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Aging unmasks adverse effects of gestational exposure to methylmercury in rats

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Abstract

The consequences of developmental exposure to methylmercury on behavior in aged animals were investigated. Methylmercury exposure was arranged by placing 0, 0.5 or 6.4 ppm Hg in the drinking water of female rats at least 4 weeks before mating and continuing until postnatal (PN) day 16. Brain Hg concentrations in cohorts of low- and high-dose offspring were 0.5 and 9.1 ppm at birth and 0.04 and 0.52 ppm at weaning (described in another report). Lever pressing of female offspring was maintained under a Multiple Differential Reinforcement of High Rate 9:4 Extinction schedule of food reinforcement (Mult DRH 9:4 EXT). Under the DRH 9:4 schedule, a food reinforcer was delivered when nine responses occurred within 4 s. Under the Extinction schedule, responding had no programmed consequences. No exposure-related differences in reinforcement rate under the DRH schedule or discrimination between the DRH and extinction components were apparent initially. At 950 days of age, the overall response rates of controls had shown a gradual decline over the previous 500 days to about 80% of their beginning levels, but, otherwise, most controls were healthy. A gradual decline in the reinforcement rate began to appear in low- and high-dose rats at about 500 and 800 days of age, respectively. Microanalyses of the nine-response burst maintained by the DRH schedule revealed that the lever-press duration increased, the inter-response time (IRT) was unaffected, and the time between response bursts increased. Overall, the nine-response burst remained intact as a coherent response unit. The increased time between response bursts caused the decline in reinforcement rate. All rats displayed these effects as they aged, but the mercury-exposed rats did so sooner.

The hypothesis of "silent damage" holds that neurotoxicants can act in undetectable ways until another event, such as aging, further challenges nervous system function [38,39]. Difficulties in detecting such damage could be due to the nervous system's extraordinary compensatory ability or because the threshold for neural damage has not been crossed. Delayed neurotoxicity, a necessary element of the hypothesis, is clearly established with methylmercury, especially in sensory–motor domains [4,10,32].

In reports that may have prompted the original hypothesis, mice exposed during gestation to high levels of methylmercury showed progressive deterioration in swimming ability and other motor endpoints as they aged [34–36]. These early reports have been supplemented by more recent studies with nonhuman primates [32]. In these, sensory–motor function [31,32] and higher-order visual function [33] deteriorated more in aging monkeys that had been exposed during development to methylmercury than in controls.

Support for the hypothesis that silent damage becomes apparent with aging is also accruing from long-term studies of individuals exposed to methylmercury. As people exposed to methylmercury during the Minamata tragedy have aged, activities of daily life become increasingly difficult to carry out, as compared with age-matched controls from neighboring fishing villages [13,14,17], even, apparently, in the absence of differences in mortality [37]. Subtle sensory or motor effects have been reported in individuals without full-blown Minamata disease [14]. Among Cree Indians who are otherwise asymptomatic, tremor is enhanced among the older people examined [2]. Hair concentrations of mercury in that study ranged from 15 to 30 ppm, levels that the authors associated with neurotoxicity during early development, not during adulthood.

The epidemiological evidence is ambiguous, however, for a number of reasons. For example, the exposure history

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Table 1
Parameters describing logistic growth from birth to food restriction

	Asymptote (g)		Maximum growth rate (g/day)		Half maximum (days)	
Exposure	M	F	M	F	M	F
Control	389 ± 26.7	246 ± 8.1	0.087 ± 0.0083	0.082 ± 0.0051	42.8 ± 2.6	37.6 ± 1.9
0.5 ppm	398 ± 16.6	240 ± 12.6	0.087 ± 0.0051	0.078 ± 0.0049	43.9 ± 1.3	38.5 ± 2.2
6.4 ppm	404 ± 24.9	255 ± 14	0.082 ± 0.0061	0.081 ± 0.0056	45.2 ± 1.8	37.6 ± 1.3

Numbers are expressed as means ± standard deviation. Each exposure group is represented by five litters.

in these studies is unknown. The people in the Japanese studies had shown frank Minamata disease so the deterioration seen in aging could have resulted either from the earlier, high-level exposure or from continuing low-level exposure throughout adulthood. Animal studies are especially important because they provide the experimental control required to resolve ambiguities inherent in epidemiological studies of environmental pollution and aging [24,25].

The present experiment was designed to examine, in aging rats, effects of methylmercury exposure that took place early in development. The subjects were the offspring of rats exposed to 0.5 or 6.4 ppm of mercury as methylmercury in their drinking water beginning 4 or 7 weeks before mating and continuing to post-natal (PN) day 16 [28]. At birth, the brain mercury levels of cohorts of the rats studied here were 0.5 or 9.1 ppm in the two exposure groups, placing them in the low to moderate range of exposure [4] although weaning these levels had dropped to 0.043 and 0.52 ppm, suggesting little mercury exposure during lactation. As adults, the rats were trained to respond under a Differential Reinforcement of High Rate (DRH) schedule of reinforcement, a procedure said to be sensitive to methylmercury [3,22] and behavior was followed until the rats were over 2 1/2 years old. Gross indicators of health, including growth and long-term survival of these rats and their cohorts were examined for comparison.

1. Methods

1.1. Subjects and exposure

Offspring of female rats exposed throughout gestation to 0, 0.5, or 6.4 ppm of Hg as methylmercuric chloride in their drinking water were used in the experiments reported here. There were a total of 295 live births from 25 litters (nine controls, seven low dose, nine high dose), but not all were used in the present study. All offspring were born within about a 2-week period. Some offspring of these 25 females were used to describe growth; others, mostly littermates, were used to describe behavior under a DRH schedule of reinforcement. Survival data also came from all litters represented. Specific numbers of subjects used are described in the appropriate sections below.

Maternal exposure began 28 days prior to mating for one group and 49 days for a second group. Maternal exposure continued until PN day 16, when pups were able to reach the water spout. Pups were weaned at 21 days of age. Premating exposure duration did not affect brain concentration of mercury in cohorts of the rats described here, so the two groups were pooled [28]. Daily mercury exposure in the 0.5-ppm group was about 40 μg/kg/day at the beginning of gestation and this increased to about 50 μg/kg/day at birth. The corresponding values for the 6.4-ppm groups were 500 and 700 μg/kg/day. At birth, mercury concentrations in the brains of female littermates from the 0.5- and 6.4-ppm groups were 0.5 and 9.1 ppm, respectively. At weaning, mercury concentration in the brain dropped to 0.04 and 0.52 ppm. See Ref. [28] for details about exposure, mercury concentration, and reproductive success.

Offspring were weighed at least weekly beginning at birth until females and males reached 240 and 300 g, respectively, when their food was restricted. Adult males (used in other studies) were maintained at 300–340 g and females at 230–250 g (depending on their age), weights that establish food as a reinforcer and maintain good health for Long–Evans rats. Rats were weighed 5 days/week when in behavioral experiments. Otherwise, they were weighed weekly, and daily feedings were adjusted to maintain their

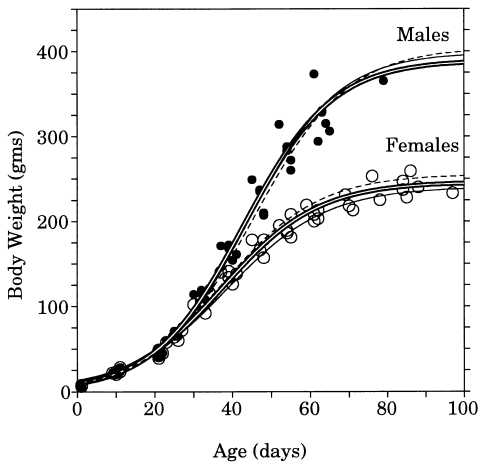

Fig. 1. Growth of male and female rats from birth to food restriction. One rat represents each litter. The curves for males and females were different from one another, but growth did not differ by exposure group. Best-fit lines for controls, 0.5- and 6-ppm groups are represented by thick, thin, and dashed lines, respectively. Points show data from individual control rats; filled points represent males; open circles represent females. The table shows the parameters used to produce the curves.

weights. All data are expressed as a function of a rat's age, which was accurate to 12 h. All deaths were recorded within 12 h. All animals were maintained under conditions meeting Public Health Service guidelines.

1.2. Growth rate

Growth-curve analyses were conducted using one male and one female pup from each of 15 litters (five/dose-group) surviving to food restriction. Rats used for the growth-curve analyses were used in experiments not reported here, but some were littermates of the ones used here. A growth curve was determined for each pup. Pup weights from PN days 1 and 10 were used, although sometimes only PN day 9 or 11 were available and these were used, with the age entered appropriately. Weights on PN days 1 and 10 were determined by weighing the litter and dividing by the number of pups. Individual weights were determined at weaning and subsequently at weekly intervals until food restriction commenced. A growth curve was estimated for each rat using linear, exponential ($y = a + b(1 - \exp(cx))$, and logistic (Eq. (1)) regression models, but the linear and exponential models were inadequate to fit the slow growth during lactation and the growth spurt after weaning. The logistic model handled each quite well; all R^2 were greater than .994 for every rat, and no systematic deviations from the best-fit line were noted.

$$\mathrm{BW} = \frac{\mathrm{BW}_{\max}}{1 + e^{(\mathrm{RATE}_{\max}(\mathrm{AGE}_{\mathrm{half}} - \mathrm{AGE}))}} \quad (1)$$

where BW = body weight in grams, $\mathrm{BW}_{\max}$ = upper asymptote of body weight in grams, $\mathrm{RATE}_{\max}$ = maximum growth rate in grams/day, and $\mathrm{AGE}_{\mathrm{half}}$ = age in days at which the rat reached its half-maximal body weight.

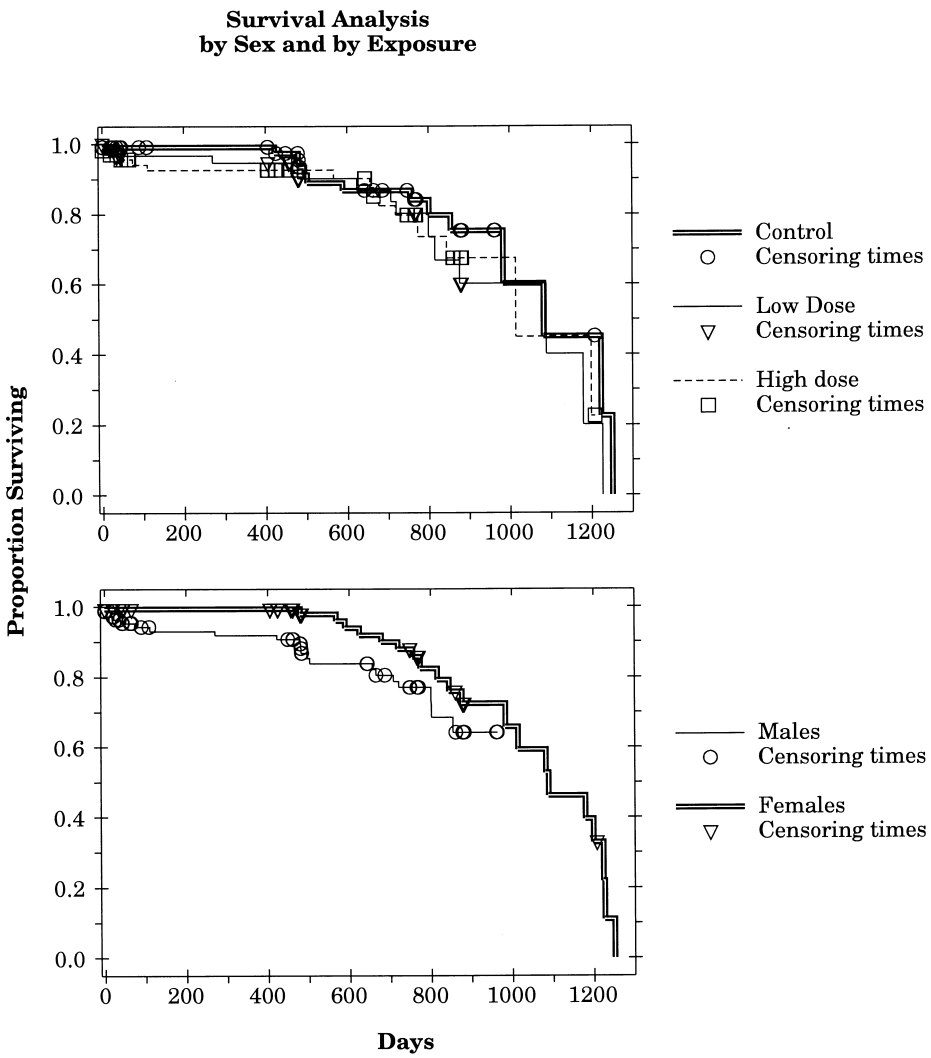

Fig. 2. Survival functions showing the probability of having survived the number of days shown on the horizontal axis for the three exposure groups (top) and for males and females (bottom). Open circles show censoring times. Cox regression on these data revealed an effect of sex ($p = .05$): Females survived longer than males. No effect of exposure was identified under any of three analyses: Exposure entered as a covariate with sex, stratified under sex, or with exposure listed as a single variable, i.e., the two exposure groups combined into a single exposure group (all p's greater than .1).

1.3. Survival

A Kaplan–Meier survival estimate, representing the probability of surviving at least t days [21], was computed for each natural death. This represents the probability of surviving at least t days and is determined by dividing the number of natural deaths by the number of animals eligible to be found dead in a group. Deaths were recorded as natural if the rat was found (1) dead, (2) moribund and therefore euthanized, or (3) missing from the litter and presumed eaten by the dam. Deaths were categorized as "censored" and subtracted from the general census if they were culled or killed at the end of an experiment but were otherwise healthy. Of 295 live births (representing nine control, seven low-dose, and nine high-dose litters), there were 43 natural deaths over the course of the study. Because the rats in the behavioral study on aging were females, more data were available on their long-term survival.

1.4. Behavior

Behavioral experiments commenced when the rats were about 120 days old and ended when the survivors were more than 900 days of age (2.5 years). One female offspring from each of 16 litters (six, five, and five from the control, low-, and high-mercury litters, respectively) was selected.

Sessions were conducted in conventional experimental chambers. Each chamber was enclosed in a sound-attenuating cubicle and equipped with two response paddles ($2.5 \times 2.5 \times 0.16$ cm) situated 5 cm from the bottom of a grid floor and 14.5 cm apart. Food pellets (45 mg Noyes pellets) were delivered through a 3.8×3.8 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 collections were accomplished with 0.01 s resolution using a DEC PDP 11/73 computer running SKED11 software (State Systems) located in an adjacent room. Sessions began at 1500 ± 0.5 on Monday through Friday.

The rats were trained to lever-press by placing them in a chamber in which a Multiple Fixed-Ratio 1 Extinction (Mult FR 1 EXT) schedule of food reinforcement was operating. During the FR 1 component, signaled by house light illumination, each lever-press resulted in pellet delivery. During EXT, signaled by extinguishing the house light, lever-pressing had no programmed consequences. Component length was 5 min and the session lasted 10 h. All rats produced at least 150 responses during this training session.

The different DRH schedules were introduced next. First, a Mult DRH 3:1 EXT schedule of reinforcement was imposed, in which three responses within 1 s resulted in pellet delivery. The reinforcement schedule increased to a Mult DRH 5:2 EXT and finally to a Mult DRH 9:4 EXT schedule, in which nine responses within 4 s was required for pellet delivery. During the DRH component, the house light was on. During the EXT component, all lights were off and no consequences were programmed to occur for pressing either lever. Schedule components alternated every 5 min and sessions lasted 30 min. The main dependent measure reported is reinforcement rate because it describes how rapidly and how effectively responding occurred. A measure of response efficiency was also examined. Efficiency is 100% (nR/r) where r = number of responses produced, R = number of reinforcers earned, and n is the number of responses required for

Table 2
Response characteristics for individual rats at baseline and toward the end of the study

		Baseline		At point of decline in reinforcer rate[a]		
Rat	Exposure	Rate[b]	Efficiency	Rate[b]	Efficiency	Comments
104	0	4.8	76	2.5	79	Last day of study
116	0	5.2	70	0.7	66	Abrupt decline at end of study
128	0	6.7	71	5.1	77	Last day of study
140	0	6.1	91	2.2	78	End of a long, slow decline
166	0	6.5	66	4.9	72	Last day of study
194	0	4.0	77	0.6	83	Last day of study
219	0.5	5.0	86	3.8	84	Abrupt decline
228	0.5	4.1	57	0.7	89	End of a long, slow decline
230	0.5	6.6	84	2.9	86	Last day of study
259	0.5	5.6	76	1.7	74	End of a long, slow decline
293	0.5	4.7	71	2.8	77	Last day of study
308	6.4	5.4	75	2.2	76	Decline, recovery, then abrupt decline
332	6.4	5.4	74	2.9	74	Abrupt decline
362	6.4	5.2	78	2.1	79	Long, slow decline
386	6.4	7.3	77	4.7	74	Abrupt decline
394	6.4	6.2	75	6.6	76	Abrupt decline

[a] Efficiency and reinforcer rate at the end of the experiment, in the midst of an abrupt decline (spanning only a few days) or toward the end of a long decline and after reinforcer rate fell below 50% for at least 5 successive days.

[b] Reinforcer rate in reinforcers/min.

a reinforcer (i.e., nine in a DRH 9:4 schedule). Note that 900 responses for 100 reinforcers would produce 100% efficiency under a DRH 9:4 schedule. Individual lever-press durations (the time that the lever remained depressed sufficiently far to close a contact) and inter-response times (IRTs, times between lever-presses, exclusive of lever-press durations) were stored and used for later analyses of more molecular aspects of responding. Pharmacological challenges (not reported here, see Ref. [30]) were conducted up to about 700 days of age, but only non-injected control days are shown here. During this period, acute injections of amphetamine, scopolamine, pentobarbital, haloperidol, and dizocilpine were administered in that order.

2. Results

2.1. Growth analyses

For the logistic model, the standard error of the estimate of the upper asymptote (BW_{max}) and age at which the rat was 1/2 the maximum weight (AGE_{half}) were less than 5.5% of the estimate. The standard error for maximum growth rate ($RATE_{max}$) was less than 12% of the estimate.

Individual parameters, describing different characteristics of growth, were analyzed as dependent measures in a repeated-measures ANOVA. Since the litter represented the statistical unit, i.e., treated as a subject, sex was treated as a repeated measure within the unit. Table 1 shows means

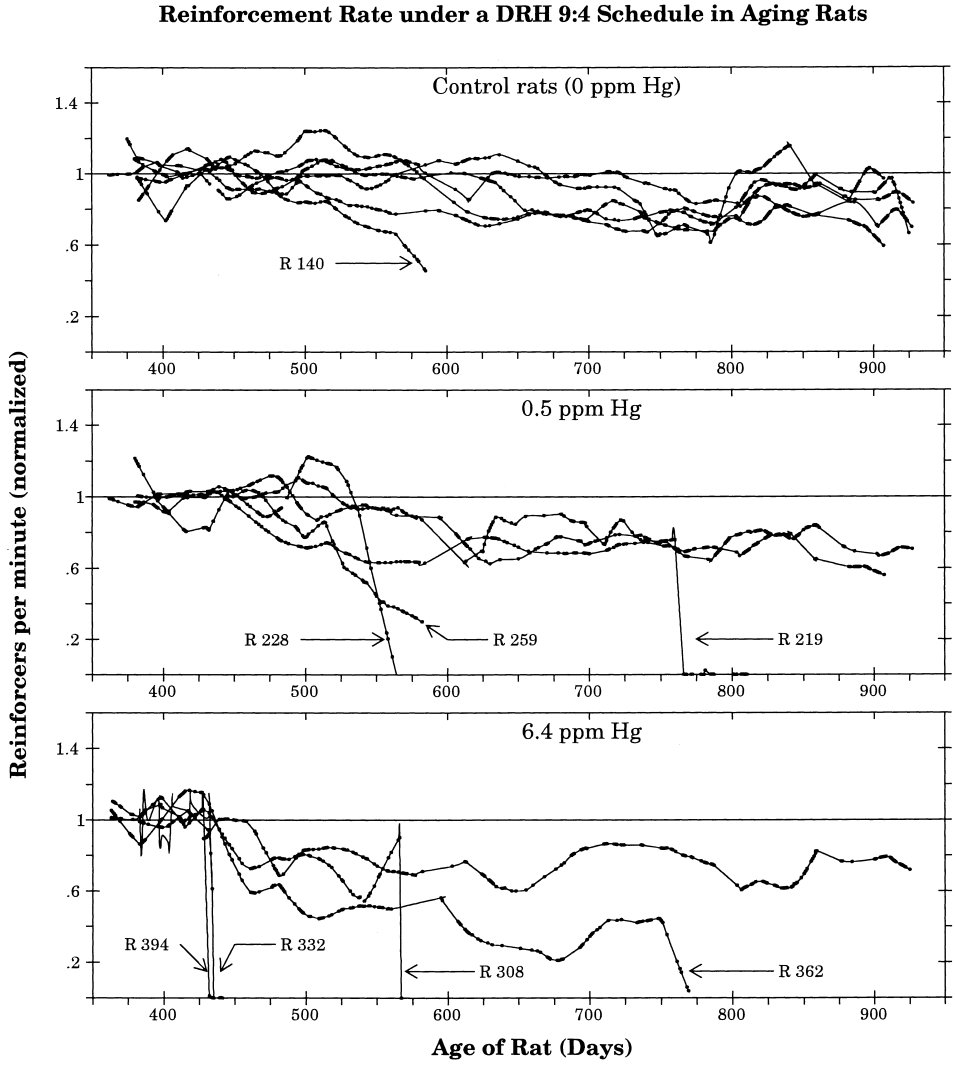

Fig. 3. Age-related changes in the number of reinforcers delivered were determined for each rat by comparing behavior during a session against the rat's baseline performance, defined as 10 consecutive sessions with no systematic change in behavior after the multiple DRH 9:4 EXT schedule was imposed. All data are expressed as a proportion of the baseline determined for each animal at the beginning of the experiment. Each line represents a separate rat. Baseline rates for the control, 0.5- and 6.4-ppm groups were 5.5 ± 1.1, 5.2 ± 0.96, and 5.8 ± 0.9 reinforcers/min (means ± 1 S.D.). The abscissa shows the age of the rats in days. The lines were obtained by smoothing data from individual sessions using a LOWESS algorithm [5,15].

Table 3
Events surrounding the decline in reinforcement rate

Rat	Group[a]	Age[b]	Events preceding decline	Subsequent events
140	0	570 (1.6)	10 mg/kg haloperidol 6 days earlier	Died at 589 days old
219	0.5	757 (2.1)	No discernable events. Abrupt decline in responding	Died at 810 days old
228	0.5	552 (1.5)	0.1 mg/kg haloperidol 1 day before	Schedule reduced to DRH 3:1, then 5:1 then back to 9:4 after 2 months
259	0.5	543 (1.5)	Gradual decline began 12 days after last amphetamine dose and continued through subsequent dose–effect determinations	Died at 625 days. Tumor on face
308	6.4	566 (1.6)	0.1 mg/kg scopolamine 4 days previously	Died abruptly at 567 days
332	6.4	434 (1.2)	No discernable event	Schedule changed to DRH 5:2 for three days, then DRH 9:4 for a month until performance declined again and schedule value changed to DRH 3:1 and gradually increased to DRH 9:4
362	6.4	495 (1.4)	Gradual decline, no discernable event	Died at 775 days
394	6.4	432 (1.2)	0.3 mg/kg d-amphetamine 3 days before	Behavior re-established on DRH 2:1. Increased to 4:2, and then after 5 months, to 8:4

[a] Shown as ppm of Hg in maternal drinking water.
[b] Age in days (years) at which reinforcement rate declined to 50% of baseline rate.

of the parameters obtained by fitting Eq. (1) to the different groups. Fig. 1 shows the average growth rates for the different groups. There was a main effect of sex on the estimate of the upper asymptote of body weight: Males were heavier than females (BW_{max} = 389 and 246 g, respectively; $F_{1,12} = 578$, $p < .0001$). There was no effect of dose ($F_{2,12} = 1.228, p = .33$) and no interaction between dose and sex ($F_{2,12} = 0.51, p = .61$) on this measure. There was a main effect of sex on the day that the rats reached half of their estimated maximum body weight (AGE_{half} = 43 and 38 days; $F_{1,12} = 138, p < .001$). No effect of dose ($F_{1,12} = 0.54$, $p = .60$) and no interaction between dose and sex ($F_{2,12} = 2.56$, $p = .12$) were noted on this measure. There was a marginal effect of sex on the maximal growth rate obtained ($RATE_{max}$ = 0.087 and 0.082 g/day; $F_{1,12} = 4.118$, $p = .065$): Males grew slightly faster than females. There was no effect of dose ($F_{2,12} = 0.733$, $p = .50$) and no sex × dose interaction ($F_{2,12} = 2.29$, $p = .14$) on this measure.

2.2. Survival

Survival was examined as a function of sex and mercury exposure (Fig. 2) Cox regression on these data revealed an effect of sex ($p = .05$); the half-lives for males and females were 1000 and 1100 days, respectively. No effect of mercury exposure was identified with any of three analyses: mercury as a covariate with sex, stratified under sex, or with both exposure groups collapsed into a single group (all p's greater than .1).

2.3. Behavior

Baseline reinforcement rates and efficiency are shown in the left columns of Table 2. The right columns are discussed below. There was no effect of methylmercury exposure on either measure (all p's > .1). Five of six control animals beginning behavioral testing were still responding at 900 days of age (Fig. 3). Among these, there was a reduction in overall reinforcement rates to about 80% of baseline. Of the 10 methylmercury exposed animals, two in the 0.5-ppm group and one in the 6.4-ppm group completed the experiment, and their reinforcement rates resembled those of controls. Two in the 0.5-ppm group (R 228 and R 259) and one in the 6.4-ppm group (R 362) showed declines in overall reinforcement rate that took place over the course of one to two weeks or more. One rat in the 6.4-ppm group (R 308) died abruptly. One rat in the 0.5-ppm group (R 219) and two in the 6.4-ppm group (R 332 and R 394) showed a relatively rapid cessation of responding.

Table 3 shows the events associated with the reinforcer rate declines illustrated in Fig. 3. While some of the declines took place as pharmacological challenges were taking place, there is no consistent evidence linking the behavioral deficits to events during this phase of the experiment. Deficits were not associated with any particular drug, nor were they closely linked in time to drug administration. In one case, death occurred abruptly (R 308), but in others, death followed performance declines by 19, 53, 82, and 280 days. In three cases, responding dropped such that no criterion response sequences, i.e., no reinforcers, were produced, but otherwise the rats appeared healthy. These animals were re-trained and eventually responding was re-established under the Mult DRH 9:4 EXT schedule (data not shown).

The age at which reinforcement rate declined to 50% of baseline for at least 5 successive days was determined for each rat (Fig. 4). The age on the last day of the experiment (900–930 days) was entered for the rats completing the experiment. A weighted, robust one-way ANOVA was conducted on the data shown in Fig. 2. Weighing (1.6,0.35,0.93 for control, 0.5 and 6.4 ppm groups, respectively) gave more weight to the control group, for which the data were more tightly clustered, and less weight to the 0.5 ppm group, in which the most variability was observed. The analysis was based on med-

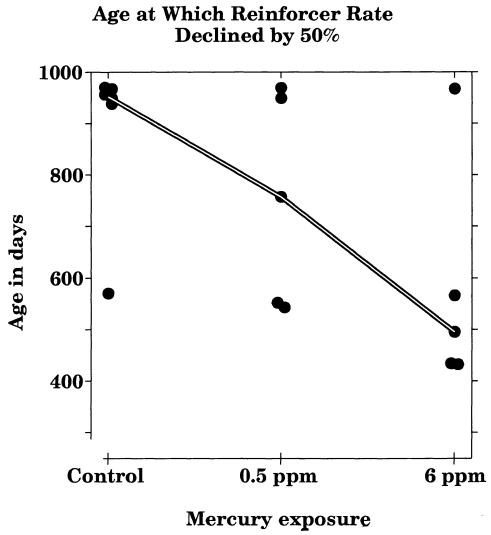

Fig. 4. The age at which the reinforcers earned fell below 50% of the baseline for at least five consecutive sessions. The age at the last day of the experiment (900–980 days) was entered for animals completing the experiment with reinforcement rates greater than 50% of baseline. Each point represents a single rat. The line connects the medians for each exposure group.

ians by scaling them by the inter-quartile range/$(1.075\sqrt{n})$, which has approximately a t distribution and can be extended to approximate an F distribution [1,7,15]. There was a main effect of concentration ($F_{2,13}$) = 18.45, p=.0002. Post hoc comparisons revealed that controls and 6.4-ppm groups differed from one another (p=.0002), but the 0.5-ppm group could not be distinguished, statistically, from either the controls or the 6.4-ppm-exposed animals.

The right columns of Table 2 show two performance measures at the end of the experiment or as reinforcer rate declined to less than 50% of control levels. The efficiency measure of performance showed little change, even in the face of rather large declines in reinforcement rates. Efficiency is a measure of the extent to which the nine-response sequence remains intact. In order to determine more directly how reinforcer rates declined while efficiency did not, the behavioral topography was dissected into three parameters of responding: IRT (criterion and non-criterion) and response duration. Under the DRH 9:4 schedule, nine responses must occur within 4 s. In a nine-response burst, there are eight IRTs, so the average time between the onset of one response and the onset of the next response must be 0.5 s (Fig. 5). This time was further broken down into a lever-press duration, the amount of time that the lever was held down, and the true IRT, which is the time between the end of one response and the beginning of the next. "Post-reinforcer pause" time, the time between completion of a reinforced nine-response burst and the beginning of the next one were not included in IRT determinations.

Reinforcement-rate declines could result from difficulty in fulfilling the high-rate criterion of nine responses in 4 s. If so, the integrity of the nine-response sequence would disintegrate. Alternatively, the response unit could remain intact, but occur less frequently. We examined this issue directly by analyzing three additional parameters of responding (Fig. 5), which were statistically analyzed with repeated-measures ANOVA (age × exposure) and are shown in Fig. 6.

Lever-press duration increased with age ($F_{1,13}$ = 7.4, p=.04), but not with mercury exposure ($F_{2,13}$ = 0.09, p=.91), and there was no interaction ($F_{2,13}$ = 0.745, p = .49). The average of criterion IRTs (those $\leq$0.38 s) did not change with age ($F_{1,13}$ = 0.29, p = 0.60) or mercury exposure ($F_{2,13}$ = 0.54 p=.59), and there was no interaction ($F_{2,13}$ = 0.238, p=.79). The average of non-criterion (those >0.38 s) IRTs did show an effect of age ($F_{1,13}$ = 12.7,

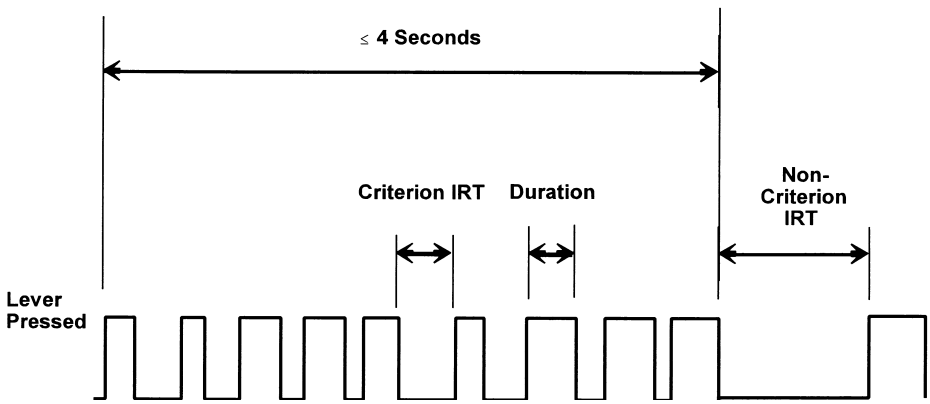

Fig. 5. A nine-response sequence can be divided into criterion IRTs, non-criterion IRTs, and lever-press durations. Under the DRH 9:4 schedule, nine responses must occur within 4 s, a requirement that generates response sequences averaging 0.5 s between the onset of one response and the onset of the next response. This time was further broken down into a lever-press duration, the amount of time that the lever was held down, and the IRT, which is the time between the end of one response and the beginning of the next. To perform this analysis, every lever-press duration and IRT was recorded with 0.01-s resolution. The nine-response sequence shown meets the 4-s criterion, and the non-criterion IRT lies between the burst illustrated here and the beginning of the next sequence.

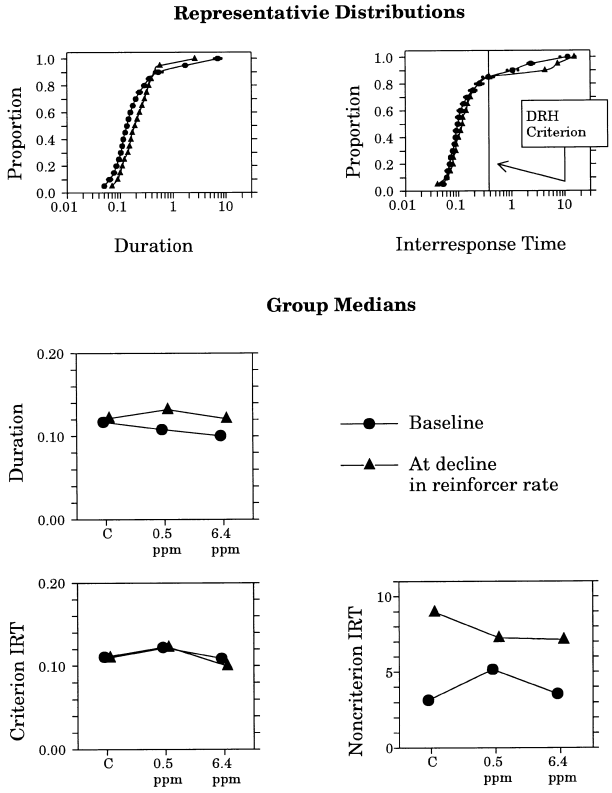

Fig. 6. The top panels show a cumulative distribution of lever-press durations (top left) and IRTs (top right) for a control rat (R 128) (averaged over 10 sessions; 95% CIs are shown as small points, but in most cases, they are obscured by the data point). The median lever-press duration increased from 0.13 s during baseline conditions to 0.19 s at the end of the experiment for this representative animal. The IRT was further analyzed into criterion and non-criterion IRTs, indicated by the vertical line in the top right panel located at 0.38 s; if all IRTs were 0.38 s and durations were 0.12 s (the overall median), then the nine-response burst would just meet criterion. Note that the portion of the distribution left of the line is much sharper than that on the right, empirical support for distinguishing the IRT distribution this way. The middle panel shows median lever-press durations across control groups during baseline sessions and under a condition called "aged" taken from the session at which reinforcement rates were close to 50% of baseline, or the last day of testing. The bottom panels show similar curves for criterion and non-criterion IRTs.

$p = .004$), but not mercury exposure ($F_{2,13} = 0.26$ $p = .77$), and there was no interaction ($F_{2,13} = 1.022$, $p = .39$).

3. Discussion

The present experiment supports and extends evidence that, with aging, the organism's behavior may reveal consequences of methylmercury exposure that had taken place early in development. There was no evidence of developmental toxicity in either exposure group based on birth weights or developmental landmarks, although the dams exposed to 0.5 ppm for 7 weeks might have experienced reproductive difficulties [28]. A detailed analysis of weight gain of all pups, revealed no mercury-related effects on this measure of growth. An analysis of mortality also revealed no mercury-related differences on survival. Mercury-exposed rats were more sensitive to amphetamine's disruption of behavior under the DRH 9:4 schedule, and less sensitive to pentobarbital's [30], but apart from these drug challenges, there was no evidence of mercury-related differences in behavior under this schedule until these animals aged.

The primary effect of developmental methylmercury exposure was a decline in overall reinforcement rate under the DRH 9:4 schedule of reinforcement. This effect was especially apparent in the time course of overall reinforcement-rate decreases, which was quite different for exposed and unexposed rats. With the DRH schedule of reinforcement, reinforcement rate is a measure of the animal's success in meeting the requirements of the DRH schedule. The progressive declines in reinforcement rate could be a reflection of a positive feedback that exists between responding and reinforcement rate under this unforgiving schedule. Even a mild reduction in the rate at which nine-response bursts occur or in the ability to produce such a burst would decrease the reinforcement rate. Such a reduction could, in turn, cause a further decline in response rate, and so on in a downward spiral. It is noteworthy, in light of this possibility, that in several cases behavior was re-established by placing the rat under a less demanding DRH schedule and thereby increasing the reinforcement density, a sort of behavior therapy (for a related application, see Ref. [29]).

It would be interesting if the reinforcement-rate decline had been triggered by specific drug challenges, but no clear connection could be detected between particular events and the decline in reinforcement rates. Sometimes, declines followed a drug injection, but no specific drug or dose preceded reinforcement-rate declines consistently. At other times, no discernable event preceded the rate decline. In one case, there was an abrupt death (R 308 from the 6.4-ppm group), but the typical case entailed a progressive drop in reinforcement rates over the course of many days to weeks. Only some cases eventually ended in the death of the rat. Since there had been no other signs, these reinforcement-rate declines could be viewed as early signs of failing health in those cases where preceded death.

A decline in reinforcement rate under this schedule could result from one of two broad classes of causes. First, it could result from a disruption of the nine-response burst of lever-presses reinforced by the DRH contingency. Alternatively, the nine-response burst could remain intact but the time between bursts could increase. In the former case, the integrity of the response unit would disintegrate and the decline in reinforcement rate might be accompanied by a decline in efficiency, a measure of the percent of responses that were part of the criterion nine-response sequence. With the exception of one rat (R 228), response efficiency exceeded 70% and went as high as 90%. These levels remained high even as reinforcement rates dropped by more than 50%.

The analysis of efficiency was supplemented with a fine-grained analysis of IRTs and lever-press durations that form the response burst. These molecular analyses were conducted for the session at which reinforcer rate was about 50% of baseline rate or, for controls, at the end of the experiment. The analyses were conducted to determine what element of the nine-response sequence contributed to the declining reinforcer rates. Both lever-press duration and non-criterion IRT increased over baseline. The increase in lever-press duration was small (about 0.02 s or about 20%) and may have lengthened the nine-response burst by 0.18 s (9 responses × 0.02 s/response).

The larger increase in non-criterion IRT is more likely to be the cause of the declining rates. There are two ways in which the non-criterion IRT could increase. First, the number of responses constituting response bursts could have decreased, leaving more long breaks between these bursts. This would have decreased efficiency since it would have increased the denominator of the equation describing efficiency. Second, the number of responses constituting bursts could have remained the same, but the recovery time between bursts could have increased. This would not change efficiency. Since efficiency was unaffected, it appears that the latter pattern occurred. The recovery time between bursts appears to have lengthened, but is not clear why. It could reflect a form of fatigue, after attempting a nine-response burst. It could reflect checking the feeder to determine if a pellet has been delivered. Nevertheless, the response burst, as indicated by the short IRTs, remained intact, and the major contributor to the decline in reinforcement rate was the time between response bursts, reflecting increased pausing between bursts of responses. The response bursts appeared to function as intact-response units.

Complex response units, including the nine-response burst here, match-to-sample problems [27] or behavior under a schedule of reinforcement [12,19,20,26], have been brought under schedule control and evaluated using behaviorally active drugs. In some cases, even these extended response units remain intact even as overall response rates are altered by drugs [27]. This seems to be the case in the present study. The response unit here is a sequence of lever-presses separated by IRTs averaging less than 1/2 s. (Note that this 1/2 s is not a direct criterion, but represents the average IRT that would meet the schedule requirement given the lever-press duration obtained.) Thus, under the conditions prevailing here, the response sequence had characteristics of being a discrete, extended response unit. The behavioral disruption was not an impairment in the simple up-and-down motion representing a lever-press, but rather a decline in the rate at which this relatively effortful response burst occurred. The advantage of using this multi-response DRH unit is that the integrity of the unit can be evaluated in its own right by looking at the degree to which the burst remains intact even as overall reinforcement rate decreases.

The behavioral disruption occurred in the absence of changes in mortality; survival rate was not influenced by exposure. Although the analyses supporting this conclusion were performed with the pup, not the litter, as the statistical unit, other evidence supports the veracity of this conclusion. Deaths in the rats from the DRH study, for which there was only one female pup/litter, were distributed across the exposure groups. The other adult deaths came from experiments in which the litter was the statistical unit, hence, there was no significant contribution by any particular litter to the overall mortality of adults. Finally, the analysis detected an expected effect of gender on mortality; females outlived males.

There are different ways by which neurotoxicant-induced deficits could interact with age. Cumulative damage could eventually reach a detectable level or latent damage may be unmasked only as age-related impairments further challenge function [13,38]. The present results suggest the latter possibility because exposure occurred only during gestation and the exposed and control animals were indistinguishable on all earlier measures taken. Moreover, morbidity was not clearly linked to mortality as no differences in survival were noted across groups, similar to a report on Minamata patients [37]. In another report, but using the same animals, animals exposed to methylmercury were more sensitive to acute administration of amphetamine or pentobarbital [30], again suggesting the presence of latent effects uncovered by another event.

The US Environmental Protection Agency has set the methylmercury Reference Dose (RfD: a level of intake considered to be without appreciable risk of adverse effect) for pregnant women to be 0.1 μg/kg/day ± 1 order of magnitude. This RfD, which has raised considerable debate [6,8,9,16] was based on effects seen in Iraqi children after *in utero* exposure to a relatively high dose. and young animals after maternal mercury exposure. The US Agency for Toxic Substances and Disease Registry established a Minimal Risk Level (MRL) of 0.3 μg/kg/day, based in large part on a study of children exposed *in utero* to chronic, low-level exposure in fish-eating populations. Pregnant rats in the low-dose group consumed an average of 40–50 μg/kg/day of mercury, a level slightly more than 2 1/2 orders of magnitude above the RfD. The exposure levels required to elevate mercury in the rat brain are about 10-fold greater than those required for primates, including humans, because mercury binds avidly to red blood cells, which are especially dense in rat blood [18,28]. Mercury concentrations were chosen to surmount this binding. After accounting for the fact that rats must achieve mercury intakes 10 times higher than primates to achieve the same brain levels, the effects seen in the aged rats place the our Lowest Observed Adverse Effect Level (LOAEL) about 1.5 orders of magnitude above the current RfD for mercury and about one order of magnitude above the MRL, uncomfortably close by risk assessment standards [11,23].

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