by the reinforcer ratios and were generally higher at the 1:1 ratios, as reported in other studies that allowed more time for stability to develop (e.g., Ref. [9].) As with the pattern of changeovers, this effect was not seen in the 2.3-year-olds.

The data are not shown, but no trends in performance were detected across experimental conditions. Insofar as we could determine, transitional or asymptotic performance toward the end of the experiment looked similar to that seen earlier in the experiment. The experiment was not designed to make such trends (i.e., “learning to learn”) readily detectable. The response ratios on the left and right lever for a particular session were assigned unpredictably for a particular daily session so it would be very difficult to identify any trends across sessions. In fact, the random assignment was used in part to minimize such trends.

The relationship between response and reinforcer ratios taken from studies of pigeons performing under concurrent schedules of reinforcement is usually described very effectively with Eq. (1), called the “generalized matching function.” Typically, slopes range from 0.8 to 1.0 and the equation typically accounts for greater than 90% of the variance [4,21]. Intercepts center on zero in those studies.

The slopes reported here are shallower than those typically reported. In most studies of behavior under concurrent schedules of reinforcement, subjects are allowed weeks to months for behavior to stabilize. Here they were allowed only 2 1/2 h. As shown in Figs. 3 and 4, slopes in the present experiment ranged from about 0.2 to 0.8 and variance accounted for was usually in the range of 0.5 to 0.8. These shallower slopes and smaller variance accounted for is consistent qualitatively and quantitatively with what has been reported on studies of choice in transition under concurrent schedules [21,37].

Hunter and Davison [37] tracked the time course of behavior change across sessions. They averaged performance over an entire session (40 reinforcers) and therefore missed the S-shaped acquisition seen in the fine-grained analysis used here. In that study, the change in session averages was modeled adequately by an exponential function with a half-life of about one session or 40 reinforcers, consistent with the examples shown in Fig. 5. It should be noted that the present experiments were designed to examine behavior in transition and we successfully captured the most important portion of the transitions in these single sessions.

The S-shaped transition function obtained here is also consistent, or at least not inconsistent, with other reports. The use of a logistic function to describe behavior change has

also been described by Myerson and Hale [43] and in the earlier experiment with squirrel monkeys [46,50].

As noted in a review of the fits obtained from different of types functions, it is not the logistic function per se that is important, but the ogive shape that permits it to fit the early portion of the transition, which is characterized by a period of slow change and then a very rapidly rising region of change [46]. Other functions, such as Gompertz equations, can also accomplish this and can do so without the constraint imposed by logistic functions that the function be symmetric.

The pattern of change reported here is also consistent qualitatively with recent examinations of how behavior changes. The snowballing pattern seen, in which behavior change begins slowly but then accelerates at a very rapid rate, may be related to observations that during the first few reinforcers of a transition, behavior is heavily influenced by the previous reinforcement contingencies. Then, the new reinforcer ratio has exceptionally large effects on subsequent behavior, and these effects ripple through several subsequent changeovers [19, 20]. Soon the discrepancy produced by the new contingencies has a large, and then diminishing effect on behavior, resulting in the S-shaped function modeled here. Modeling behavior change according to the cumulative reinforcers delivered indicates that the number of reinforcers required to initiate and complete the transition is consistent across different reinforcement contingencies and is even similar across rats (this study), pigeons [37], and squirrel monkeys [46,50].

#### 4.2. Effects of methylmercury on the acquisition of choice

The results of the present experiments replicate those of an earlier experiment [50] in which prenatal exposure to methylmercury affected the course of behavior change later in life. In that experiment, the subjects were three young squirrel monkeys exposed to methylmercury during gestation such that maternal blood levels reached about 0.85 ppm. There were also many monkeys exposed prenatally to lead that were affected similarly. The lead-exposed group offers some assurance that this approach can identify effects of a neurotoxicant known to alter learning. That experiment involved delayed consequences of prenatal exposure and not, as is much more common in the lead literature, effects of contemporaneous or postnatal exposure.

The present experiment extends to rodents observations that prenatal methylmercury can influence the acquisition of choice. This is so even if methylmercury exposure does not have demonstrable effects on the eventual asymptote that presumably approximates steady-state performance. After sufficient training, exposed animals eventually performed like controls did, but more reinforcers were required for them to reach this steady state. This was seen clearly in the 2.3-year-olds and there was good evidence for an effect on the smaller transition (1:4) in the 1.7-year-olds. The lack of an effect on asymptotic behavior is visible both in the analyses

of matching relationships (Fig. 4) and in the lack of an effect of methylmercury exposure on the term  $B_{\max}$ . The effect on behavior change was most strongly visible in the half-maximal reinforcers. This measure characterizes the rapidity with which choice, as indicated by response allocation, shifts from approximately equal responding on each lever to most behavior occurring on the richer lever. As discussed below, this can be viewed as sensitivity of behavior to the source of reinforcement, or sensitivity to discrepancies in the richness of two different reinforcement sources.

The value of half-maximal reinforcers is not independent of the slope term,  $k$ , since shallow slopes necessarily place the half-maximal reinforcers farther to the right. It is possible that the slope term conveys additional information about the rate of behavior change, but this is not clear at present. Interpretation of this term must wait for a fuller understanding of behavior change under this schedule. It should also be noted that the slope term was the most difficult of all three terms to estimate with nonlinear regression, sometimes yielding fairly high standard errors.

The present report also confirms what has been noted in numerous reports of developmental neurotoxicity: the phenomenon of individual susceptibility. Clearly, in the 1.7-year-olds, and somewhat in the older rats, the effects were characterized by the appearance of large effects in some rats rather than by a wholesale shift in the distribution.

#### 4.3. Interactions of methylmercury and age

It appears that there was an interaction between methylmercury and age on the course of transitions in the present experiments, but there are reasons to be cautious about this conclusion. The presence of an interaction with age is supported by the appearance of a mercury effect following both sets of transitions for the older rats. This is further supported by a report by Newland and Rasmussen [48] describing an age-related effect on high-rate behavior in littermates of the rats described here. In that experiment, performance under a DRH 9:4 schedule of reinforcement (nine responses had to occur within 4 s to be eligible for reinforcement) began to deteriorate in methylmercury-exposed rats at about 2 years of age. The ages used here approximately straddle that age. Delayed neurotoxicity has also been described in aging Minamata victims exposed to methylmercury as adults [39] and in sensory function in nonhuman primates exposed developmentally [61,63].

The above conclusions should be tempered both by a statistical and by a methodological consideration. The statistical consideration is that the direct evaluation of an interaction was not conducted, so the conclusion of an interaction with aging is based on a failure to reject the null hypothesis in 1.7-year-old rats performing under the larger (9:1) transition coupled with a somewhat weaker effect on the performance of these animals.

The methodological consideration is that the baseline portions of the sessions were different between the two ages.

For the 1.7-year-old rats, the reinforcer ratios were obtained randomly during the baseline session and the overall allocation of reinforcement did not always remain between 0.4 and 0.6. Any effects on response bias were handled by normalizing ratios during transition to the mean ratio from the last 10 min of the baseline portion of the session. Normalization was relatively unimportant for the older group, but sometimes biases appeared in the younger rats because the reinforcer ratio was so free to vary and it may have affected the fit of Eq. (2) to the data.

We suspect that this methodological difference affected variability more than the parameter estimates, which may have reduced statistical power for the 1.7-year-olds. In light of these considerations, we interpret Fig. 6 as showing that there are clear effects of prenatal methylmercury exposure on transitions in the 2.3-year-old rats. In the 1.7-year-olds, there may have been either a more subtle effect or no effect that could be detected by the procedures used.

#### 4.4. Behavioral mechanisms

A behavioral mechanism parsimoniously accounts for complexities in behavior by invoking such fundamental behavioral processes as reinforcement or discrimination. The important point is that the mechanism, reinforcement, for example, takes place at the same level of observation as the phenomenon to be explained. Some degree of explanation is accomplished without incorporating neural mechanisms like neuronal activity or neurotransmitter function. An accurate account of the behavioral mechanism is helpful in finding links to the appropriate neural mechanism [41]. The effects reported here and in other experiments on methylmercury's behavioral toxicity can be understood by recalling the three-term contingency of reinforcement that is so important in understanding operant, or voluntary, behavior. Any behavior, however simple or complex, can be called "operant" if it is sensitive to its previous consequences, as was certainly the case here. The response–reinforcer contingency (two of the three terms) always occurs in a context, the third term, which includes discriminative stimuli present or previously presented. Contextual control over behavior is seen in discrimination, generalization, or memory processes [18,23,44]. This framework helps organize the effects of methylmercury at the level of the behaving animal.

Apparently, developmental methylmercury exposure does not impair discrimination, the acquisition of discrimination, discrimination reversals [59], or information processing speed in primates [62]. Methylmercury exposure had no detrimental effect on remembering and sometimes even increased the number of correct responses on delayed spatial alteration tasks [31]. In rodents, too, there is no evidence of effects of developmental exposure to methylmercury on performance (other than overall activity) in mazes [26,38], except at a very high exposure level [71] or on measures of memory and discrimination that are relatively nonspecific [6,27,32]. Interestingly, developmental methylmercury

impairs object permanence in young monkeys [7] and affects recognition of faces or other stimuli [35,36]. While these monkeys were screened for general visual acuity, these latter effects could still be interpreted as a consequence of impaired high-order visual function or a developmental delay [49]. Alternatively, they could be seen as related to differences between recognition, which the Fagan Test taps, and recall processes that are tapped by the other procedures listed. In view of the evidence that methylmercury has little effect on discrimination processes, that it accumulates in visual cortices, and has significant effects on visual function, the prospect that this represents delayed development or poor high-order visual processing remains a possibility.

It is difficult, if not impossible, to conceive of a procedure that completely separates discrimination from reinforcement processes (but see Refs. [18,23] for interesting theoretical attempts). It is possible, however, to emphasize one set of processes at the expense of the other. The procedure used here is well suited to the direct examination of the acquisition of a response–reinforcer relationship with little involvement of discrimination processes. There are relatively few opportunities for discrimination to influence choice in transition under concurrent schedules of reinforcement as presented here. The lever associated with the richer schedule of reinforcement, and even the ratio between the rich and lean schedules, varies unpredictably. There is no discriminative stimulus consistently associated with the richer lever, other than reinforcement density. Eventually, lever position does become a potential discriminative stimulus, but only after the reinforcer ratios have changed. The reinforcement density early in the transition can also serve as a discriminative stimulus, but this is perfectly confounded with the actual density.

As reported in animal studies, methylmercury's effects, at least at low exposure levels, are sometimes viewed as being motor or sensory, but not cognitive. This is an insufficiently precise picture both of methylmercury and of the process of behavior change. In fact, methylmercury presents an interesting case because it supports other behavioral analyses indicating that reinforcement and discriminative processes are separable [18,23]. Behavior change can be retarded by mechanisms acting on reinforcement processes, but not memorial processes, the domain often implied by the phrase "cognitive effects." The mechanisms identified here could also be referred to as "attentional" insofar as attention denotes behavioral sensitivity to differences in sources of reinforcement. This idea is entirely consistent with the results here [45] and with evidence that methylmercury influences monoamine pathways, as discussed below.

#### 4.5. Neural mechanisms

The present studies present no direct information about neural mechanisms, but the results point to some potential sources. It seems possible to rule out sensory or motor mechanisms since there is little opportunity for either class

of effects to be experienced here. The reinforcement schedules do not place a high demand on the motor abilities and there was no consistent evidence of effects on response rate or even of changeover rates. The single exception might be seen in the lower changeover rates in the 40 lower dose rats at 2.3 years of age. The absence of a dose–effect relationship or of an indication that this occurred in the 1.7-year-olds or in the earlier report [51] suggests that this effect should be viewed with considerable caution until it can be replicated.

Developmental exposure to methylmercury in humans, nonhuman primates, and rodents results in mercury accumulation in many regions of the central nervous system, including the cerebellum, striatum, and virtually all cortical regions [1,11,58]. In the cortex, developmental mercury accumulation is associated with reduced cell density and deranged and reduced size in cortical laminae, especially in the neocortex [1]. Accumulation in motor and sensory cortices, spinal cord, and cerebellum could account for sensory and motor effects frequently noted, but neither region seems likely to be involved in the effects described here. Cortical or mesolimbic sources of methylmercury's effects seem plausibly related to the effects reported here.

Regions of the frontal cortex and striatum have been linked to behavior associated with choice, decision making, and the tracking of reinforcement magnitude and quality and are important in operant and respondent conditioning [67,68,70]. In rats, the medial frontal cortex participates in choosing between reinforcers that are readily available and those accessible only by way of a barrier [72]. Cortical lesions influence sensitivity to probabilistic reinforcers [42], with monoamine pathways perhaps playing an important role in choice [66], delayed choice, and attention [53–55]. The striatum also plays an important role when reinforcement conditions change [67,68]. Interestingly, methylmercury exposure affects control of dopamine and noradrenergic function [2,3,30,56] in cortex [52] and striatum [28,29] in vitro. Drug challenges with behaving, adult rats exposed during gestation supports these observations by demonstrating selective sensitivity to *d*-amphetamine, a catecholamine agonist [57].

Methylmercury appears to diminish the sensitivity to reinforcing events and this, in turn, appears to retard behavior change in situations that require a choice, even a continuously changing choice, between two response alternatives. The literature referred to above suggests that impaired cortical and striatal function influences sensitivity to changes in the source of reinforcement, in choice, and in the actions of reinforcing events. While still speculative, the possible neural and behavioral mechanisms behind the effects reported here seem to have a common source, and both point to the possibility that one aspect of methylmercury's neurotoxicity lies in disturbances in the sensitivity or speed by which choice is influenced by changing reinforcement contingencies. These effects are mediated through the role of consequences and especially the response–reinforcement relationship.

## Acknowledgements

Supported by ES06466 and ES10865 from NIEHS.

## References

- [1] S. Barone Jr., N. Haykal-Coates, D.K. Parran, H.A. Tilson, Gestational exposure to methylmercury alters the developmental pattern of trk-like immunoreactivity in the rat brain and results in cortical dysmorphology, *Brain Res. Dev. Brain Res.* 109 (1998) 13–31.
- [2] J. Bartolome, W.L. Whitmore, F.J. Seidler, T.A. Slotkin, Exposure to methylmercury in utero: effects on biochemical development of catecholamine neurotransmitter systems, *Life Sci.* 35 (1984) 657–670.
- [3] J. Bartolome, P. Trepanier, E.A. Chait, F.J. Seidler, R. Deskin, T.A. Slotkin, Neonatal methylmercury poisoning in the rat: effects on development of central catecholamine neurotransmitter systems, *Toxicol. Appl. Pharmacol.* 65 (1982) 92–99.
- [4] W.M. Baum, Matching, undermatching, and overmatching in studies of choice, *J. Exp. Anal. Behav.* 32 (1979) 269–281.
- [5] M. Bornhausen, H.R. Musch, H. Greim, Operant behavior performance changes in rats after prenatal methylmercury exposure, *Toxicol. Appl. Pharmacol.* 56 (1980) 305–310.
- [6] J. Buelke-Sam, C.A. Kimmel, J. Adams, C.J. Nelson, C.V. Vorhees, D.C. Wright, V. St. Omer, B.A. Korol, R.E. Butcher, M.A. Geyer, Collaborative behavioral teratology study: results, *Neurobehav. Toxicol. Teratol.* 7 (1985) 591–624.
- [7] T.M. Burbacher, K.S. Grant, N.K. Mottet, Retarded object permanence development in methylmercury exposed *Macaca fascicularis* infants, *Dev. Psychol.* 22 (1988) 771–776.
- [8] T.M. Burbacher, P.M. Rodier, B. Weiss, Methylmercury developmental neurotoxicity: a comparison of effects in humans and animals, *Neurotoxicol. Teratol.* 12 (1990) 191–202.
- [9] A.C. Catania, Concurrent performances: reinforcement interaction and response rate independence, *J. Exp. Anal. Behav.* 6 (1963) 253–263.
- [10] J.M. Chambers, W.S. Cleveland, B. Kleiner, P.A. Tukey, *Graphical Methods for Data Analysis*, Wadsworth, Belmont, CA, 1983.
- [11] L.W. Chang, Z. Annau, *Developmental neuropathology and behavioral teratology of methylmercury*, *Neurobehavioral Teratology*, Elsevier, New York, 1984, pp. 405–431.
- [12] N.R.C. Committee on the Toxicological Effects of Methylmercury, *Toxicological Effects of Methylmercury*, National Academy Press, Washington, DC, 2000.
- [13] K.S. Crump, T. Kjellstrom, A.M. Shipp, A. Silvers, A. Stewart, Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort, *Risk Anal.* 18 (1998) 701–713.
- [14] P.W. Davidson, J. Kost, G.J. Myers, C. Cox, T.W. Clarkson, C.F. Shamlaye, Methylmercury and neurodevelopment: reanalysis of the Seychelles child development study outcomes at 66 months of age, *JAMA* 285 (2001) 1291–1293.
- [15] P.W. Davidson, D. Palumbo, G.J. Myers, C. Cox, C.F. Shamlaye, J. Sloane-Reeves, E. Cernichiari, G.E. Wilding, T.W. Clarkson, Neurodevelopmental outcomes of Seychellois children from the pilot cohort at 108 months following prenatal exposure to methylmercury from a maternal fish diet, *Environ. Res.* 84 (2000) 1–11.
- [16] P.W. Davidson, G.J. Myers, C. Shamlaye, C. Cox, P. Gao, C. Axtell, D. Morris, J. Sloan-Reeves, E. Cernichiari, A. Choi, D. Palumbo, T. Clarkson, Association between prenatal exposure to methylmercury and developmental outcomes in Seychellois children: effect modification by social and environmental factors, *Neurotoxicology* 20 (1999) 833–842.
- [17] M. Davison, D. McCarthy, *The Matching Law: A Research Review*, Erlbaum, Hillsdale, NJ, 1988.
- [18] M. Davison, J.A. Nevin, Stimuli, reinforcers, and behavior: an integration, *J. Exp. Anal. Behav.* 71 (1999) 439–482.

- [19] M. Davison, W.M. Baum, Choice in a variable environment: every reinforcer counts, *J. Exp. Anal. Behav.* 74 (2000) 1–24.
- [20] M. Davison, W.M. Baum, Choice in a variable environment: effects of blackout duration and extinction between components, *J. Exp. Anal. Behav.* 77 (2002) 65–89.
- [21] M.C. Davison, I.W. Hunter, Concurrent schedules: undermatching and control by previous experimental conditions, *J. Exp. Anal. Behav.* 32 (1979) 233–244.
- [22] P.A. De Villiers, Choice in concurrent schedules and a quantitative formulation of the law of effect, in: W.K. Honig, J.E.R. Staddon (Eds.), *Handbook of Operant Behavior*, Prentice-Hall, Englewood Cliffs, NJ, 1977, pp. 233–287.
- [23] J.W. Donahoe, J.E. Burgos, D.C. Palmer, A selectionist approach to reinforcement, *J. Exp. Anal. Behav.* 60 (1993) 17–40.
- [24] J. Elsner, Testing strategies in behavioral teratology: III. Microanalysis of behavior, *Neurobehav. Toxicol. Teratol.* 8 (1986) 573–584.
- [25] J. Elsner, Tactile-kinesthetic system of rats as an animal model for minimal brain dysfunction, *Arch. Toxicol.* 65 (1991) 465–473.
- [26] J. Elsner, R. Looser, G. Zbinden, Quantitative analysis of rat behavior patterns in a residential maze, *Neurobehavioral Toxicology 1 (Suppl. 1)* (1979) 163–174.
- [27] J. Elsner, B. Hodel, K.E. Suter, D. Oelke, B. Ulbrich, G. Schreiner, V. Cuomo, R. Cagiano, L.E. Rosengren, J.E. Karlsson, Detection limits of different approaches in behavioral teratology, and correlation of effects with neurochemical parameters, *Neurotoxicol. Teratol.* 10 (1988) 155–167.
- [28] L.R. Faro, J.L. Do Nascimento, M. Alfonso, R. Duran, Acute administration of methylmercury changes in vivo dopamine release from rat striatum, *Bull. Environ. Contam. Toxicol.* 60 (1998) 632–638.
- [29] L.R. Faro, J.L. Do Nascimento, M. Alfonso, R. Duran, Mechanism of action of methylmercury on in vivo striatal dopamine release. Possible involvement of dopamine transporter, *Neurochem. Int.* 40 (2002) 455–465.
- [30] L.R. Faro, J.L. Do Nascimento, J.M. San Jose, M. Alfonso, R. Duran, Intrastratial administration of methylmercury increases in vivo dopamine release, *Neurochem. Res.* 25 (2000) 225–229.
- [31] S.G. Gilbert, T.M. Burbacher, D.C. Rice, Effects of in utero methylmercury exposure on a spatial delayed alternation task in monkeys, *Toxicol. Appl. Pharmacol.* 123 (1993) 130–136.
- [32] E.S. Goldey, J.P. O’Callaghan, M.E. Stanton, S. Barone Jr., K.M. Crofton, Developmental neurotoxicity: evaluation of testing procedures with methylazoxymethanol and methylmercury, *Fundam. Appl. Toxicol.* 23 (1994) 447–464.
- [33] P. Grandjean, P. Weihe, R.F. White, F. Debes, Cognitive performance of children prenatally exposed to “safe” levels of methylmercury, *Environ. Res.* 77 (1998) 165–172.
- [34] P. Grandjean, P. Weihe, R.F. White, F. Debes, S. Araki, K. Yokoyama, K. Murata, N. Sorensen, R. Dahl, P.J. Jorgensen, Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury, *Neurotoxicol. Teratol.* 19 (1997) 417–428.
- [35] V.M. Gunderson, K.S. Grant-Webster, T.M. Burbacher, N.K. Mottet, Visual recognition memory deficits in methylmercury-exposed *Macaca fascicularis* infants, *Neurotoxicol. Teratol.* 10 (1988) 373–379.
- [36] V.M. Gunderson, K.S. Grant, T.M. Burbacher, J.F.D. Fagan, N.K. Mottet, The effect of low-level prenatal methylmercury exposure on visual recognition memory in infant crab-eating macaques, *Child Dev.* 57 (1986) 1076–1083.
- [37] I. Hunter, M. Davison, Determination of a behavioral transfer function: white-noise analysis of session-to-session response-ratio dynamics on concurrent VI VI schedules, *J. Exp. Anal. Behav.* 43 (1985) 43–59.
- [38] C.Y. Kim, K. Nakai, Y. Kasanuma, H. Satoh, Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury, *Neurotoxicol. Teratol.* 22 (2000) 397–403.
- [39] Y. Kinjo, H. Higashi, A. Nakano, M. Sakamoto, R. Sakai, Profile of subjective complaints and activities of daily living among current patients with Minamata disease after 3 decades, *Environ. Res.* 63 (1993) 241–251.
- [40] R.E. Kirk, *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole, Belmont, CA, 1968.
- [41] M.J. Marr, Behavioral pharmacology: issues of reductionism and causality, in: J.E. Barrett, T. Thompson, P.B. Dews (Eds.), *Advances in Behavioral Pharmacology*, Erlbaum, Hillsdale, NJ, 1990, pp. 1–12.
- [42] S. Mobini, S. Body, M.Y. Ho, C.M. Bradshaw, E. Szabadi, J.F. Deakin, I.M. Anderson, Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement, *Psychopharmacology* 160 (2002) 290–298.
- [43] J. Myerson, S. Hale, Choice in transition: a comparison of melioration and the kinetic model, *J. Exp. Anal. Behav.* 49 (1988) 291–302.
- [44] M.C. Newland, Motor function and the physical properties of the operant: applications to screening and advanced techniques, in: L.W. Chang, W. Slikker (Eds.), *Neurotoxicology: Approaches and Methods*, Academic Press, San Diego, CA, 1995, pp. 265–299.
- [45] M.C. Newland, S.K. Shapiro, Studying the neurotoxicity of indoor air with nonhuman species: issues in experimental design and interpretation, in: R.B. Gammage, B.A. Bervin (Eds.), *Indoor Air and Human Health*, CRC Lewis, Boca Raton, FL, 1996, pp. 225–248.
- [46] M.C. Newland, P.A. Reile, Learning and behavior change as neurotoxic endpoints, in: H.A. Tilton, J. Harry (Eds.), *Target Organ Series: Neurotoxicology*, Raven Press, New York, 1999, pp. 311–338.
- [47] M.C. Newland, P.A. Reile, Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats, *Toxicol. Sci.* 50 (1999) 106–116.
- [48] M.C. Newland, E.B. Rasmussen, Aging unmasks adverse effects of gestational exposure to methylmercury in rats, *Neurotoxicol. Teratol.* 22 (2000) 819–828.
- [49] M.C. Newland, E.M. Paletz, Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology* 21 (2000) 1003–1027.
- [50] M.C. Newland, S. Yezhou, B. Logdberg, M. Berlin, Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state, *Toxicol. Appl. Pharmacol.* 126 (1994) 6–15.
- [51] M.C. Newland, S. Yezhou, B. Logdberg, M. Berlin, In utero lead exposure in squirrel monkeys: motor effects seen with schedule-controlled behavior, *Neurotoxicol. Teratol.* 18 (1996) 33–40.
- [52] J.R. O’Kusky, E.G. McGeer, Methylmercury-induced movements and postural disorders in developing rat: high-affinity uptake of choline, glutamate, and gamma aminobutyric acid in the cerebral cortex and caudate-putamen, *Neurochemistry* 53 (1989) 999–1006.
- [53] F. Passetti, Y. Chudasama, T.W. Robbins, The frontal cortex of the rat and visual attentional performance: dissociable functions of distinct medial prefrontal subregions, *Cereb. Cortex* 12 (2002) 1254–1268.
- [54] F. Passetti, J.W. Dalley, T.W. Robbins, Double dissociation of serotonergic and dopaminergic mechanisms on attentional performance using a rodent five-choice reaction time task, *Psychopharmacology* 165 (2003) 136–145.
- [55] F. Passetti, L. Levita, T.W. Robbins, Sulpiride alleviates the attentional impairments of rats with medial prefrontal cortex lesions, *Behav. Brain Res.* 138 (2003) 59–69.
- [56] B. Rajanna, M. Hobson, Influence of mercury on uptake of [3H]dopamine and [3H]norepinephrine by rat brain synaptosomes, *Toxicol. Lett.* 27 (1985) 7–14.
- [57] E.B. Rasmussen, M.C. Newland, Developmental exposure to methylmercury alters behavioral sensitivity to *d*-amphetamine and pentobarbital in adult rats, *Neurotoxicol. Teratol.* 23 (2001) 45–55.
- [58] K.R. Reuhl, L.W. Chang, Effects of methylmercury on the development of the nervous system: a review, *Neurotoxicology* 1 (1979) 21–55.
- [59] D.C. Rice, Effects of pre-plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance, *Neurotoxicology* 13 (1992) 443–452.

- [60] D.C. Rice, Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents, *Neurotoxicology* 17 (1996) 139–154.
- [61] D.C. Rice, Evidence for delayed neurotoxicity produced by methylmercury, *Neurotoxicology* 17 (1996) 583–596.
- [62] D.C. Rice, Lack of effect of methylmercury exposure from birth to adulthood on information processing speed in the monkey, *Neurotoxicol. Teratol.* 20 (1998) 275–283.
- [63] D.C. Rice, Age-related increase in auditory impairment in monkeys exposed in utero plus postnatally to methylmercury, *Toxicol. Sci.* 44 (1998) 191–196.
- [64] D.C. Rice, S. Hayward, Effects of postnatal exposure of monkeys to a PCB mixture on concurrent random interval–random interval and progressive ratio performance, *Neurotoxicol. Teratol.* 21 (1999) 47–58.
- [65] D.C. Rice, S. Hayward, Effects of exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on behavior (concurrent random interval–random interval and progressive ratio performance) in rats, *Neurotoxicol. Teratol.* 21 (1999) 679–687.
- [66] N.R. Richardson, A. Gratton, Changes in medial prefrontal cortical dopamine levels associated with response-contingent food reward: an electrochemical study in rat, *J. Neurosci.* 18 (1998) 9130–9138.
- [67] W. Schultz, L. Tremblay, J.R. Hollerman, Reward processing in primate orbitofrontal cortex and basal ganglia, *Cereb. Cortex* 10 (2000) 272–284.
- [68] W. Schultz, L. Tremblay, J.R. Hollerman, Changes in behavior-related neuronal activity in the striatum during learning, *Trends Neurosci.* 26 (2003) 321–328.
- [69] H. Tamashiro, K. Fukutomi, E. Lee, Methylmercury exposure and mortality in Japan: a life table analysis, *Arch. Environ. Health* 42 (1987) 100–107.
- [70] L. Tremblay, W. Schultz, Relative reward preference in primate orbitofrontal cortex, ([comment])*Nature* 398 (1999) 704–708.
- [71] C.V. Vorhees, Behavioral effects of prenatal methylmercury in rats: a parallel trial to the collaborative behavioral teratology study, *Neurobehav. Toxicol. Teratol.* 7 (1985) 717–725.
- [72] M.E. Walton, D.M. Bannerman, M.F. Rushworth, The role of rat medial frontal cortex in effort-based decision making, *J. Neurosci.* 22 (2002) 10996–11003.