

Developmental exposure to methylmercury alters behavioral sensitivity to d-amphetamine and pentobarbital in adult rats

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Abstract

Female rats were exposed to 0, 0.5, or 6.4 ppm methylmercury in their drinking water before mating, and throughout gestation and lactation. When the female offspring were 4–6 months old, they were trained to respond under a multiple differential reinforcement of high rate (DRH) 9:4 — Extinction schedule of reinforcement. No differences among exposure groups were apparent in steady-state behavior. Drug challenges were conducted with multiple doses of d-amphetamine, scopolamine, pentobarbital, haloperidol, and dizocilpine, drugs selected for their different pharmacological effects. The ED₅₀ values for amphetamine's reinforcement rate-reducing effects for the control, 0.5-, and 6.4-ppm groups were 3.1, 1.9, and 0.9 mg amphetamine/kg body weight, respectively, demonstrating an increased sensitivity to d-amphetamine in methylmercury-exposed rats. Rats in the 6.4-ppm group also demonstrated a relative insensitivity to pentobarbital. Further, these exposed rats exhibited an inverted U-shaped dose–effect curve under the pentobarbital dose–effect determination, while controls showed only a declining curve. Exposed rats did not respond differentially to haloperidol, scopolamine, or dizocilpine, suggesting specificity. The present data suggest an involvement of catecholaminergic and GABAergic activity in methylmercury's neurotoxicity. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Methylmercury; Developmental exposure; Catecholamine sensitivity; GABA sensitivity

1. Introduction

Pharmacological challenges are useful in uncovering subtle toxicant-induced behavioral effects and revealing potential neurochemical mechanisms of action [1,38]. Under this preparation, drugs with well-established neurochemical effects are administered to toxicant-exposed and unexposed animals, and differences in drug sensitivity are examined. A single drug alone, and certainly a single dose, is insufficient for characterization because such an approach cannot reveal whether the effect is specific to the drug examined or represents a general change in sensitivity to any behaviorally active agent. Instead, a full dose–effect profile using drugs specific to several neurochemical substrates is necessary to reveal pharmacological specificity and dose-related effects. Such a design can uncover much about the behavioral relevance of putative neurochemical substrates of neurotoxicity [1,38].

Acute administration of d-amphetamine has revealed methylmercury-related effects on endpoints such as locomotor activity [10], acquisition of a differential reinforcement of a low rate (DRL) schedule [16], and acquisition and rates of lever pressing [20]. These effects were not evident in the absence of the drug challenge. Since d-amphetamine promotes the release of dopamine and norepinephrine [6], the reports implicate involvement of the catecholamine systems in methylmercury's effects. Another report, using a single dose of apomorphine [12], supports these suggestions. Most of these studies, however, relied on only a single drug or, sometimes, only a single dose. It is not clear whether methylmercury's effects were specific to dopamine or norepinephrine systems, and whether the effects were related to the dose of d-amphetamine. A generic sensitivity to any behaviorally active drug could exist.

In the present study, female rats were exposed to 0, 0.5, or 6.4 ppm of methylmercury (MeHg) in their water before, during, and after gestation resulting in exposure of about 0, 40, and 500 µg/kg/day, respectively, during gestation. The offspring were trained as adults to perform under a differential reinforcement of high rate (DRH) schedule. The

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behavior of animals exposed to methylmercury did not differ from controls in acquisition or performance. In an attempt to uncover possible silent effects, haloperidol, d-amphetamine, pentobarbital, scopolamine, and dizocilpine challenges were administered acutely. These drugs were chosen because they act through different neurotransmitter systems and could indicate specificity.

2. Method

2.1. Subjects and exposure

In this study, six, six, and five rats, each representing a litter, were exposed prenatally to one of three conditions: control, 0.5, and 6.4 ppm MeHg. Female Long–Evans rats purchased from Harlan Sprague–Dawley were bred and randomly assigned to one of three conditions: 0, 0.5, or 6.4 ppm of mercury as methylmercuric chloride, dissolved in their only source of drinking water. Exposure began at least 4 weeks prior to mating with an unexposed male, and continued throughout gestation and lactation. Exposure terminated on postnatal day 16, when the pups were able to drink from the waterspout. The only source of mercury for the pups, then, was via the mother during gestation or lactation, although lactational exposure was insignificant (see Ref. [26]).

Water bottles were weighed daily to monitor intake. Mercury concentrations were determined by atomic absorption spectrophotometry at 4-week intervals. Throughout exposure, mercury concentrations remained within 3% of the mean level. For the 0.5-ppm group, daily exposure ranged from about 40 $\mu\text{g}/\text{kg}/\text{day}$ before gestation to 50 $\mu\text{g}/\text{kg}/\text{day}$ before birth. Corresponding values for dams in the 6.4-ppm group were 500 and 700 $\mu\text{g}/\text{kg}/\text{day}$. Concentrations of mercury in the pup brain were determined by atomic absorption spectrophotometry. Values for the 0.5- and 6.4-ppm groups were 0.5 and 9.5 ppm, respectively at birth and 0.04 and 0.53 ppm at weaning. Methylmercury exposure did not affect food or water consumption, or weight gain (see Ref. [26]).

All rats were housed individually in wire cages which were hung over corn-cob bedding in a room maintained on a 12-h light–dark cycle (lights on at 7:30 a.m.). All conditions met US PHS and USDA standards. The experiment was approved by the Auburn University Animal Care and Use Committee.

2.2. Apparatus

Eight Lehigh Valley Electronics experimental chambers were used, each equipped with two levers on the front panel, a grid floor, a 28-V houselight located above the food dispenser, and white noise generated from a speaker placed above the food dispenser. Each chamber was enclosed in a sound-attenuating cubicle. Reinforcement contingencies

were controlled and data collection was achieved with 0.01 s resolution by SKED-11 software on DEC PDP 11/73 computer, all located in a room separate from the chambers.

2.3. Procedure

At 4 to 6 months, 34 female rats (representing 17 litters from the three exposure groups) were trained to respond under a multiple DRH $n:t$ extinction schedule of reinforcement. The rats in the present study were selected from this group. Initially, each rat was placed inside a chamber for one 10-h session. A fixed ratio-1 (FR1) schedule of reinforcement, in which one lever press on the right lever would produce a food reinforcer, was placed in effect. When 100 responses occurred, the schedule was then changed to a multiple DRH $n:t$ EXT schedules of reinforcement. Under the differential reinforcement of high rate (DRH $n:t$) component (signaled by houselight illumination), n lever presses emitted within t seconds produced a 45-mg food pellet, a reinforcement contingency that selects high-rate “bursts” of responses. In this schedule, *each* response initiated a separate timer and the next n responses were timed to ensure that they occurred within t seconds. If so, then a reinforcer was delivered. Thus, every n -response burst was timed, making this implementation a free operant and not a discrete-trials procedure. Under the extinction (EXT) component (signaled by no illumination), there were no programmed consequences. These components alternated every 5 min for 30 min. After the FR1 training schedule, rats’ behavior was placed under mult DRH 3:1 EXT (under DRH, three responses in 1 s produced a food pellet) until stable responding developed. Behavior was then placed under mult DRH 5:2 EXT until stable responding occurred. Finally, a mult DRH 9:4 schedule, in which nine responses in 4 s produced a food pellet, was imposed. Sessions were conducted in the late afternoon at the same time (± 30 min) from Monday to Friday.

There were no exposure-related differences in acquisition of behavior.

2.4. Drug challenges

Healthy rats (six, six, and five, each representing litters from the control, 0.5, and 6.4 ppm groups, respectively) that were responding robustly were selected from the acquisition study. The determination of acute dose–effect curves commenced when the rats were between 13 and 15 months of age, and after behavior stabilized under the multiple DRH 9:4 EXT schedule; that is, no systematic change in reinforcement rate, response rate, and the number of unreinforced responses occurred over 10 consecutive sessions. Drugs were administered acutely in the following order: d-amphetamine (0.3–10 mg/kg), scopolamine (0.1–3 mg/kg), pentobarbital (1–20 mg/kg), haloperidol (0.03–1 mg/kg), and dizocilpine (0.01–0.3 mg/kg). Scopolamine, d-amphetamine, and pentobarbital were dissolved in saline and admi-

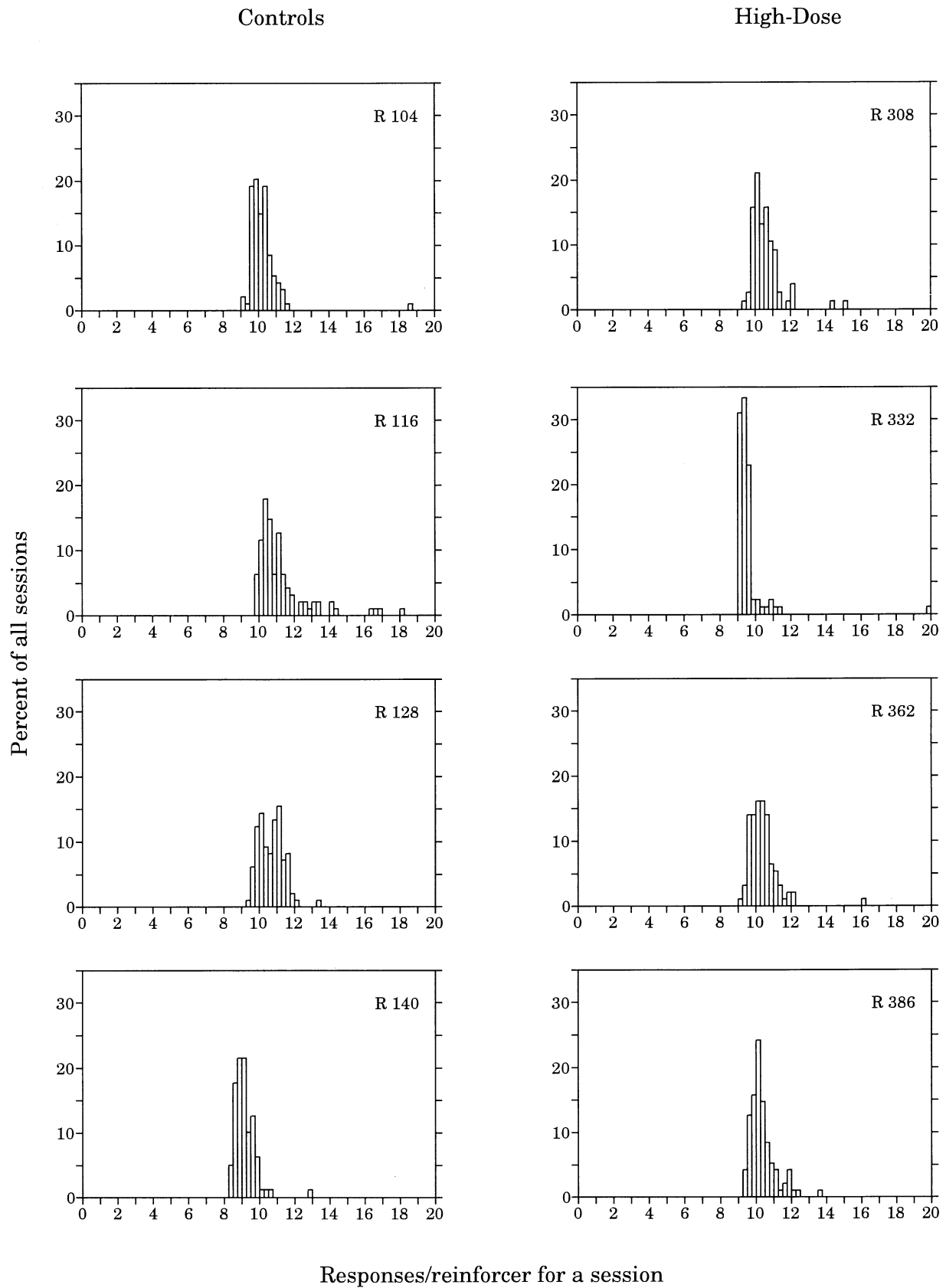


Fig. 1. Frequency histograms of responses/reinforcer taken across control sessions (range, 72–98 sessions) for individual rats. The horizontal axis describes the ratio of lever presses made to reinforcers delivered for individual sessions. The vertical axis shows the percentage of sessions that fell into a bin shown on the horizontal axis. Perfect performance under a DRH 9:4 schedule would yield a value of nine responses per reinforcer.

nistered intraperitoneally in a 1-ml/kg volume. Dizocilpine was dissolved in saline and administered subcutaneously. Haloperidol was dissolved in a saline solution with 3% lactic acid and a buffering agent and administered intraperitoneally. After an injection, the rat was placed in its home cage for 20 min (40 min after haloperidol and dizocilpine) before being placed in the experimental chamber for a session. d-Amphetamine, scopolamine, pentobarbital, and dizocilpine were administered on Tuesdays and Fridays. Haloperidol injections were separated by at least 5 days. Doses were administered in an ascending sequence. Subsequently, some intermediate doses were repeated after an initial ascending dose–response determination. If a dose suppressed behavior by greater than 50% for a particular rat, then a higher dose was not administered to that animal. Hence, not all rats received the highest doses. Vehicle injections (1 ml/kg) were administered on Thursdays (or 1 day before an injection during the haloperidol determination).

At the termination of the experiment, rats were 20 to 22 months of age.

2.5. Data analysis

The following behavioral measures were monitored: responses on the active (right) lever during the DRH and EXT components and the number of reinforcers earned per

session. Reinforcement rate best characterized the behavior required by the DRH schedule, so this measure served as the primary datum in this study. The relation between reinforcement rate and response rate was also examined. All dose-effect data are presented as percent of nondrug control sessions, as determined from the five control sessions immediately preceding the first dose of each drug averaged separately for each animal.

A two-way ANOVA with repeated measures (using SPSS) was conducted for all dependent variables under each drug, with methylmercury exposure group as the between-subjects variable and drug dose as the within-subjects variable. The repeated measured ANOVA requires an entry for every subject under every condition, or all data from that subject are dropped. Recall that higher doses were not administered to all rats (due to different drug sensitivities), therefore an accommodation was necessary. In order to satisfy the demands of the repeated measures ANOVA, a reinforcer rate of zero was included for doses higher than the maximal dose for any rat. This can be justified by observing that the descending limb of the dose–response curve for behavior is always monotonic. Nevertheless, two additional checks on the veracity of the analyses were conducted and are presented. First, graphical presentation of the dose–effect curves includes only rats actually tested at each dose; in the figures shown below, an artificial zero was not

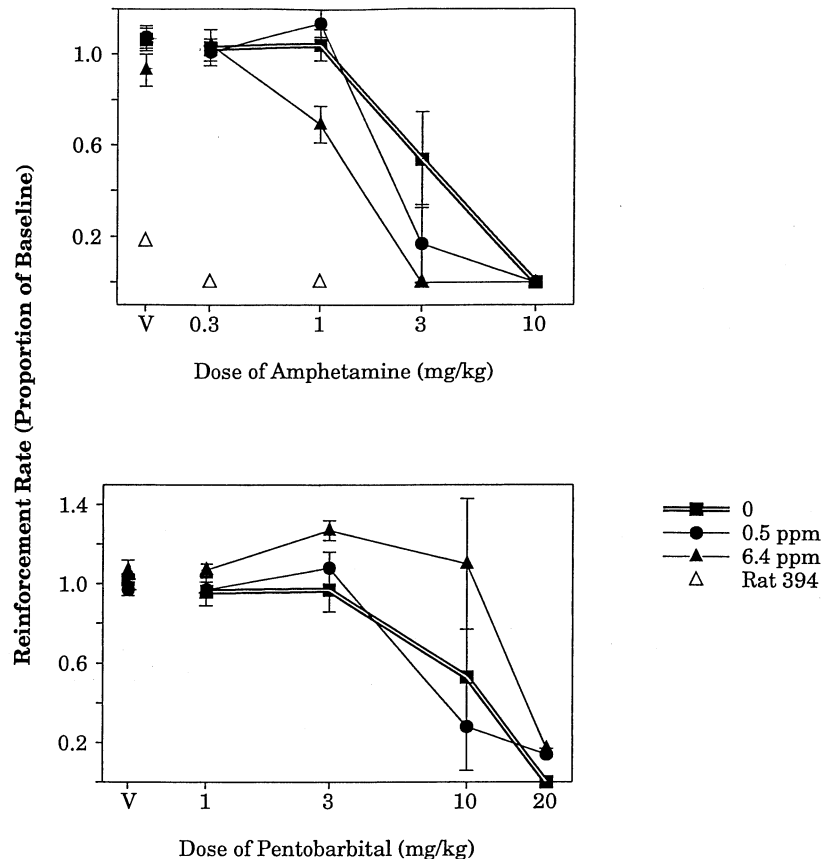


Fig. 2. Dose–response functions for reinforcement rate under d-amphetamine (top panel) and pentobarbital (bottom panel). Error bars = 1 S.E.M.

included when calculating the means and standard errors shown in the dose–effect curves. Second, sensitivity was reanalyzed using logit analyses that applied a binary measure (response rate below or above 50% of control values) at each dose.

3. Results

Histograms in Fig. 1 show the number of responses per reinforcer across all control sessions for selected rats. For each session, the number of responses under the DRH component was divided by the number of reinforcers delivered per session. Under the DRH 9:4 schedule, perfect performance would yield a result of exactly nine responses/reinforcer; imperfect or inefficient performance would yield a higher value. Note that for one animal (R 140), some values were less than 9; this animal, at times, did not pause to collect the delivered food pellet, but rather responded through the reinforcer cycle, “banking” some pellets. Thus, the ninth response of one DRH “burst” also counted as the first response of the next burst, and fewer than nine responses per reinforcer occurred. Overall, the figure indicates that the relationship between response rate and reinforcement rate is characterized by very little variability, with most values falling between 9 and 12 responses per reinforcer. Further, the medians for the animals fall within one to two responses per reinforcer of each other, showing that the 6.4-ppm group’s behavior does not differ from controls.

3.1. *d*-Amphetamine

d-Amphetamine decreased reinforcement rate in a dose-related fashion (Fig. 2, upper panel) across all groups. There was a leftward shift in the *d*-amphetamine dose–response curve for the MeHg-exposed rats. The differentiation is apparent at 1 mg/kg of *d*-amphetamine in which reinforcement rate was reduced by about a third in the 6.4-ppm group, but unaffected in the other exposure groups. At 3 mg/kg *d*-amphetamine, all of the methylmercury-exposed rat’s reinforcement rates (in both exposure groups) were reduced by at least 50%. Only half of the control rats’ reinforcement rates were reduced by at least 50% by the same dose (data not shown). A two-way ANOVA with repeated measures revealed a main effect of dose ($F_{4,52}=86.11$, $P<.01$), an effect of methylmercury ($F_{2,13}=4.5$, $P=.03$), but no interaction between the two variables ($F_{8,52}=2.44$, $P=.09$; Greenhouse–Geisser adjustment for sphericity violation).

Rat 394’s (from the 6.4-ppm group) behavior was inconsistent across control sessions between injections. Further, its behavior was sensitive to injection per se, so its data were not included in the statistical analyses reported above. Nevertheless, statistical analyses were re-conducted including this animal and the outcomes were similar. Its data are included in the figures as open triangles, and did not contribute to calculations of means.

3.2. Pentobarbital

Pentobarbital affected behavior in a dose-dependent manner ($F_{4,52}=26.82$, $P<.01$). The effects may have been biphasic for the 6.4-ppm group, and monophasic for the others (Fig. 2, lower panel). There was a main effect of methylmercury ($F_{2,13}=5.31$, $P=.02$), but no interaction between pentobarbital and methylmercury exposure ($F_{8,52}=1.42$, $P=.21$). A rightward shift in the dose–response curve was apparent in the 6.4-ppm group. The 3-mg/kg dose significantly increased reinforcement rates in the 6.4-ppm group ($F_{1,9}=30.254$, $P<0.01$), but no rate increase was observed in the control group or in most of the rats of the 0.5-ppm group at any dose. It took a dose almost twice as high (cf. 10 vs. 20 mg/kg) to suppress reinforcement rate by at least 50% in the 6.4-ppm group as compared to the control and 0.5 ppm groups.

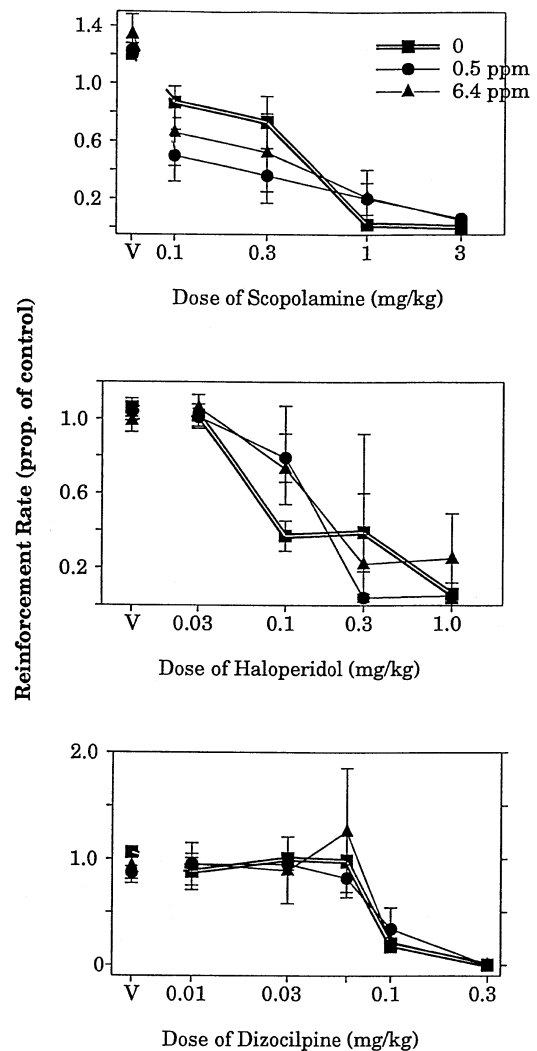


Fig. 3. Dose–response functions for reinforcement rate under scopolamine (top), haloperidol (middle), and dizocilpine (bottom). Error bars = 1 S.E.M.

Rat 207 from the 0.5-ppm group died after the d-amphetamine injections, reducing the 0.5-ppm group to five rats. The behavior of Rat 394 (6.4 ppm) could not be maintained under the mult DRH 9:4 EXT schedule during previous sessions, so it was placed on a mult DRH 5:2 EXT schedule after the amphetamine challenges. This rat's data

are included in the rest of the analyses and figures since behavior stabilized under this schedule and drug effects were expressed as a proportion of an individual animal's baseline. Nevertheless, the statistical analyses were conducted without this rat's data but the main conclusions did not change.

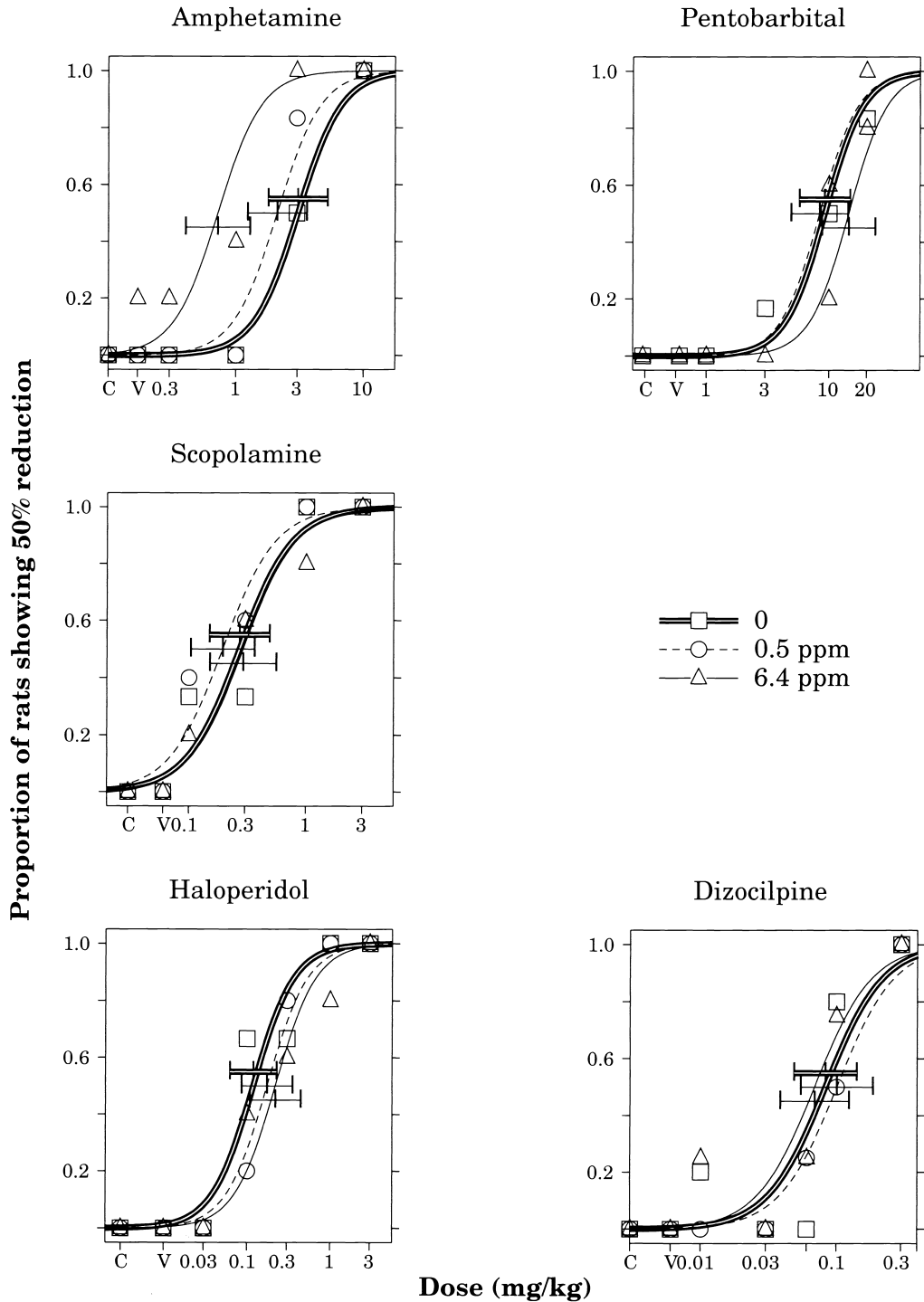


Fig. 4. Quantal dose–effect curves for each exposure group under each drug. Dose is represented on the x-axis and the percentage of animals whose behavior was suppressed by half is represented on the y-axis. ED₅₀ values are represented as the dose at which 50% of the animals were behaviorally affected by the drug. Error bars = 95% confidence intervals. (□ Control, ○ 0.5 ppm, △ 6.4 ppm).

3.3. Haloperidol

Haloperidol (Fig. 3, middle panel) reduced reinforcement rate in a dose-dependent fashion ($F_{5,60}=62.29$, $P<.01$). There was neither a main effect of methylmercury ($F_{2,12}=0.18$, $P=.84$), nor an interaction ($F_{10,60}=1.82$, $P=.08$). From Fig. 3, there appears to be a Mercury \times Haloperidol interaction at 0.1 mg/kg haloperidol; the 0.5- and 6.4-ppm groups seem to have been less affected than controls. An ANOVA conducted on that dose alone revealed no significant effect ($F_{2,14}=2.96$, $P=.09$).

Rat 228's behavior declined abruptly to near-zero levels during the haloperidol challenges, so its behavior was placed first under the mult DRH 3:1 EXT schedule to stability, and then the mult DRH 5:2 EXT schedule. This rat's data were not included in the haloperidol analyses.

3.4. Scopolamine

Fig. 3 (top panel) shows that scopolamine reduced reinforcement rate in a dose-dependent manner ($F_{4,52}=40.87$, $P<.01$). There was no main effect of methylmercury exposure on reinforcement rate ($F_{2,13}=0.46$, $P=.64$), and no interaction between scopolamine and methylmercury exposure ($F_{8,52}=1.12$, $P=.36$).

3.5. Dizocilpine

About 4 weeks elapsed between the haloperidol and dizocilpine challenges. During this time, one rat from each exposure group (Rats 140, 259, and 308) died or was moribund and euthanized. Hence, each group's n was reduced by one during the dizocilpine challenges (five

control, four at 0.5 ppm, and four at 6.4 ppm). Rat 228's behavior had stabilized under the mult DRH 9:4 EXT schedule, and dizocilpine challenges for this rat were administered while the rat was under this schedule. Since data are reported as percent of control, these rats' data are included in the figures and analysis.

Dizocilpine decreased reinforcement rate in a dose-dependent manner ($F_{5,50}=2.67$, $P=.03$), but only at the higher doses (0.1 and 0.3 mg/kg) that were associated with behavior incompatible with bar-pressing (stereotypies, such as circling). There was no main effect of methylmercury exposure ($F_{5,10}=1.46$, $P=.28$) nor an interaction between dizocilpine and methylmercury exposure ($F_{10,50}=0.32$, $P=.98$). Fig. 3 (lower panel) illustrates reinforcement rate under dizocilpine.

3.6. ED_{50}

ED_{50} values were established for each exposure group for each drug to analyze differences in the descending limb of the dose–response curve. These were estimated by entering the number of rats whose reinforcement rate was reduced by 1/2 of its own baseline rate at each dose of each drug. A logistic regression was conducted and a quantal dose–response curve was determined for each exposure group (Fig. 4). The dose at which half of the animals demonstrated 50% suppression of behavior was the ED_{50} . The 95% confidence intervals (CI's) for each ED_{50} overlapped greatly among all three exposure groups for the drugs scopolamine, dizocilpine, and haloperidol, confirming that no methylmercury interactions existed with these challenges.

The amphetamine ED_{50} values for the control, 0.5, 6.4 ppm groups were 3.1, 1.9, and 0.9 mg/kg, respectively. The

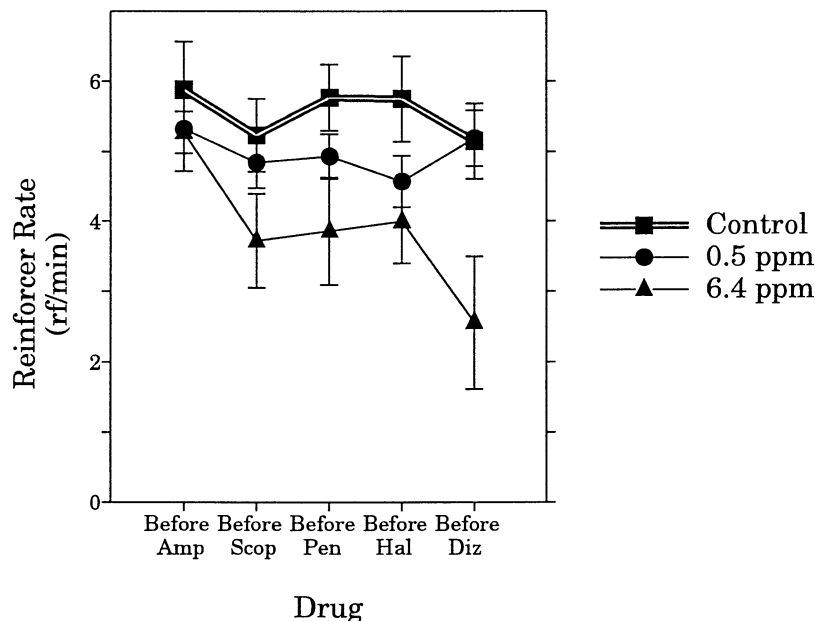


Fig. 5. Baseline reinforcement rates before each drug challenge. The y-axis represents average reinforcement rates for each exposure group (three control conditions were averaged for each animal, then the data for the animal were averaged within exposure group). The x-axis refers to the drug determinations.

95% CI for the ED₅₀ values overlapped for the control and 0.5-ppm groups, but the 6.4-ppm group's 95% CI did not overlap with the control group or with the 0.5-ppm group; thus, the 6.4-ppm group's ED₅₀ was significantly different from the other two.

The ED₅₀ values for pentobarbital were 9.12, 8.32, and 14.45 for the control, 0.5-, and 6.4-ppm groups, respectively. The 95% CIs surrounding the ED₅₀ values almost fully overlapped for the control and 0.5-ppm groups under pentobarbital. The 6.4-ppm group's CI overlapped with these groups, but only slightly; nonetheless, there were no significant differences on this endpoint.

3.7. Baseline reinforcement rates

Baseline reinforcement rates before the commencement of each drug determination were compared across groups. Fig. 5 shows that before the first drug challenge (amphetamine), the groups' means were indistinguishable. During the amphetamine determination, the two methylmercury exposure groups' baselines gradually began to diverge in an exposure-related fashion from the control's baseline with each progressive drug determination. While the 0.5-ppm group's baseline recovered, the 6.4-ppm group's baseline continued to decline. A two-way repeated measures ANOVA was conducted (drug as the within-group variable, exposure group as between-group variable), and revealed a main effect of drug on baseline ($F_{4,40}=5.19$, $P=.02$), no main effect of MeHg exposure ($F_{2,10}=3.0$, $P=.10$), and an interaction between drug and MeHg ($F_{8,40}=2.5$, $P=.03$).

Fig. 5 includes only animals that underwent all five drug determinations (control = 5, 0.5 group = 4, 6.4 ppm group = 4), since different n 's under each drug would make comparison difficult. When data were re-examined using all animals, however, the pattern remained the same.

4. Discussion

4.1. General considerations

Pharmacological challenges in behaving animals can be used both to test and to generate hypotheses about neurochemical substrates underlying the actions of neurotoxic substances. In the present study, the challenges were selected to test hypotheses derived from two general sources. The first was the delayed actions of methylmercury exposure on neurotransmitter systems, especially catecholnergic and GABAergic, as assessed in younger animals and higher exposure levels than in the present study. The second source constituted *in vitro* studies of interactions between methylmercury and certain neurotransmitter systems, or *in vitro* interactions between acute drug administration occurring in close proximity to methylmercury exposure. The latter class of investigations is helpful in identifying neuro-

transmitter systems that are potentially vulnerable to methylmercury exposure.

The present investigation extends previous studies of neurochemical substrates of methylmercury's neurotoxicity along several dimensions: dosing regimen, daily dose, and age of the animals tested. During gestation, the rats described in the present study were exposed continuously to relatively low levels of methylmercury via maternal drinking water. This stands in contrast to dosing protocols in which methylmercury is administered by injection or gavage to a pregnant rat for a few days during gestation. With injection, or even by gavage, there can be a relatively brief period of high methylmercury concentration that then tapers off. With drinking water exposure, especially when exposure begins weeks before mating as in the present study, fetal exposure is more constant and brief pulses of high concentrations are nonexistent. Consequently, maternal blood and brain levels of methylmercury are likely to remain stable throughout gestation.

The lower daily dose used in the present study, 40 $\mu\text{g}/\text{kg}/\text{day}$, was selected to approximate the highest dose administered, by gavage, to rats from gestational days 6–9 in a study [8] that described the lowest behaviorally active dose of methylmercury in the rodent literature [31]. The higher dose, about 500 $\mu\text{g}/\text{kg}/\text{day}$, approximated an exposure level shown to have subtle behavioral effects on sensory–motor function using an exposure regimen similar to that used in the present study [18]. Because species differences in the brain/blood ratio of methylmercury can vary by more than an order of magnitude [23], the concentration of methylmercury in the brain is a more precise biomarker of exposure than raw intake when making comparisons among animal studies or between animal and human exposures [9]. Brain levels at birth, about 0.5 and 9.5 ppm for the two groups described here, were categorized at exposure levels described as “low” (<3 ppm) and “moderate” (3–11 ppm), respectively [9], and by weaning all brain levels fell to “low” levels.

The rats in the present study were older than those described in earlier studies in which rats were tested either close to weaning (e.g., Refs. [12,28]) or close to attaining adult body weight (about 3 months) [10,28,36]. Rats in the present study were about 13–15 months old at the beginning of the drug challenges, and the challenges continued until they were about 20–22 months. Thus, the present report extends findings of altered sensitivity to catecholamines and barbiturates to fully adult rats, suggesting that the disruption in these neurotransmitter systems is irreversible.

The experimental design used in the present study permitted a comprehensive dose–effect profile to be drawn with respect to methylmercury and to the drugs examined. Two levels of methylmercury exposure were used so trends associated with methylmercury exposure could be identified across a 10-fold range of exposure. A full dose–effect profile, bracketed by a behaviorally inactive dose and a dose that reduced responding by at least 50%, was also

determined for each drug challenge. The latter criterion was determined on an individual basis, so the highest dose used differed across rats, an approach that permitted the statistical estimate of the drug dose that reduced overall reinforcer rate by 50% on an individual basis without risking overdose in sensitive animals. Thus, intersubject variability could be determined. The use of drugs representing different pharmacological classes permits some assessment of specificity to be made.

4.2. *Effects of amphetamine and haloperidol*

Amphetamine decreased rate in a dose-dependent fashion. This effect is consistently observed when amphetamine interacts with behavior under a fixed-ratio schedule of reinforcement (e.g., Refs. [14,17,19,24,39]), which, like a DRH schedule, selects high response rates. Rats exposed to methylmercury were more sensitive to d-amphetamine than were unexposed rats, and this sensitivity was related to the degree of methylmercury exposure. Reinforcement rates were reduced by at least 50% at 1 mg/kg of d-amphetamine in the 6.4-ppm-exposed rats. In contrast, a dose of 3 mg/kg of d-amphetamine was required to produce this level of suppression in the control and 0.5-ppm-exposed rats. Four of six control rats exhibited some behavior after 3 mg/kg of d-amphetamine; only two rats from the 0.5-ppm group and none of the 6.4-ppm rats responded at this dose. Differential sensitivity was also evident in the ED₅₀ values, defined here as the dose that reduced reinforcer rate by 50% in half of the animals; the 6.4-ppm group's ED₅₀ was three times lower than that from the control group. This sensitivity to amphetamine extends effects reported by others [10,16,20] to lower levels of mercury exposure and to a longer time after exposure.

Two sets of mechanisms have been described that could underlie the sensitivity to amphetamine provoked by the presence of methylmercury in the nervous system. The first set involves direct effects of methylmercury on synaptic function. Methylmercury at low micromolar ranges has been reported to disrupt calcium flux across the neural membrane [34], and inhibits the production of monoamine oxidase [11]. The former mechanism might be reflected in challenges with a broad range of drugs while the latter would appear more specifically in monoamine neurotransmitter systems, which amphetamine acts upon. Tissue levels of methylmercury in the present study were probably below those examined in the *in vitro* studies examining mercury's actions on neural function, however. By weaning, methylmercury concentrations in whole brain were about 0.5 ppm in the high-dose group [26]. In the absence of postweaning exposure and an additional 10-fold weight gain, it can be estimated that mercury concentrations in the brain were 100-fold less than seen at birth. This places possible whole-brain concentrations in the 50-ppb range or 0.25 μ M, assuming no loss of mercury, for adults in the high-exposure group. By

contrast, the studies referred to above described mercury's effects at concentrations greater than 2 μ M, so little is known about the concentrations likely present during the drug challenges.

A second set of possibilities derives from observations that prenatal developmental methylmercury exposure produces dynamic changes in noradrenergic transmitter systems in cerebellum, cortex, kidney, and liver [35]. Changes in noradrenergic systems are quite complex and depend on the region examined (cerebellum or cortex), dose, timing of exposure (pre- vs. postnatal exposure), and age. Noradrenergic binding in cerebellum was shown to be especially sensitive to prenatal methylmercury exposure (500–1000 μ g/kg/day sc, from GD8 to birth at GD21) [4]. It is not known how long-lasting these effects are, but in several of the conditions reviewed, methylmercury-induced alterations in noradrenergic function had not stabilized by 41 days of age [35].

The observation that developmental methylmercury exposure produced sensitivity to amphetamine but not to haloperidol could indicate that noradrenergic neurotransmitter systems are more sensitive to methylmercury exposure than are dopaminergic systems [35]. Haloperidol is a relatively specific antagonist at the dopamine D2 receptor, while d-amphetamine is an indirect agonist in both noradrenergic and dopaminergic systems [6]. However, developmental methylmercury exposure has also been reported to increase the number of dopamine binding sites in the striatum [10,12] with corresponding sensitivity to apomorphine [12] or amphetamine [10], albeit transiently and at high exposure levels.

4.3. *Pentobarbital*

The shape of the dose–effect curve describing the relationship between reinforcement rate and pentobarbital dose was biphasic for the 6.4-ppm rats; moderate doses of pentobarbital increased response rates while higher doses decreased rates. Such a biphasic dose–effect curve is sometimes seen with barbiturates and high-rate operant behavior [14,22].

There was a slight rightward shift in the 6.4-ppm group's quantal dose–response curve (Fig. 4), reflecting a somewhat diminished sensitivity in the mercury-exposed animals to the rate-reducing effects of pentobarbital. The dose–response analysis was used to estimate the dose at which behavior was reduced by 50% in half of the animals, so the rate increase would not be detected by such an analysis. It is not clear why methylmercury exposure would result in such a biphasic dose–effect profile to pentobarbital, except to note that barbiturates sometimes produce such an increase in high-rate behavior in other species [14,22].

The present report of an interaction between methylmercury exposure and acute barbiturate exposure is not an isolated one. Methylmercury-exposed mice were less sensitive to hypnotic actions of hexobarbital, an effect ascribed to

neural mechanisms rather than alterations in liver enzymes [36]. There may be an interaction between GABA systems and developmental methylmercury exposure, although the picture is quite complex. Methylmercury decreases the uptake of GABA in the cerebellum, leaving more in the synapse [2]. One way by which such a mechanism could result in diminished sensitivity in adult behavior would be for a form of down-regulation to occur, resulting in diminished sensitivity in the GABA neurons, or fewer of them. It has been reported that neonates exposed prenatally to methylmercury have fewer GABA receptors [28], but whether this is seen in the adult is not known.

4.4. Other drugs

No interaction was noted between methylmercury and scopolamine, or dizocilpine on any of the variables examined. *In vitro* studies of the cholinergic systems [2,7,15,21,29,37] and of glutamatergic systems [3,13,27,30] have suggested the possibility of methylmercury-induced changes in sensitivity. This suggests some vulnerability of these receptor systems to methylmercury exposure, although this was observed only with concurrent methylmercury and drug exposure. The present report suggests that any vulnerability with these systems that may exist during development is not reflected in high-rate operant behavior in adulthood.

Methylmercury alters the function of calcium channels, which, in turn, may alter the release of transmitter non-specifically, as has been demonstrated *in vitro* [5,32,33]. While not conclusive, the specificity seen in the present study suggests that effects of organic mercury on presynaptic membrane transport of ions such as calcium may not appear in adults exposed only during development, since such a mechanism should enhance sensitivity to a broad range of drugs.

4.5. An analysis of the high-rate behavior

The dependent measure chosen for analysis, reinforcer rate, was selected in order to capture two important features of behavior: the amount of responding during the session and the ability of the behavior to meet the demands of the DRH schedule. Histograms of responses/reinforcer taken from control (no acute drug administration) sessions indicated that behavior was tightly controlled by the DRH 9:4 schedule contingency in all three exposure groups. The number of responses/reinforcer peaked sharply at about 9–10 and the distribution trailed off sharply after that. The sharpness of the distribution indicates that the measure of reinforcer rate was closely related to overall response rate. The relationship was not perfect, as indicated by the right tail of the distribution; there were occasions in which nine successive responses did not meet the speed criterion of 4 s. Thus, there were two dimensions of responding to be captured here, and reinforcer rate did so without grossly misrepresenting the overall response rate. Histograms could not be conducted for indi-

vidual rats during those drug sessions that substantially lowered response rate because the number of events to tabulate was too small. Analyses of response “efficiency” (a measure of the percent of response sequences that produce reinforcers) during these sessions, however, indicated that declining reinforcer rates largely reflected declining rates of nine response bursts (data not shown).

When examining behavior under control conditions across all five drug challenges, a rate decline was observed in the 6.4-ppm group, but not in the control group (Fig. 5), and only transiently in the 0.5-ppm group. The changing baseline was accommodated in analyses of drug effects by expressing all drug effects as a percent of the animals’ behavior during control sessions conducted immediately prior to and during the determination of the acute dose–effect curves. An attempt to link the baseline change to a particular drug challenge or even nonspecifically to drug administration was not successful, and is described in another report [25]. In that report, which followed these animals until survivors were about 2.5 years old, it appeared that this declining baseline represented an interaction between aging and prenatal methylmercury exposure, even though at the end of the drug challenges the rats were only about 20–22 months old.

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