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Prenatal methylmercury exposure increases responding under clocked and unclocked fixed interval schedules of reinforcement

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Abstract

Recent experiments have suggested that developmental methylmercury exposure produces perseverative behavior in adulthood. In the present experiment, interactions between developmental low-level methylmercury (MeHg) and nutritionally relevant dietary selenium (Se) on operant behavior and its persistence were examined in aged animals. Female rats were exposed, *in utero*, to 0, 0.5, or 5 ppm mercury as MeHg via drinking water, approximating mercury exposures of 0, 40, and 400 μg/kg/day. They also received both pre- and chronic post-natal exposure to a diet that was marginal (0.06 ppm) or rich (0.6 ppm) in Se, a nutrient believed to protect against MeHg's toxicity. This created a 2 (chronic Se) × 3 (gestational MeHg) full factorial design, with 6–8 female rats per cell. At eleven months of age, a multiple schedule consisting of alternating fixed interval (FI) and clocked FI (CFI) components was arranged. The CFI component was divided into 5, 24-second bins, each associated with a different auditory stimulus, providing a "clock." Low and high response rates were evaluated using the initial 40% (bins 1 and 2) and last 20% (bin 5) of the FI and CFI components, respectively. Rats exposed to 5 ppm Hg made more responses than the other two groups during the last 20% of the intervals, regardless of selenium exposure or presence of the clock stimuli. They did not differ from the other groups during the initial 40% of the FI and CFI components. Following reinforcement omission for half of the intervals at 21 months of age, the 5 ppm Hg group continued to respond at higher rates than the other groups in both components.

Keywords: Methylmercury; Selenium; Fixed interval; Developmental; Operant behavior

1. Introduction

Methylmercury (MeHg) is a known developmental neurotoxicant found in fish and marine mammals. Fish, however, are also an important source of nutrients such as selenium and long-chain polyunsaturated fatty acids. In rodent studies examining adult-onset MeHg exposure, selenium (Se) ameliorated some of the effects of chronic, high-level MeHg exposure [9,12,21]. For developmental MeHg exposure, however, evidence that Se confers protection is less pronounced. With developmental MeHg exposure, a diet severely deficient in Se enhanced MeHg's fetolethality [30], as well as MeHg's detrimental effects on the development of gait, thermal preference, and open-field activity, but these effects did not persist into adulthood [46]. In contrast, a Se-sufficient diet has not attenuated MeHg's neurobehavioral toxicity [4,36] with the exception of one study [46]. In that study, Se excess attenuated MeHg-induced hypoactivity in animals exposed to 6 mg/kg MeHg by gavage on days 6–9 of gestation and examined at two months of age [8]. Thus, while Se ameliorates some of MeHg's effects following adult-onset exposure, the picture is less clear for developmental exposure.

A recent study focused on the potential interactions between low-level developmental MeHg exposure and nutritionally relevant dietary Se on spatial discrimination reversals in adulthood [36]. Although all rats acquired the original discrimination similarly, MeHg-exposed rats, regardless of Se exposure, made more errors than controls on the first and third reversals, which were away from the lever that was reinforced in the original discrimination. MeHg-exposed rats also had shorter choice latencies than controls, implying an impulsive or perseverative response pattern. Rats consuming a low-Se diet, regardless of their MeHg exposure, made more omissions (trials

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without a response) during the first reversal and required more sessions to complete this reversal than rats exposed to a high-Se diet. Thus, while there were main effects of both MeHg and Se, on no measure was there an interaction between Se and MeHg exposure. However, behavioral procedures that permit greater variation in response rate, such as the fixed-interval schedule of reinforcement, might be sensitive to such an interaction.

The behavioral effects produced by MeHg exposure could be viewed as reflecting a reduced sensitivity to changing reinforcement contingencies or an increased reinforcer efficacy [23]. Disentangling these two ideas is difficult, since each would result in persistent, or even perseverative, responding when reinforcement contingencies are altered. Both interpretations are consistent with findings that gestational MeHg exposure slowed transitions during a choice in-transition procedure [27,28], resulted in more rapid acquisition of lever-pressing and lack of ratio strain under large fixed ratio schedules of reinforcement [32], and a tolerance for higher ratios under a progressive ratio procedure [32,35].

The behavioral patterns seen in MeHg-treated animals in previous studies allow us to make predictions about the behavior of exposed animals under other reinforcement schedules. For example, response rates under fixed interval (FI) schedules are positively related to the reinforcement magnitude of food pellets [19,20], sucrose solution [42], and cocaine [2]. If a reinforcer's efficacy is increased for animals exposed to MeHg, then increased response rates under the FI schedule, especially in the last portion of the interval, would be expected.

Previous studies of developmental MeHg exposure have not identified deficits in discrimination [e.g. 5,39,41], an observation that supports a second prediction. If exteroceptive stimuli are correlated with the passage of time in an FI schedule, a "clocked" FI (CFI) [16,31], then we would expect animals to make fewer responses, particularly in the first portion of the interval, as compared with the typical FI. Since there is little evidence that developmental MeHg exposure affects discrimination processes [5,24,39,41], then we might expect no differential effect of MeHg on clocked performance. However, MeHg-exposed animals might be expected to have greater response rates during the latter portion of the interval in both components if reinforcer efficacy is altered by MeHg exposure. Finally, if the reinforcer is omitted at the end of the FI and CFI components, but responses continue to be recorded, then we would expect MeHg-exposed animals to make more responses than controls.

The present study was designed to examine the role of exteroceptive stimuli and reinforcement omission in rats exposed developmentally to MeHg and chronically to a diet either marginal or rich in Se, a nutrient hypothesized to protect against MeHg's neurotoxic effects [18,34,44,45,48]. The experiments were conducted using a 2 (chronic Se)×3 (gestational MeHg) full factorial design, which allows for the direct examination of the interactions between MeHg and Se, as well as the main effects of either element. The MeHg concentrations chosen produce levels spanning the low to moderate range [6,26], as determined by brain mercury [25]. Likewise, the Se diets were at the low and high end of recommended intakes. The 0.06 ppm Se concentration is lowest possible with a casein-based diet and is still a nutritionally adequate level for rodents [22,38]. The higher, 0.6 ppm, concentration is at the high end of adequate and represents an excess over the AIN-93 formulation, which contains 0.15 ppm of Se [37,38], but is below that thought to be toxic [1].

Upon reaching adulthood, female offspring were trained to respond under a *Mult* FI 120″, Clock FI (CFI) 120″ schedule of reinforcement. When the FI schedule was in effect, the first lever-press after 120″ produced sucrose. When the CFI was in effect, five distinct auditory stimuli were presented sequentially for 24″ each, resulting in a 120″ interval, and the first lever-press after 120″ produced sucrose. Rats experienced twenty-two sessions of the *Mult* FI CFI schedule with auditory stimuli before group comparisons of baseline responding were made at 13 months of age. After this first comparison, drug challenges began (to be described elsewhere) with multiple doses of cocaine, desipramine, SKF-38393, quinpirole, SCH-23390 and sulpiride. At 20 months of age, thirty days after completing the last dose-effect determination, responding under the *Mult* FI CFI schedule was reassessed and compared with their performance at 13 months of age. Finally, reinforcement omission trials were instated for 10 sessions at 21 months of age: responses at the end of the interval were not followed by sucrose for half of the FI and CFI components, but responding continued to be monitored for an additional 240″. The responses at the end of the interval in the remaining FI and CFI components were followed by sucrose as in previous sessions.

2. Methods

2.1. Subjects

The subjects were 42 female Long–Evans rats (F_1 generation) housed in a temperature- and humidity-controlled, AAALAC-accredited colony room that was maintained on a 12-hour light-dark cycle (lights on at 7:00 a.m.). Subjects were bred at the Biological Research Facility at Auburn University (described below), and each was randomly selected from a separate litter, so the litter served as the statistical unit for all analyses. These rats were exposed *in utero* to MeHg via maternal consumption of drinking water containing 0, 0.5, or 5 ppm of mercury (Hg) as methylmercuric chloride (Alfa Aesar, Ward Hill, MA) and a diet containing approximately 0.06 and 0.6 ppm Se throughout life, forming a 2 (chronic Se)×3 (developmental MeHg) factorial design, as described below. There were six to eight rats per experimental group (N=42) at 13 months of age and five to eight rats per experimental group (N=37) remained at 20 months of age.

After weaning on post-natal day (PND) 21, the subjects were injected subcutaneously with an electronic identification chip (Biomedic Data Systems, Seaford, DE). Subjects were housed in standard 22.9 cm×45.7 cm×19 cm plastic "shoebox" cages with a wire top and solid bottom. They were housed two per cage but were separated by a transparent divider diagonally placed in the cage so that feeding could be tailored to each

individual rat's requirement while maintaining adequate space requirements for each rat. During adulthood, after PND 90, their food was rationed to approximately 10 g/day so as to maintain their body weight at 250 g. Rats that shared a home cage also received the same diet, so that diets were never mixed. To prevent excessive tooth growth, a cleaned, nylon chew "bone" was freely available in the home cage. Rats were 11 ± 1 months of age at the beginning of the present experiment and 21 ± 1 months of age when the omission procedure began.

2.2. Breeding

Beginning at approximately 23.5 weeks of age and continuing to 42 weeks of age, 58 male and 114 female Long–Evans rats (F_0 generation; Harlan, Indianapolis, IN) were bred. Breeding commenced after 5.5 weeks of exposure to the appropriate Se diet and 2.5 weeks of MeHg exposure (see Sections 2.3 and 2.4; see Fig. 1). Breeding cages contained the female's Se diet and tap water, so males were never exposed to MeHg. Each Long–Evans male was paired with a single female during every other dark cycle. Most males were paired with a second female during alternating dark cycles. A male was paired with the same female(s) throughout breeding. When a male was bred with two females, the females were always members of different exposure groups. Breeding of females continued until a sperm plug or systematic increases in daily body weight were observed, suggesting gravidity. Births before 5:00 pm were assigned to PND 0 for that day. All births after 5:00 pm were assigned to PND 0 for the subsequent day. Large litters were culled to produce 8 F_1 pups including at least three females when possible, but only one female from some of the litters were used in the present study. Behavior of the F_0 rats will not be described here.

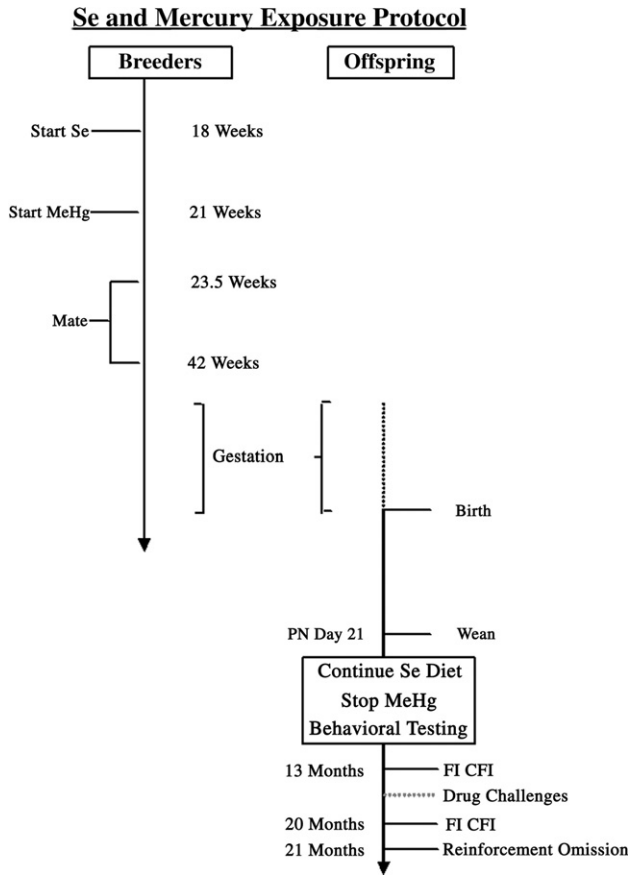

Fig. 1. Timeline for breeding and exposure for F_0 breeders and F_1 offspring. Note exposure to methylmercury ended for offspring at weaning. Breeders were not included in the present experiment. See text for details.

All rats were monitored daily by the research staff and personnel from the Department of Laboratory Animal Health at Auburn University and were inspected by veterinary staff at least twice a week. Sentinel rats exposed to the same air and to bedding taken from selected rats used on the study were inspected semiannually for infectious diseases. All experiments were approved by the Auburn University Institutional Animal Care and Use Committee. The colony was housed in an AAALAC-accredited facility that also met PHS guidelines for animal care.

2.3. Selenium exposure: F_0 and F_1 generation

At 18 weeks (125 days) of age, mothers (F_0 generation; Harlan, Indianapolis, IN) of the rats used in the present experiment were placed on one of two diets, each based on the AIN-93 formula for laboratory rodents but customized for Se concentration (see Fig. 1). The "low selenium" diet contained Se from casein only and can vary around the nominal concentration of 0.06 ppm. The "high selenium" diet was supplemented with sodium selenite to produce 0.6 ppm. Selenium content of the diets was analyzed with each shipment using inductively coupled plasma mass spectrometry (ICP-MS). Analyses revealed actual concentrations between 0.05 and 0.07 ppm and, in one shipment used when the subjects were adults, 0.1 ppm in the low-Se diet, and 0.6 and 0.9 ppm in the high-Se diets. Between mating and lactation, the base diet was an AIN 93 growth diet containing 7% fat from soybean oil. A maintenance diet of an AIN 93 diet with 4% fat was used at all other times. Both diets were obtained from Research Diets Inc (New Brunswick, NJ). Dietary Hg was below the detectable level of 50 ppb. Male breeders were maintained on a standard chow diet, except when briefly exposed to the F_0 female's diet during breeding (see Section 2.2). All F_1 offspring received the same diet as their maternal dams throughout life.

2.4. Methylmercury exposure: F_0 generation only

At approximately 21 weeks (145 days of age), after three weeks (20 days) on the custom Se diets, each Se group of F_0 breeders was further divided into three MeHg exposure groups, counterbalancing bodyweight, to create 6 experimental groups. Methylmercury was added to drinking water of F_0 breeders in concentrations of 0, 0.5, or 5 ppm of Hg as methylmercuric chloride, (Alfa Aesar, Ward Hill, MA). Sodium carbonate (<5 nanomolar), which can buffer the MeHg [43], was added to all three water mixtures. These concentrations produce exposures of about 0, 40 and 400 μg/kg/day respectively, based on average daily consumption, with some elevation during gestation due to

increased fluid consumption [26]. Fluid consumption reported in the earlier paper [26] was confirmed by taking periodic measurements of water intake. Drinking water was prepared from a stock solution containing 15 ppm of Hg as MeHg. Every time a new dilution was created, actual Hg concentration was determined by atomic absorption and found to be within 10% of the target values.

Maternal exposure to the MeHg-containing water was discontinued on post-natal day 16 when the F_1 pups were capable of reaching the waterspout. Because there is little exposure via breastmilk, MeHg exposure functionally terminated at birth [26,43]. Throughout the remainder of life, all F_1 rats received plain tap water to drink. Male breeders received exposure to plain tap water only.

2.5. Testing apparatus

The experiments were conducted in 16 commercially purchased operant chambers (Med-Associates Inc. model #Med ENV 007) containing two front levers (each calibrated so that 0.20 neuton registered a press), a pellet dispenser situated between the two front levers and filled with 45 mg sucrose pellets (Research Diets, Inc., New Brunswick, NJ), Sonalert tones™ (2900 and 4500 Hz, nominally; calibrated to an amplitude of 70 dbC), a house light (28 V 100 ma), and a light emitting diode (LED) above each lever. Dimensions of the chamber were 12 in. L × 9 1/2 in. W × 11 1/2 in. H. The standard grid floor was covered with a secured piece of plexiglas, which covered all but the back inch of the floor. This was used because chronic MeHg exposure for rats in other experiments sometimes caused them to fall through the bars. No rat in the present experiment displayed such signs. Each chamber was surrounded by a sound-attenuating cabinet with built-in ventilating fan that circulated air into the experimental environment and provided masking white noise. Programs for experimental procedures and data collection were written using MED-PC IV (Med-Associates, Georgia, VT). Session events were recorded with 0.01″ resolution.

2.6. Behavioral methods

At the beginning of the study and throughout experimental testing, body weights did not differ among any of the exposure groups. Three squads of subjects were conducted daily at different, but consecutive and regular, times; assignment of subjects to squads and chambers was distributed across exposure groups. Fans, lights, tones, levers, and pellet dispensers were tested before and after sessions for each squad of rats to ensure that equipment was functioning properly. Electronic identification chips were used to track subjects, and rats were scanned prior to each session to insure they were placed in the appropriate chamber and home cage.

2.6.1. Training

Upon reaching adulthood, rats were trained to lever-press on the right-lever using autoshaping [7,27]. After the lever was pressed 10 times, the autoshaping procedure ended, and a fixed ratio (FR) 1 schedule was in effect. A single lever-press resulted in the delivery of a 45 mg sucrose pellet as a 0.5 s, 4500 Hz tone was sounded. The stimulus light over the right-lever remained lit. Sessions ended after 100 lever presses in the free-operant arrangement or 12 h elapsed, which ever occurred first. Right lever presses were placed on a fixed interval (FI) schedule of food reinforcement that increased each session until behavior could be maintained under an FI 120″ schedule. The first day of training began with an FR1 schedule of reinforcement. The subsequent days used one of the following FIs in ascending order: 5″, 15″, 30″, 60″, and 90″.

2.6.2. Mult FI CFI condition

A multiple schedule consisting of alternating FI and Clocked FI (CFI) components was then arranged. When the FI schedule was in effect, the first lever-press after 120″ produced food. When the CFI was in effect, five stimuli were presented for 24″ each, resulting in a 120″ interval, and the first lever-press after 120″ produced food. Initially, visual stimuli were used for each of the five 24″ bins. These visual stimuli did not bring behavior under adequate stimulus control, however, so the CFI stimuli were changed to auditory stimuli. These stimuli consisted of a 0.25″ flickering 2900 Hz tone (bin 1), a steady 2900 Hz tone (bin 2), a flickering 4500 Hz tone (bin 3), a steady 4500 Hz tone (bin 4), and alternating 2900 and 4500 Hz tones with each flickering for 0.25″ (bin 5).

Each session began with a 5-min chamber blackout. Following this, the houselight was turned on, and the components alternated, beginning with the FI. Components were not separated by a blackout, and each component was presented 8 times per session. Reinforcement consisted of a 45 mg sucrose pellet. Rats experienced twenty-two sessions of the *Mult* FI CFI schedule with auditory stimuli before comparisons between groups were made at 13 months of age. After this first comparison, drug testing began (to be described elsewhere) with multiple doses of cocaine, desipramine, SKF-38393, quinpirole, SCH-23390 and sulpiride. At 20 months of age, thirty days after completing the last dose-effect determination, responding under the *Mult* FI CFI schedule was reassessed and compared with their performance at 13 months of age.

2.6.3. Reinforcement omission trials

At 21 months of age, an intervention resembling the “peak interval” procedure [33] was arranged. In this procedure, half of the 16 *Mult* FI CFI components (4 FI and 4 CFI) were food (F) trials, and the other half (4 FI and 4 CFI) were nonfood (NF) or extinction trials, with trials occurring in the following order: FI_F CFI_{NF} FI_{NF} CFI_F FI_F CFI_{NF} FI_{NF} CFI_F FI_{NF} CFI_F FI_F CFI_{NF} FI_{NF} CFI_F FI_F CFI_{NF}. On food trials, the conditions and stimuli were identical to those of the *Mult* FI CFI condition: the first response after 120″ was followed immediately by food. On nonfood trials, food was not delivered for the first response after 120″, but responses continued to be recorded for an additional 240″, resulting in a total interval time of 360″. For the nonfood CFI trials, the auditory stimuli associated with the first four bins continued to be presented for 24″ each, but the

alternating 2900 Hz and 4500 Hz tones associated with bin 5 did not stop after 24″ but continued throughout the additional 240″.

2.7. Data and statistical analyses

All statistical analyses were performed using SYSTAT® 11 (SYSTAT Software Inc. Richmond, CA, USA). The Type I error rate (α) was set at 0.05 for all omnibus and post hoc tests. Specific tests are described below.

2.7.1. FI CFI condition

Each segment of the CFI component (and the corresponding FI component) was divided equally into five bins. The number of responses occurring in each bin was accumulated across individual trials and averaged for the FI and CFI components separately. In order to provide a full characterization of behavior, four dependent variables were analyzed separately for each component:

a. Overall response rate — the total number of responses throughout the interval divided by 120″
b. Response rate in bins 1 and 2 averaged — the total number of responses for bins 1 and 2 divided by the time available to respond (48″). Bins 1 and 2 were combined due to the exceptionally low-rate of responding in bin 1 under the CFI component. This variable was used to examine Se and MeHg effects on low-rate responding.
c. Response rate in bin 5 — the total number of responses in the last 24″ of each interval. This variable was used to examine Se and MeHg effects on high-rate responding.
d. Quarterlife — percent of the interval at which the first 25% of responses occurred. This was used to represent the temporal patterning of responding through the interval. A quarterlife of greater than 30″, or 25%, occurs when the response rate is higher at the end of the interval than at the beginning, as usually occurs in behavior under FI schedules of reinforcement.

For each animal, data for the four dependent variables were obtained by averaging across five consecutive sessions under baseline conditions before and after drug exposure at 13 and 20 months of age, respectively. A three-way analysis of variance (ANOVA) using component (FI vs. CFI), Se, and MeHg as factors was performed for each dependent variable at 13 months of age. To assess the influence of prolonged schedule exposure, aging, or experiences with acute drug administrations, a repeated-measures analysis of variance (RMANOVA) was performed for each dependent variable of the FI and CFI components at 13 and 20 months of age. MeHg (0. 0.5, 5 ppm) and Se (0.06 ppm, 0.6 ppm) served as the two between-subjects factors, with 5–8 rats per cell. Age served as the within-subject factor.

Significant ANOVAs were followed by pairwise, Tukey post hoc comparisons among the three MeHg dose groups to determine which differed from each other; post hoc comparisons were not necessary for Se, as it involves only a single comparison. *F*-ratios, degrees of freedom and *P*-values were reported for all RMANOVAs and two-way ANOVAs, and *P*-values were reported for post hoc contrasts and comparisons.

2.7.2. Reinforcement omission trials

Data were collected for the first ten sessions of the omission condition; food trials were not analyzed. For nonfood or omission trials, the total number of responses occurring in the additional 240″ after the end of the 120″ interval was accumulated across individual trials and averaged for the FI_{NF} and CFI_{NF} components separately. This value served as the dependent variable for a repeated-measures analysis of variance (RMANOVA). MeHg (0. 0.5, 5 ppm) and Se (0.06 ppm, 0.6 ppm) served as the two between-subjects factors. The ten sessions and two components (FI_{NF} and CFI_{NF}) served as the within-subject factors.

3. Results

3.1. FI CFI condition: 13 months

There was a within-subject effect of the clock on all response rate measures. As seen in Fig. 2, overall response rates were slightly higher in the FI component, [$F(1,36)=4.180$, $P=.048$], as were FI rates in bins 1 and 2 averaged [$F(1,36)=6.943$, $P=.012$], and bin 5 [$F(1,36)=7.943$, $P=.008$]. This effect is hard to see in the figure. It occurred because each rat had a higher FI than CFI rate, but the figure shows group averages.

There was a between-subjects effect of MeHg on overall rate [$F(2,36)=4.871$, $P=.013$] and bin 5 rate [$F(2,36)=5.173$, $P=.011$], but not on bins 1 and 2 averaged [$F(2,36)=2.070$, $P=.141$]. For both overall rate and rate in bin 5, the 5 ppm Hg group responded more than the controls and the 0.5 ppm Hg groups in the FI and CFI components (Ps<0.05 see Fig. 2).

There was no interaction between Se and MeHg for overall rate [$F(2,36)=.797$, $P=.458$], bin 5 rate [$F(2,36)=1.266$, $P=.294$], or bins 1 and 2 averaged [$F(2,36)=0.12$, $P=.887$].

For quarterlife, there was a within-subject interaction between component (FI vs. CFI) and Se exposure [$F(1,36)=9.386$, $P=.004$; Fig. 3, left panel]. Low Se rats displayed a lower quarterlife in the FI component than in the CFI, but for the high Se animals, quarterlife was approximately the same for the two components.

There was a between-subjects interaction of MeHg and Se on quarterlife [$F(2,36)=4.182$, $P=.023$; Fig. 3, left panel]. For both the FI and CFI components, the High Se controls had lower quarterlife values than all other exposure groups (Ps>0.1 for both components).

3.2. FI CFI condition: effects of age

For both overall response rate and response rate in bin 5, performance at 13 months did not differ from that at 20 months (Ps>0.1; not shown). For response rate in bins 1 and 2 averaged, there was an age by MeHg interaction for the CFI component [$F(1,31)=4.067$, $P=.027$]. The 5 ppm Hg groups,

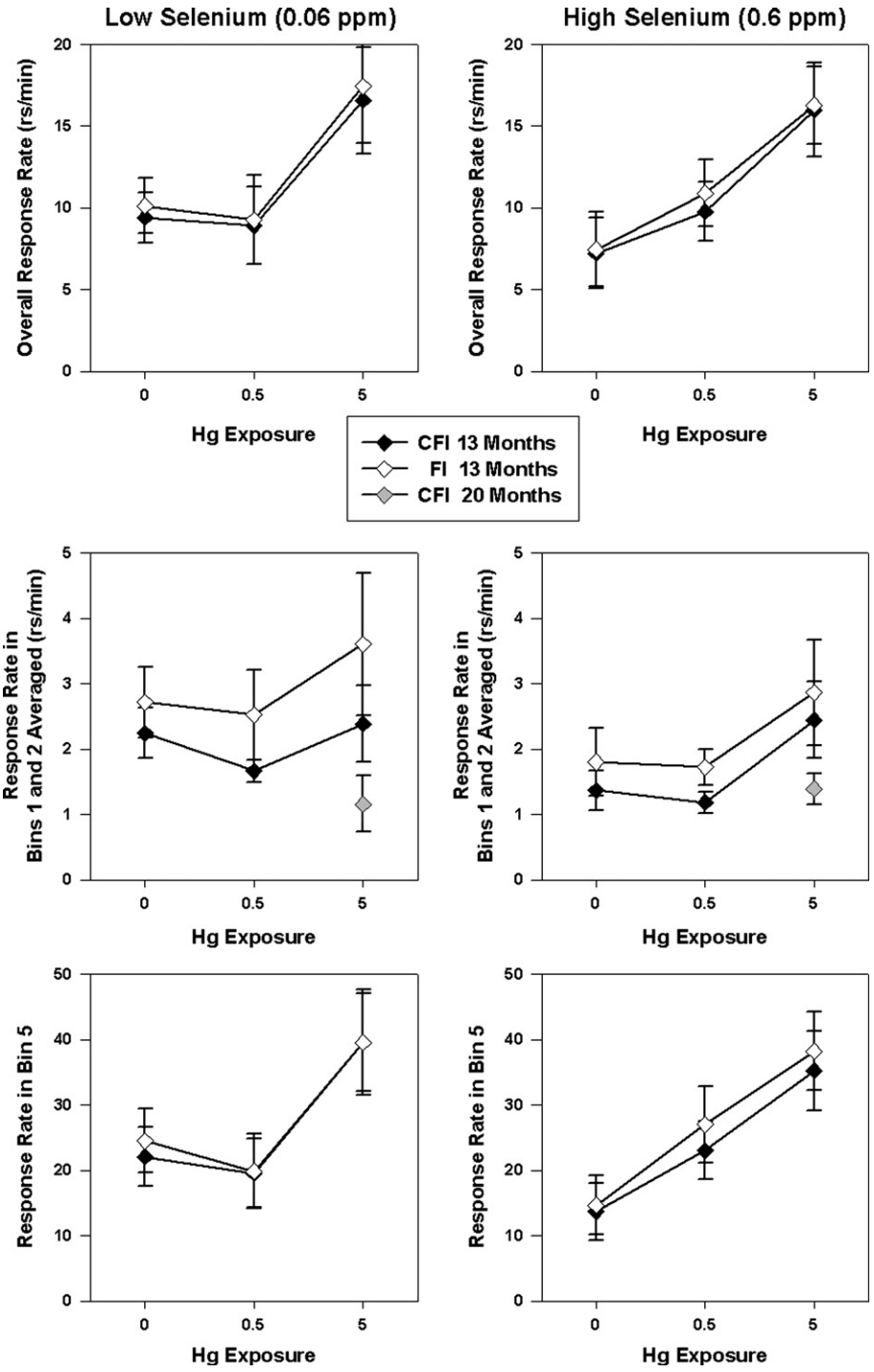

Fig. 2. *Mult* FI CFI performance at 13 months of age. Overall response rate (top), rate in bins 1 and 2 averaged (middle), and bin 5 rate (bottom) in the CFI (filled diamond) and FI (open diamond) components for the low Se (left) and high Se (right) MeHg groups. The gray diamond represents response rate in bins 1 and 2 averaged at 20 months of age for the 5 ppm Hg groups. No other age-related effects were detected and, to facilitate presentation, are not shown. Error bars represent ± 1 SEM.

regardless of Se exposure, had lower response rates at 20 months of age for the CFI, but not the FI, component (see lone gray diamonds in the middle panels of Fig. 2).

For quarterlife, there was interaction between age and Se for the CFI component [$F(1,31)=4.317$, $P=.046$; Fig. 3]. At 13 months of age, the low Se animals had greater quarterlife values in the CFI than in the FI component, indicating sharper stimulus control, but at 20 months of age, the CFI and FI quarterlife values were indistinguishable from one another, due mostly to a drop in the CFI quarterlife. A different pattern was obtained in the high Se group. At 13 months of age, the FI and CFI quarterlife values were almost identical, indicating little influence by the clock, but at 20 months of age, the quarterlife of the CFI increased and was greater than that of the FI, indicating

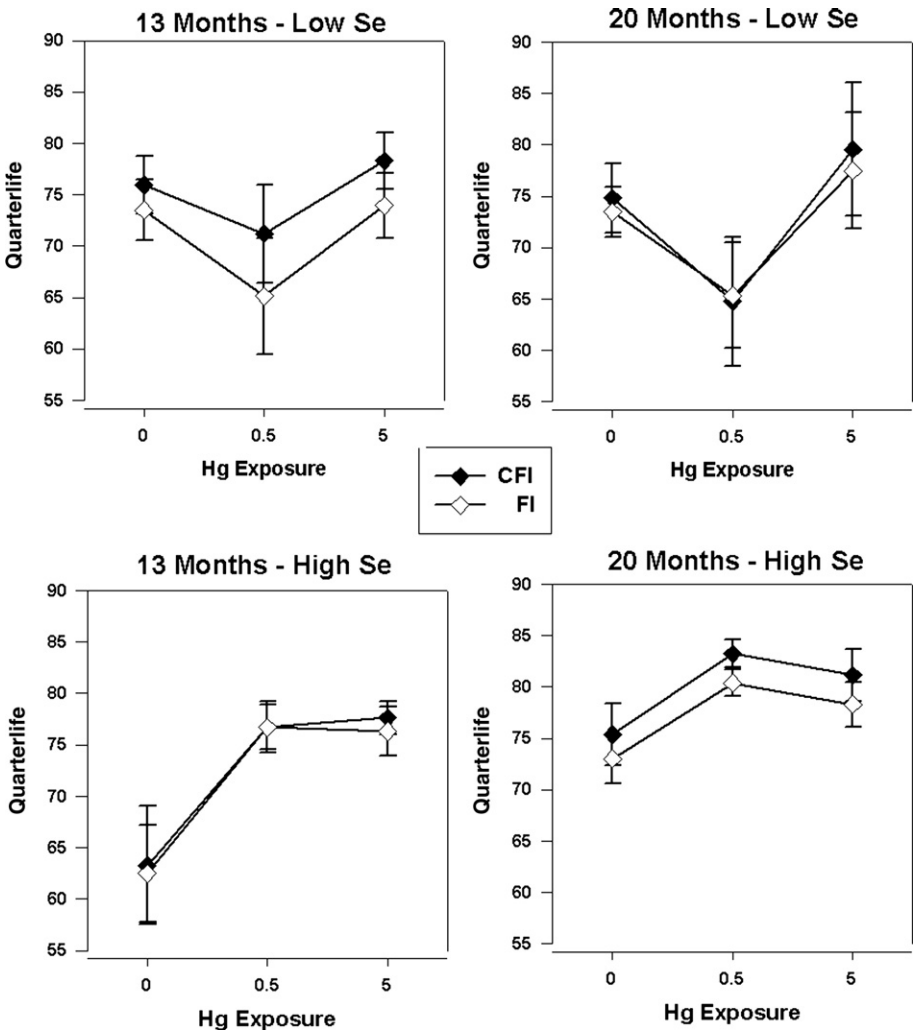

Fig. 3. *Mult* FI CFI performance at 13 and 20 months of age. Quarterlife for the CFI (filled diamonds) and FI (open diamonds) components for the low Se (top) and high Se (bottom) MeHg groups at 13 (left) and 20 months (right) of age. Error bars represent±1 SEM.

an improvement in stimulus control. Thus, there was sharper temporal control in the older high Se rats than in the older low Se rats, but the opposite was true when the rats were younger.

For no measure was there a between-subjects interaction of Se and MeHg (Ps>0.1), and no other statistically significant within-subjects effects of age or interactions with age were observed (Ps>0.1).

3.3. Reinforcement omission trials

After omission of the reinforcer, responding was greater in the CFI_{NF} than in the FI_{NF} component for all animals. The difference between the two components was largest during the initial sessions. This difference diminished across sessions; CFI_{NF} responding declined while FI_{NF} responding started low and declined relatively less, producing a within-subject interaction between the component and session number [$F(1,30)=144.4$, $P<.001$; Fig. 4].

There was a between-subjects effect of MeHg [$F(2,30)=3.6$, $P=.04$; Fig. 5]. The 5.0 ppm Hg group responded more in both the CFI_{NF} and FI_{NF} as compared with controls and the 0.5 ppm

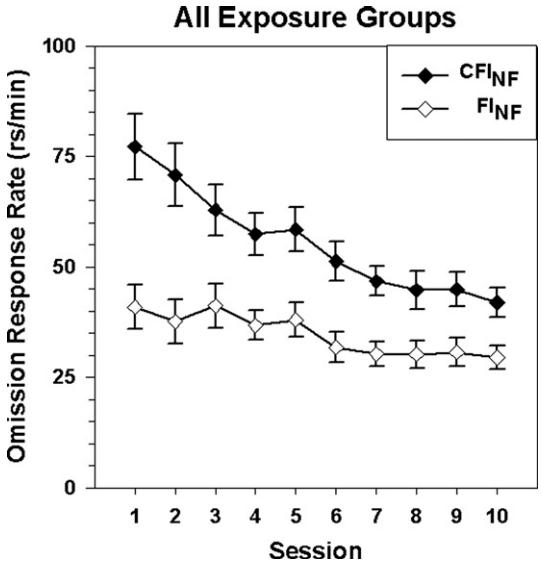

Fig. 4. Reinforcement omission procedure. Responses made during the last 240″ of the CFI_{NF} (filled diamonds) and FI_{NF} (open diamonds) components across sessions for all groups combined. Error bars represent±1 SEM.

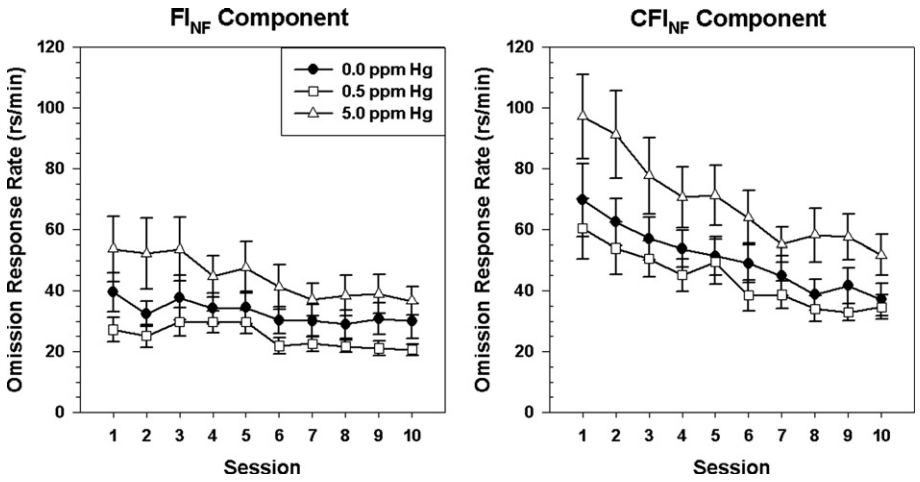

Fig. 5. Reinforcement omission procedure. Responses made during the last 240″ of the FI_{NF} (top) and CFI_{NF} (bottom) components across sessions for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles) combined across selenium exposure. Error bars represent ±1 SEM.

Hg groups (Ps<0.05). There was no Se by MeHg between-subjects interaction [$F(2,30)=.401$, $P=.943$], no interaction among session, Se and MeHg [$F(18,270)=.423$, $P=.982$], and no other statistically significant main effects or interactions were observed (Ps>0.1).

4. Discussion

Prenatal exposure to MeHg and a lifelong diet that was either marginal or rich in Se were manipulated in a 2 (Se)×3 (MeHg) factorial design, a design that allowed for the assessment of the main effects of both elements, as well as their potential interaction. Since little is known about the behavioral effects of Se, the ability to examine this trace element is a particular advantage of this design.

The fixed interval schedule was selected because both low- and high-rates of responding can be examined in the first and last portion of the interval, respectively. The clock procedure was added to determine whether behavior under strong stimulus control is relatively resistant to MeHg's developmental neurotoxicity. It has been noted in previous studies that stimuli correlated with response requirements provide a "behavioral prosthesis" that blunts the disruptive action of drugs [13,14] or adult-onset, chronic MeHg exposure [15]. The reinforcement omission trials permitted the evaluation of MeHg's effects on the resistance to change when intermittent components of the *Mult* FI, CFI were not reinforced.

4.1. Effects of the clock

Overall rate, bins 1 and 2 averaged rate, and bin 5 rate were all greater in the FI component than in the CFI component. For both overall rate and bin 5 rate, the error bars for the two components overlapped, graphically suggesting that the clock stimuli were ineffective. However, when individual rats' performance on the two components was examined (not shown), there was a consistent pattern of greater responding in the FI component for almost every rat. This consistent increase in the FI across subjects greatly reduced the within-subject variability and led to a statistically significant effect of the clock in the repeated-measures analyses.

The effect of the clock was clearest during the first 40% of the FI (bins 1 and 2 averaged). This is the portion of the interval in which the clock effect was predicted to be greatest based on the work with clocked FIs and pigeons [e.g. 16,17]. However, unlike the pigeon studies, in which zero rates of responding commonly occurred in first portion of the CFI, the rats in the current study made approximately two responses per minute early in the CFI component. To date, no other study has examined the effects of a clocked FI on rats' response rates, but the present study suggests that the influence of the clock is weaker in rats than in pigeons. This could be due to either species differences or the stimulus modality tested. Both visual and auditory stimuli were tested in the present study, and although data from the visual clock component are not shown here, the stimulus control was not substantial in that phase either. Perhaps some other modality, such as olfaction, would work better for the rat, since odors exert stronger stimulus control over a rat's behavior than visual and auditory stimuli [29].

Although the influence by the clock stimuli was weaker in rats than previously reported in pigeons, control was still manifested and was even amplified during the reinforcement omission trials: there was far greater responding in the CFI_{NF} component as compared with the FI_{NF} component, especially during the first few sessions, for all animals. Because the bin 5 stimulus remained on during the additional 240″, responding was expected to be high during omission trials, since there had previously been a consistent association between the bin 5 stimulus and the delivery of a reinforcer. The 2-fold increase in responding seen during the CFI_{NF} component demonstrates that the rats' behavior in the present study was under the control of these clock stimuli. However, as sessions progressed with the reinforcement omission procedure and rats experienced more

bin 5 stimulus presentations without reinforcement, the stimulus became less predictive and responding decreased rapidly. In the FI_{NF} component, there was no stimulus change associated with the passage of time, and responding was lower throughout the omission sessions.

4.2. Effects of MeHg

For both overall rate and rate in bin 5, rats exposed to 5 ppm Hg responded more than both controls and the 0.5 ppm Hg group in the FI and CFI components, effects that were seen at 13 and 20 months of age and that did not depend on Se exposure. The absence of an interaction between MeHg and the clock stimuli supports previous findings that MeHg does not disrupt discrimination processes [5,24,39,41]. The elevated responding by the 5 ppm Hg rats during bin 5, which persisted during the reinforcement omission trials when it was introduced at 21 months of age, is consistent with the hypothesis that MeHg enhances the reinforcer's efficacy. During the omission portion, response rates declined in all groups in both the FI and CFI components, but response rates were always greater in the 5 ppm Hg group compared to the other groups for all ten sessions.

There was no main effect of MeHg on quarterlife or on bins 1 and 2 averaged, though there was a nonsignificant trend towards greater responding by the 5 ppm Hg group during bins 1 and 2 averaged. The MeHg-induced increase in response rates and lack of MeHg effects on quarterlife are in contradiction to the findings of previous studies using pre- and post-natal exposure to MeHg in monkeys [10,39]. There are several potentially important procedural differences, however. The first is the timing and duration of MeHg exposure. The rats in the present study were exposed only *in utero*, which is comparable to exposure during the first two trimesters of human and monkey brain development but not the third trimester, which for rats occurs during the first days after birth [47]. The monkeys in the previous studies [10,39] were exposed during the third trimester. Since neurogenesis of the cerebellum and hippocampus dentate granule cells occurs primarily during the third trimester [40], the exposure regimens of the current and previous studies may result in different degrees of neurological damage to these areas. The second difference between the studies is the dependent measure. Acquisition of an FI schedule was examined in the previous studies, whereas the current study focused on steady-state performance. Regardless of the differences in findings, the FI schedule does appear to be sensitive to MeHg-induced changes in response patterns, and the findings of response rate increases in the present study are in accordance with previous findings [10,27,28,32,36,39] of MeHg-induced alterations in reinforcer efficacy and a resultant perseverative response pattern.

Thus, the present study supports previous findings of perseveration following developmental MeHg exposure [10,27,28,32,36,39]. Since the presence or absence of the clock stimuli did not influence MeHg's effects on response rates, the present study also supports previous studies that discrimination deficits [5,24,39,41] are not associated with developmental MeHg exposure. The latter finding is intriguing since responding has been modulated by the presence of external stimuli following adult-onset, chronic MeHg exposure in pigeons [15].

4.3. Effects of Se

At 13 months of age, there was little difference between the quarterlife values in the FI and CFI components for the high Se animals, suggesting a lack of stimulus control, whereas for the low Se animals, the quaterlife values for the CFI component were greater than those of the FI component. Selenium's effect on quarterlife reversed at 20 months of age, however. At 20 months of age, the low Se animals' quarterlifes for the two components became indistinguishable due to a drop in the CFI quarterlife, whereas the quarterlife of the CFI component increased for the high Se animals, suggesting an increase in stimulus control. There are few studies examining the behavioral effects of Se, so the interpretation of these findings is difficult. A previous study [36] from this lab examined the interaction between MeHg and Se on a spatial discrimination reversal procedure and found that a low-Se diet resulted in more trials without a response and more sessions to complete a reversal than a diet high in Se. Thus, low Se exposure was detrimental to performance in that procedure, making the interpretations of the young Se rats' performance in the present study even more difficult.

4.4. MeHg and Se interactions

In the present study, a diet rich in Se did not protect against the MeHg-induced rate increases seen in the 5 ppm Hg groups during the FI or the CFI component. In addition, Se did not influence the rate increases in the 5 ppm Hg group during the FI_{NF} and CFI_{NF} components. Se is hypothesized to protect against MeHg toxicity by binding Hg, thereby making it inert [34]. This hypothesis implies that protection would be conferred if the molar content of selenium exceeds that of mercury in the brain. Based on the molar ratio of Hg:Se in the brains of the current rats littermates [25], the lack of protection in the 5 ppm Hg group might be expected: both 5 ppm Hg groups had a 20-fold excess of Hg over Se in the brain [25]. However, for the 0.5 ppm Hg groups, the mean Hg:Se ratios in the low and high Se groups were 1.2 and 0.45, respectively. Thus, the High Se, 0.5 ppm Hg group had more Se than Hg, so MeHg effects might not be expected. For the Low Se, 0.5 ppm group, the Hg:Se ratio was close to 1.0, so effects are less predictable for this group. It remains possible that no interaction was detected in the 0.5 ppm exposure group because the range of Hg:Se ratios was too narrow. This restricted range is due to the difficulty in perturbing brain Se content, even in the face of far greater ranges of dietary Se [3,11] than the 10-fold range used here [25].

Thus, a failure to detect an interaction between developmental MeHg and Se could be due to any of several causes. There could be no interaction to detect. There could be an interaction but the difficulty of producing wide ranges of brain Se content makes it difficult to detect it. In these cases, MeHg–Se interactions are of minor relevance to public health considerations. It also remains possible that there are MeHg–

Se interactions earlier in development [8] that disappear in older animals or that are not apparent in fixed-interval responding or reinforcement omission procedures.

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