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Gestational Methylmercury Exposure Selectively Increases the Sensitivity of Operant Behavior to Cocaine

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Developmental methylmercury (MeHg) exposure alters dopamine neurotransmitter systems, but the selectivity of this and the effects of low, environmentally relevant MeHg exposure regimens are poorly understood. In previous reports, some including littermates of animals studied here, chronic, low-level exposures affected performance on reversal tasks and enhanced reinforcer efficacy. Using high- and low-rate operant behavior under a fixed interval (FI) schedule, sensitivity was examined to drugs that target noradrenergic and dopaminergic neurotransmitter systems. Female rats were exposed in utero to 0, 0.5, or 5 ppm of mercury, as MeHg, via maternal drinking water. Selenium (Se) is thought to attenuate MeHg's neurotoxicity, so animals consumed a diet containing 0.06 or 0.6 ppm of Se. At 11 months, they lever-pressed under a FI 120" schedule of sucrose reinforcement. Acute dose-effect curves were generated with cocaine, desipramine, SKF-38393, quinpirole, SCH-23390, and sulpiride. As compared with unexposed animals, those exposed to 5 ppm mercury, regardless of Se exposure, were 2 to 3 times more sensitive to the rate-reducing effects of high doses of cocaine and did not show increased responding earlier in the interval following moderate cocaine doses. Cocaine's effects in the 0.5 ppm Hg groups depended on dietary Se: low Se diet resulted in a rightward shift in the DEC compared to controls, whereas a high Se diet did not. No differential effects of MeHg were seen with the other drugs. Gestational MeHg exposure produces irreversible sensitivity to dopamine, but not norepinephrine, reuptake inhibitors and not to drugs that target D_1 or D_2 receptors.

Keywords: methylmercury, development, cocaine, dopamine, operant behavior

Acute administration of *d* amphetamine to animals exposed to methylmercury (MeHg) during early development has unmasked or amplified MeHg-induced changes in the acquisition of responding under a differential reinforcement of a low rate (DRL) reinforcement schedule (Eccles & Annau, 1982), acquisition and asymptotic rates of lever pressing during autoshaping (Hughes & Sparber, 1978), lever pressing under a differential reinforcement of high rate (DRH) schedule (Rasmussen & Newland, 2001) self-injurious behavior as adults (Wagner, Reuhl, Ming, & Halladay, 2007), and general locomotor activity (Cagiano et al., 1990). In addition, developmental exposure to MeHg causes transient increases in receptor sensitivity (Cagiano et al., 1990), reduces monoamine oxidase (MAO) activity in the brainstem (Beyrouty et al., 2006) and increases dopamine (DA) neurotransmitter levels, DA uptake, and DA turnover (Bartolome et al., 1982; Bartolome, Whitmore, Seidler, & Slotkin, 1984). These studies suggest that disruption of DA neurotransmission is a consequence of developmental exposure to MeHg.

Dopaminergic, noradrenergic, and serotonergic neurotransmitter systems all provide inputs to the prefrontal cortex and play important roles in choice and reinforcement processes (for review, see Robbins & Everitt, 1996; Wise, 2004). Low, clinically relevant, doses of methylphenidate are involved in attention tasks (Berridge et al., 2006), and reversal learning procedures (Seu, Lang, Rivera, & Jentsch, 2008) that involve prefrontal cortex activity, implicating a role for both DA and norepinephrine. DA systems are activated by unpredicted changes in reinforcer delivery, reinforcer preference, and the acquisition of reinforced behavior (Schultz, Tremblay, & Hollerman, 2000). The D_1 and D_2 receptor subtypes play different roles in mediating these forms of behavioral plasticity depending on the neuroanatomical region examined and the task being performed (Andrzejewski, Spencer, & Kelley, 2005; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Robbins, 2005). Some recent reports suggest that simultaneous activation of D_1 and D_2 receptors may be required to provoke changes in postsynaptic pulsing of action potentials in nucleus accumbens (Hopf, Cascini, Gordon, Diamond, & Bonci, 2003) and in reversal learning procedures (Calaminus & Hauber, 2008; Floresco et al., 2006).

Similar behavioral processes are modified by developmental MeHg exposure (Newland, Donlin, Paletz, & Banna, 2006). Visual and spatial reversal learning are significantly retarded, especially during the first reversal (Paletz, Craig-Schmidt, & Newland, 2006; Paletz, Day, Craig-Schmidt, & Newland, 2007; Reed, Paletz, & Newland, 2006), but an extradimensional shift, from spatial to visual reversal learning, and back to spatial, is unaffected (Paletz et al., 2007). The acquisition of choice is also slowed, especially in older animals (Newland, Reile, & Langston, 2004). More interesting, the efficacy of primary reinforcers, as detected with progressive ratio procedures, is enhanced, as indicated by the tolerance of

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larger response:reinforcer ratios in adulthood (Paletz et al., 2006; Reed, Banna, Donlin, & Newland, 2008). We hypothesize that these behavioral effects of developmental MeHg exposure are associated with changes in DA neurotransmitter function. This hypothesis is tested here using drug challenges conducted with littermates of some of the rats examined in other studies (Reed et al., 2006).

MeHg may also alter selenium (Se) function in the central nervous system by forming an insoluble Se-Hg complex in the brain (Ralston, Blackwell, & Raymond, 2007). This diverts Se from selenoprotein synthesis (Vahter et al., 1995) and suppresses the activity of glutathione peroxidase, a selenoenzyme (Nishikido, Furuyashiki, Naganuma, Suzuki, & Imura, 1987). Reductions in Se levels cause an increase in DA levels, DA turnover, and various DA metabolites (Castano et al., 1997; Castano, Cano, & Machado, 1993). Consequently, the neurobehavioral toxicity of MeHg could arise from the decreased bioavailability of Se in the brain due to its binding to Hg (Watanabe, Yin, Kasanuma, & Satoh, 1999), thereby leading to altered DA functioning and increased sensitivity to DA agonists (Cagiano et al., 1990; Eccles & Annau, 1982). Increasing dietary Se levels could alleviate MeHg's toxicity (Ralston et al., 2007), a second hypothesis tested here.

The present experiment was designed to (a) examine interactions between MeHg and Se on low- and high-rate operant behavior using a fixed interval (FI) schedule of reinforcement, (b) extend previous reports of MeHg–DA interactions to more specific agonists, and (c) determine whether particular DA receptor subtypes are responsible for these interactions. Pregnant rats consumed drinking water containing methylmercuric chloride (0, 0.5, or 5 ppm of Hg) throughout gestation until Postnatal Day 16, and a diet that was either marginal (0.06 ppm) or rich (0.6 ppm) in Se. This can be considered a gestational-only MeHg exposure regimen because there is negligible mercury exposure via nursing (Newland & Reile, 1999). Their female offspring, who were exposed to MeHg only during gestation but continued their respective Se diet, were examined. The MeHg concentrations chosen produce brain concentrations of 0.1 to 0.3 ppm (low dose) to about 20to 30 ppm (high dose), or approximately 1 to 20 micromolar concentrations in the brain, levels that are relevant to estimating human exposures (Burbacher, Rodier, & Weiss, 1990; Newland, Reed, LeBlanc, & Donlin, 2006). The Se diets were at the low and high end of recommended intakes: the 0.06 ppm Se concentration is a nutritionally adequate level for rodents (National Research Council, 1995; Reeves, Nielsen, & Fahey, 1993), and the 0.6 ppm concentration represents an excess over the AIN-93 formulation, which contains 0.15 ppm of Se (Reeves, 1997; Reeves et al., 1993), but is below that thought to be toxic (Abdo, 1994).

As adults, the offspring were trained to lever press under a FI schedule of reinforcement. A FI schedule of reinforcement was selected because both low and high rates of responding can be examined in the first and last portion of the interval, respectively. Drug challenges with cocaine, desipramine, SKF-38393, quinpirole, SCH-23390, and sulpiride commenced after behavior stabilized. Cocaine, a DA reuptake inhibitor and desipramine, a noradrenergic reuptake inhibitor, were used to investigate the involvement of those neurotransmitter systems. SKF-38393, a D_1 agonist, and SCH-23390, a D_1 antagonist, were used to examine alterations of the D_1-like receptor subtype. Quinpirole, a D_2 agonist, and sulpiride, a D_2 antagonist, were used to access variations in the D_2-like receptor subtype.

Method

Subjects

The subjects were 42 female Long-Evans rats (F_1 generation) bred (described below) and housed in a temperature- and humidity-controlled, AAALAC-accredited colony room that was maintained on a 12-hr light–dark cycle (lights on at 7 a.m.). Females were used to facilitate comparisons with adult-onset MeHg exposures conducted with the dams after parturition in other experiments. Each subject was randomly selected from a separate litter, so the litter served as the statistical unit for all analyses. The rats were exposed in utero to MeHg via maternal drinking water containing 0, 0.5, or 5 ppm of Hg as methylmercuric chloride (Alfa Aesar, Ward Hill, MA) and a diet containing approximately 0.06 or 0.6 ppm Se, which continued throughout life (detailed below) forming a 2 (chronic Se) $\times$ 3 (developmental MeHg) factorial design. There were 5 to 8 rats per experimental group.

After weaning on postnatal day (PND) 21, the subjects were injected subcutaneously with an electronic identification chip (Biomedic Data Systems, Seaford, DE) and housed in standard 22.9 cm $\times$ 45.7 cm $\times$ 19 cm plastic "shoebox" cages with a wire top and solid bottom. Rats were weighed 5 days a week from the day of weaning until the end of the experiment. 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 (individually adjusted) so as to maintain their body weight at 250 g. Rats that shared a home cage also received the same Se diet (see Exposures), so diets were never mixed. To prevent excessive tooth growth, a cleaned, nylon chew "bone" was freely available in the home cage. The rats were 11 $\pm$ 1 month of age ($\sim$335 days of age) at the beginning of the present experiment. This study was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996).

SE Exposure: F_0 and F_1 Generation

At 18 weeks (125 days) of age, female F_0 breeders (mothers 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 Figure 1). The "low-Se" diet contained Se from casein only at a nominal concentration of 0.06 ppm. The "high-Se" diet was supplemented with sodium selenite to produce 0.6 ppm. The lower concentration is the lowest possible with a casein-based diet; and the actual concentration can vary somewhat. Se content was analyzed with each diet shipment using inductively coupled plasma mass spectrometry (ICP-MS). The actual concentrations were usually between 0.05 and 0.07 ppm (one shipment used for adult consumption contained 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

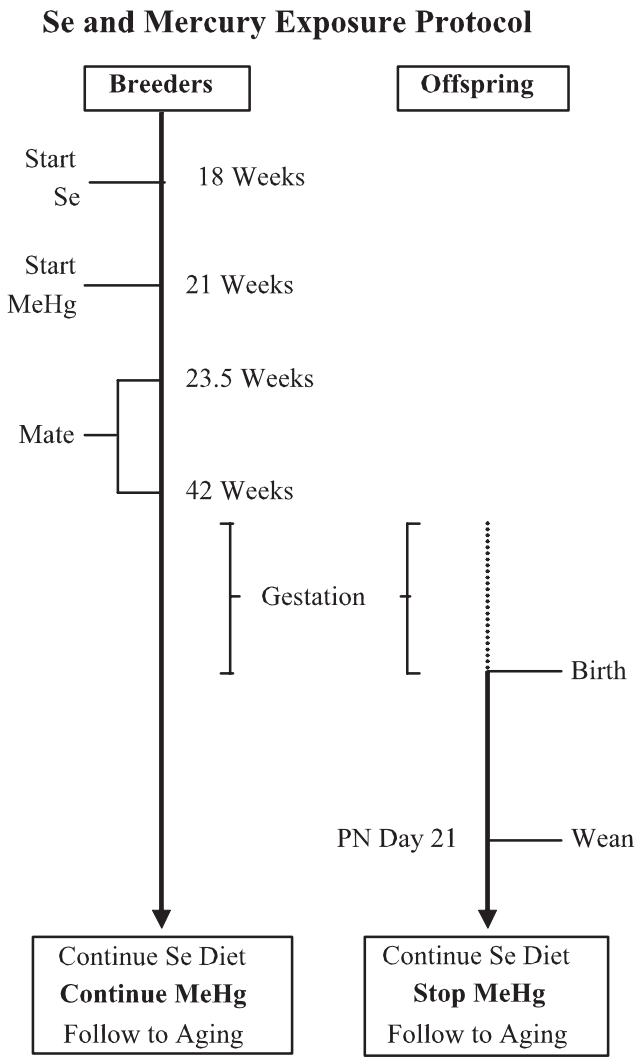

Figure 1. Timeline for breeding and exposure for F_0 breeders and F_1 offspring. Note that maternal exposure to methylmercury (MeHg) ended at 16 days and was reinstated after weaning. Functionally, exposure to the F_1 offspring ended at birth (Newland & Reile, 1999; Stern, Cox, Cernichiari, Balys, & Weiss, 2001). Breeders were not included in the present experiment.

obtained from Research Diets, Inc. (New Brunswick, NJ). Dietary mercury was below the detectable level of 50 ppb. Male breeders were maintained on the chow diet, except when briefly exposed to the F_0 female's diet during breeding (see Breeding). All F_1 offspring received the same diet as their maternal dams throughout life.

Methylmercury Exposure: F_0 Generation Only

At approximately 21 weeks (145 days of age), after 3 weeks (20 days) on the custom Se diets, each Se group of F_0 breeders was further divided into three MeHg exposure groups, matched for bodyweight, to create 6 experimental groups. MeHg was added to the breeders' drinking water in concentrations of 0, 0.5, or 5 ppm of mercury (Hg) as methylmercuric chloride, (Alfa Aesar, Ward Hill, MA; hereafter the MeHg groups are referred to as 0, 0.5, or 5 ppm Hg). 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 (Newland & Reile, 1999). Fluid consumption reported in the earlier paper was confirmed by taking periodic measurements of water intake. Drinking water was prepared from a stock solution containing 15 ppm of Hg as MeHg. Actual Hg concentrations were determined by atomic absorption when a new dilution was created and were found to be within 10% of the target values.

Maternal exposure to the MeHg-containing water was discontinued on PND 16 when the F_1 pups were capable of reaching the waterspout. Throughout the remainder of life, all F_1 rats received plain tap water to drink. Male breeders only drank tap water.

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. 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 was confirmed by the presence of sperm in the vagina, and continued until systematic increases in daily body weight were observed, suggesting gravidity. Births before 5 p.m. were assigned to PND 0 for that day. All births after 5 p.m. were assigned to PND 0 for the subsequent day. Large litters were culled to produce eight F_1 pups including at least three females when possible, but only one female, randomly chosen from some of the litters, was used in the present study.

All rats were monitored daily by the research staff or personnel from the Department of Laboratory Animal Health at Auburn University. 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.

Experimental Chamber

The experiments were conducted in 16 commercially purchased operant chambers (Med Associates, Inc. model Med ENV 007, St. Albans, Vermont) containing two front levers (each calibrated so that 0.20 N registered a press), a 45 mg sucrose pellet dispenser situated between the two front levers, Sonalert tones (2,900 and 4,500 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"L × 91/2"W × 111/2"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 exper-

iments 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.

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 16 rats were tested sequentially beginning at 9 a.m. with each squad being tested at approximately the same time every day Monday through Friday. 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 ensure they were placed in the appropriate chamber and home cage.

Training. On reaching adulthood, pressing the right lever was established using autoshaping (Newland et al., 2004). After sucrose-maintained responding was robustly maintained, an FI 15", FI 30", FI 90", and FI 120" schedule was introduced over four sessions. The reinforcement cycle comprised a 0.5" 4,500 Hz tone and delivery of a 45 mg sucrose pellet (Research Diets, Inc., New Brunswick, NJ).

FI schedule. A multiple schedule was arranged in which an FI 120" and clocked FI (CFI) 120" schedule alternated through the course of the session. When the FI 120" schedule was in effect, the first lever-press after 120" produced a sucrose pellet. The CFI, which involved presenting distinct auditory stimuli during different portions of the FI schedule, will not be described in the current paper (see Reed & Newland, 2007, for a review of the CFI effects). Before the present study began, these rats were in a closely related study in which visual stimuli (distinct lights) were used in the clock FI component. No drugs were administered during that study.

Drug challenges. After animals' response rates stabilized, which required approximately 2 months, acute dose-effect curves were determined for all 42 rats in the following order: cocaine (1 to 40 mg/kg), desipramine (0.3 to 17 mg/kg), SKF-38393 (1 to 17 mg/kg), quinpirole (0.03 to 1 mg/kg), SCH-23390 (5.6 to 100 μg/kg), and sulpiride (3 to 100 mg/kg). Cocaine, desipramine, quinpirole, and SCH-23390 were dissolved in saline and administered intraperitoneal in a 1 ml/kg volume. SKF-38398 was administered in a 2 ml/kg volume. Sulpiride was dissolved in a 0.1 N HCl and saline solution and administered intraperitoneal in a 1 ml/kg volume.

After injection, the rat was placed into the experimental chamber. Ten minutes elapsed between an injection and the onset of the session. Cocaine, desipramine, SKF-38398, quinpirole, and low doses of the DA antagonists, SCH-23390 and sulpiride, were administered Tuesdays and Fridays. Higher doses of the DA antagonists were administered once weekly. Generally, an ascending sequence of doses was used, but sometimes intermediate doses were repeated after the dose-effect curve was determined. Mondays and Wednesdays were noninjection control days. Vehicle injections were administered on Thursdays. If a drug reduced response rates to nearly zero for a particular animal, the next dose of that drug was not administered to reduce the risk of drug toxicity. Therefore, not all rats received the highest dose. There was one week of nondrug sessions between drugs. The rats were tested in age-matched squads, so they were between 13 and 14 months of age at the beginning of drug challenges and 18 to 19 months of age by the end of the experiment.

Data and Statistical Analyses

The FI 120" schedule was divided equally into five, 24" bins. Session averages within the bins were computed. Three dependent variables were analyzed:

1. *Overall response rate (rs/min)*—The total number of responses throughout the interval divided by 2 min.

2. *Response rate in the first two bins*—The total number of responses for Bins 1 and 2 divided by the time available to respond (0.8 min). Bins 1 and 2 were combined due to the low rate of responding in Bin 1. This variable was used to examine Se and MeHg effects on low-rate responding.

3. *Response rate in Bin 5*—The total number of responses in the last 24" of each interval divided by 0.4 min. This variable was used to examine Se and MeHg effects on high-rate responding.

Log transforms were performed on response rate in the first two bins because variability increased proportionally with magnitude. Data representing noninjected controls came from four or five control sessions and a separate control was used for each round of drug injections. All saline and drug effects were then expressed as a proportion of this control value. Proportion of control was used, instead of raw rates, because response rates for the groups differed (Reed & Newland, 2007). This allowed sensitivity to drug dose to be evaluated while adjusting for baseline response rate differences.

All statistical analyses were performed using SYSTAT 11 (SYSTAT Software Inc. Richmond, CA). The Type I error rate (α) was set at .05 for all omnibus tests and Tukey's post hoc comparisons. A repeated-measures analysis of variance (RMANOVA) was performed for each dependent variable. MeHg (0. 0.5, 5 ppm) and Se (0.06 ppm, 0.6 ppm) served as the two between-subjects factors, with 5 to 8 rats per cell. Drug dose served as the within-subject factor. F ratios, degrees of freedom, and p values are reported for all significant RMANOVAs, and p values are reported for nonsignificant RMANOVAs and selected post hoc contrasts and comparisons. Where appropriate, usually where the adjustment to the degrees of freedom was less than 0.8, Huyn–Feldt or Greenhouse–Geiser adjustments to degrees of freedom were used to account for lack of sphericity in the dataset.

With the experimental design used, many statistical tests can, and often should, be conducted. The drug challenges were conducted to test specific hypotheses regarding the sensitivity of MeHg-exposed animals' behavior to drugs selected to represent specific neurotransmitter actions. For cocaine, all effects are re-

ported with an emphasis on interactions between drug dose and MeHg or Se exposure. For other drugs, analyzes were conducted to detect effects different from those seen with cocaine because the question of interest was whether any specific receptor subtype is responsible for the effects seen following cocaine administration. Thus, these interactions are emphasized in describing the results and in selecting those to emphasize graphically.

Results

Baseline

Effects on nondrug sessions effects have been described elsewhere (Reed & Newland, 2007) and will be summarized briefly here (see Table 1 for baseline rates). For both overall rate, $F(2, 36) = 4.871$, $p = .013$, and rate in Bin 5, $F(1, 36) = 7.943$, $p = .008$, the 5 ppm Hg group responded more than the controls and the 0.5 ppm Hg groups in the FI during baseline sessions. Response rates did not differ among the groups during the first two bins during baseline, $F(2, 36) = 2.070$, $p = .141$. 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 the first two bins, $F(2, 36) = 0.12$, $p = .887$.

Cocaine

For all dependent measures, there was a main effect of cocaine dose ($ps < .001$). For overall rate (see Figure 2), there was an interaction among cocaine dose, Se, and MeHg, $F(12, 216) = 2.267$, $p = .028$, which was due primarily to the 0 ppm and 0.5 ppm Hg groups. The low Se, 0.5 ppm Hg group responded more than the high Se, 0.5 ppm group for doses 5.6 to 17 mg/kg of cocaine ($ps < .05$), producing an inverted U-shaped dose-effect curve. As the large error bars show for this group, there was heterogeneity across subjects in their responsiveness to cocaine. Some animals showed very high response rates at these intermediate doses. For the controls, the high Se, 0 ppm Hg group responded more than the low Se, 0 ppm Hg group at the highest dose of cocaine ($p < .01$). The elevated responding and variability in the high Se, 0 ppm Hg group reflected individual differences in responsiveness to cocaine; some animals showed rate increases even at this high dose and others showed rate decreases. The 5 ppm Hg groups did not differ from each other ($ps > .1$).

For measures of low-rate behavior (see Figure 3), there was an interaction between cocaine dose and MeHg, $F(12, 210) = 2.388$, $p = .048$. The higher cocaine doses produced a substantial cocaine-induced rate increase, sometimes as high as 10 to 15-fold, for all but the 5 ppm Hg group. For high-rate behavior in Bin 5 (see Figure 4), there was a between-subjects effect of MeHg, $F(2, 36) = 6.556$, $p = .004$. The 5 ppm Hg groups, regardless of Se exposure, responded less than the controls and 0.5 ppm Hg group ($ps < .05$).

Table 1
Baseline Response Rates

	MeHg	Overall rate	Bins 1 and 2	Bin 5
Low Se	0.0 ppm	13.1 ± 1.7	2.7 ± 0.5	29.5 ± 4.9
	0.5 ppm	9.3 ± 2.7	2.5 ± 0.7	19.8 ± 5.6
	5.0 ppm	17.4 ± 3.5*	3.6 ± 1.1	39.5 ± 8.1*
High Se	0.0 ppm	7.5 ± 2.3	1.8 ± 0.5	14.6 ± 4.6
	0.5 ppm	10.9 ± 2.1	1.7 ± 0.3	27.0 ± 5.8
	5.0 ppm	16.3 ± 2.4*	2.9 ± 0.8	38.2 ± 6.0*

Note. Given as responses/minute ($M \pm SEM$). MeHg = methylmercury; Se = selenium.
* $p < .05$ (ANOVA 5 ppm Hg group vs. 0 ppm and 0.5 ppm Hg groups).

Desipramine

Desipramine decreased overall rate, rate in the first two bins, and Bin 5 rate ($ps < .001$). There were no effects of MeHg nor were there any interactions between MeHg and desipramine dose on overall rate (not shown), low-rate behavior in the first two bins (not shown), or high-rate behavior in Bin (Figure 5; $ps > .1$).

Specific Agonists

Figure 6 shows the effects of the specific D1 and D2 agonists and antagonists on overall response rate. Effects on low- and high-rate behavior are described in the narrative when relevant.

SKF-38393, a D_1 agonist, reduced overall response rate and high-rate responding in Bin 5, ($ps < .001$) but had no effect on low-rate responding in the first two bins, $F(5, 180) = 1.946$, $p = .092$. There were no interactions between MeHg and SKF-38393 dose, Se and SKF-38398, or an interaction among MeHg, Se, and SKF-38398 dose on overall rate and low- and high-rate responding ($ps > .1$).

There was an effect of quinpirole dose, a D_2 agonist, on all measures ($ps < .001$). Quinpirole produced a complex dose-effect relationship in all exposure groups. For overall rate, there appeared to be elevated responding in the low Se, 0.5 ppm Hg group compared to the other groups (see Figure 6), but statistically there was no interaction among MeHg, Se, and quinpirole dose, $F(14, 245) = 1.578$, $p = .165$, and no interaction between MeHg and quinpirole dose, $F(14, 245) = 1.254$, $p = .287$. Because graphically there appears to be an interaction with Se that was not detected in statistical tests, it seemed possible that there was inadequate power to detect a three-way interaction. An exploratory analysis was conducted with the low Se animals only to see if a two-way interaction between MeHg and quinpirole could be detected. Even with the high Se animals removed, there was still no interaction between MeHg and quinpirole dose by conventional standards, $F(14, 126) = 2.21$, $p = .064$. In addition, there were no interactions between MeHg and SKF-38398 dose, Se and SKF-38398, or an interaction among MeHg, Se, and SKF-38398 dose ($ps > .1$) for any of the three measures.

Specific Antagonists

For all three measures, there were rate-decreasing effects of SCH-23390, a D_1 antagonist ($ps < .001$), but no interactions between MeHg and SCH-23390 dose, Se and SCH-23390, or an interaction among MeHg, Se, and SCH-23390 dose ($ps > .1$, Figure 6). On all three measures, sulpiride, a D_2 antagonist, also caused a dose-related decrease in responding ($ps < .001$), but on no measure was there an interaction between MeHg and quinpirole dose, Se and quiniprole, or an interaction among MeHg, Se, and quinpirole dose ($ps > .1$, Figure 6).

Discussion

Gestational MeHg exposure selectively increased the sensitivity of behavior to the DA reuptake inhibitor, cocaine, in fully adult

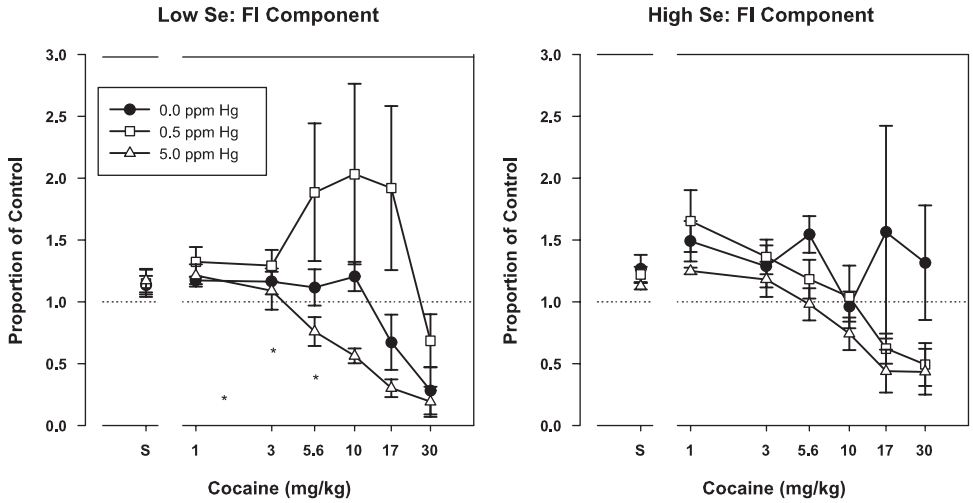

Figure 2. Overall response rate (responses/minute) for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles) for the low Se (left) and high Se (right) across cocaine doses. Error bars represent ± 1 *SEM*. ($p < .05$ post hoc comparison (∗) low Se, 0.5 ppm Hg vs. high Se, 0.5 ppm Hg (^) low Se, 0 ppm Hg versus high Se, 0 ppm Hg).

rats. This is evidence that MeHg exposure while the nervous system is developing causes a change in DA function that is irreversible, and that has important behavioral consequences. For high-rate behavior, the magnitude and direction of the MeHg-induced sensitivity to cocaine was nearly identical to that seen with amphetamine in an earlier study (Rasmussen & Newland, 2001). The increase in low-rate responding during the first portion of the FI interval, which is typically associated with lower, and more clinically relevant, doses of psychomotor stimulants, did not appear in the animals exposed to 5 ppm MeHg but was amplified in the 0.5 ppm-exposed animals. The present study extends earlier ones (Cagiano et al., 1990; Eccles & Annau, 1982; Hughes & Sparber, 1978; Rasmussen & Newland, 2001) by providing detailed dose-effect relationships across a wide, more than an order of magnitude, range of doses (see also Rasmussen & Newland, 2001) and by using cocaine, a different DA transporter blocker than used previously. As in an earlier study (see also Rasmussen & Newland, 2001), the present study uses an environmentally relevant MeHg exposure regimen that produces only subtle signs of behavioral toxicity.

The enhanced sensitivity to DA transporter blockers and the absence of effects with the other drugs narrows the range of

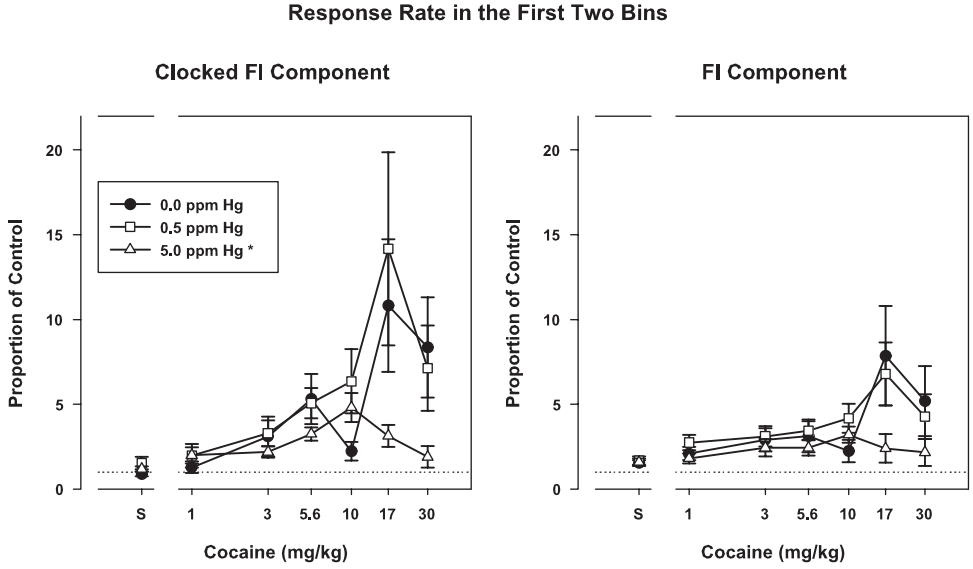

Figure 3. Low-rate behavior for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles), combined across selenium exposure, across cocaine doses. Error bars represent ± 1 *SEM*. ($^{*}p < .05$ ANOVA comparison 5 ppm Hg vs. 0 ppm and 0.5 ppm Hg group).

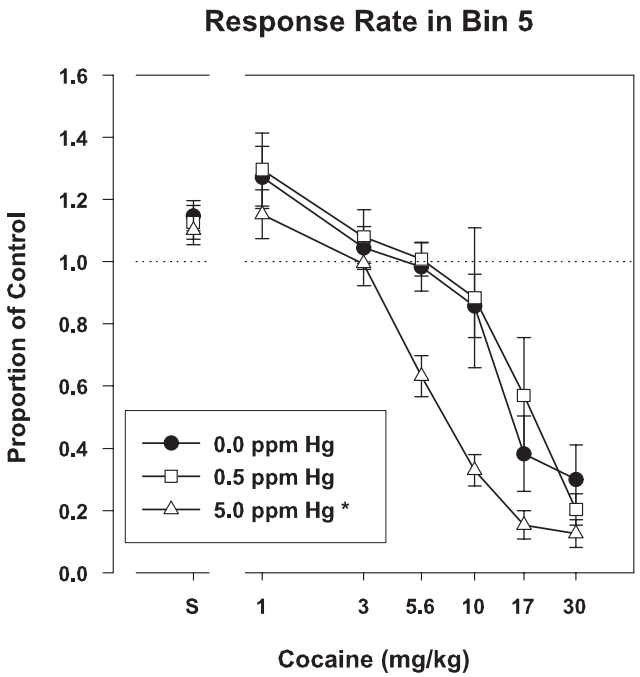

Figure 4. High-rate behavior for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles), combined across selenium exposure, across cocaine doses. Error bars represent ± 1 *SEM*. ($p < .05$ ANOVA comparison (∗) 5 ppm Hg vs. 0 ppm and 0.5 ppm Hg group). Reprinted with permission from "Methylmercury and nutrition: Adult effects of fetal exposures in experimental models," by M. C. Newland, E. M. Paletz, and M. N. Reed, 2008, *NeuroToxicology, 29,* pp. 783–801. Copyright 2008 by Elsevier.

possible mechanisms by which MeHg-induced neurotoxicity might arise and suggests others. Developmental MeHg exposure impairs the development of the frontal cortex (Barone, Haykal-Coates, Parran, & Tilson, 1998), which is rich in DA receptors. Insofar as this neuropathology interferes with the formation of mesocortical DA pathways or reduces the number of DA receptors, diffuse supersensitivity of remaining receptors might be hypothesized (Cagiano et al., 1990; Wagner et al., 2007). If such supersensitivity appears, however, then it is not specific to D1 of D2 receptors because MeHg-exposed rats responded similarly to controls when administered direct D_1 and D_2 agonists and antagonists. Enhanced sensitivity to amphetamine in young rats has been related to DA receptor density, but those effects did not persist into adulthood (Cagiano et al., 1990). In adult mice, amphetamine-induced stereotypy and self-injurious was enhanced by developmental MeHg exposure, albeit at a higher dose than used here, and this was accompanied by MeHg-induced changes in cortical and striatal dopamine activity (Wagner et al., 2007).

With regard to presynaptic mechanisms, it can be noted that MeHg alters calcium homeostasis in the nerve terminal (Atchison, Joshi, & Thornburg, 1986; Sirois & Atchison, 1996, 2000). The resulting calcium excess would increase neurotransmitter release, which in term, could result in greater sensitivity to reuptake inhibitors such as cocaine or amphetamine. Evidence against this hypothesis comes from the absence of an interaction with desipramine, a NE reuptake inhibitor. Alterations in calcium stores or influx would be expected to produce nonselective sensitivity to reuptake inhibitors, but the effects are specific to cocaine and DA. Moreover, this process requires the presence of MeHg in nerve terminals, and there is no detectable MeHg in the brains of adults exposed during gestation (Newland & Reile, 1999).

Another possibility comes from reports that developmental MeHg exposure produces lasting changes in brain-stem concentrations of MAO (Beyrouty et al., 2006). If this occurs in other regions then it could result in enhanced sensitivity to cocaine and amphetamine. However, It would likely increase sensitivity to desipramine, too and no such increase was seen here, so this mechanism seems unlikely.

It is noteworthy that the MeHg-exposed animals were sensitive to DA reuptake inhibitors here, and in earlier reports, but were not differentially sensitive to direct agonists. It is unlikely that an effect of these receptor-subtype specific drugs was somehow missed because the dose-effect curve spanned doses too low to be behaviorally active and doses sufficiently high that they significantly reduced responding. Coactivation of D_1 and D_2 receptors in the basal ganglia and the nucleus accumbens result in an increase of postsynaptic intracellular calcium levels via a signaling pathway that is not activated by either receptor alone (Lee et al., 2004). In addition, there is a DA-induced enhancement of spike firing in nucleus accumbens neurons that requires both receptor subtypes (Hopf et al., 2003). Thus, sensitivity to cocaine and amphetamine, but not the specific agonists, may be because these drugs increase synaptic DA and thereby activate both D_1 and D_2 receptors. This suggests that MeHg's developmental neurotoxicity involves postsynaptic signaling pathways that are initiated by coactivation of D_1 and D_2 receptors.

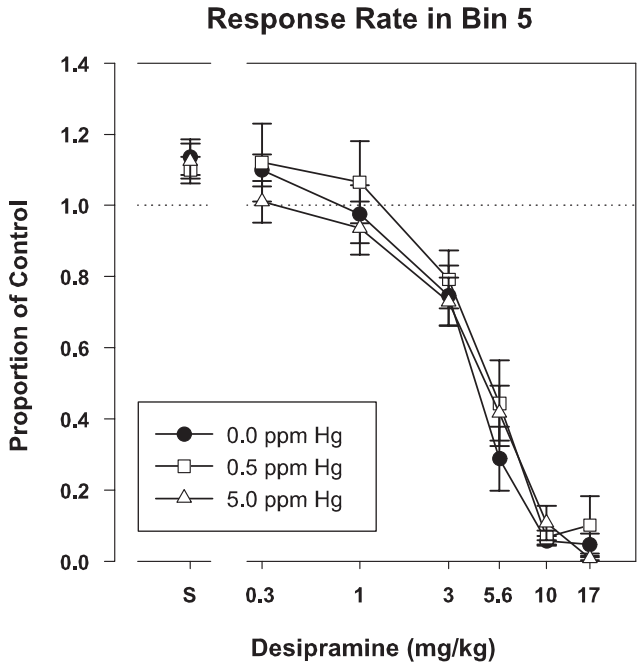

Figure 5. High-rate behavior for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles), combined across selenium exposure, across desipramine doses. Error bars represent ± 1 *SEM*.

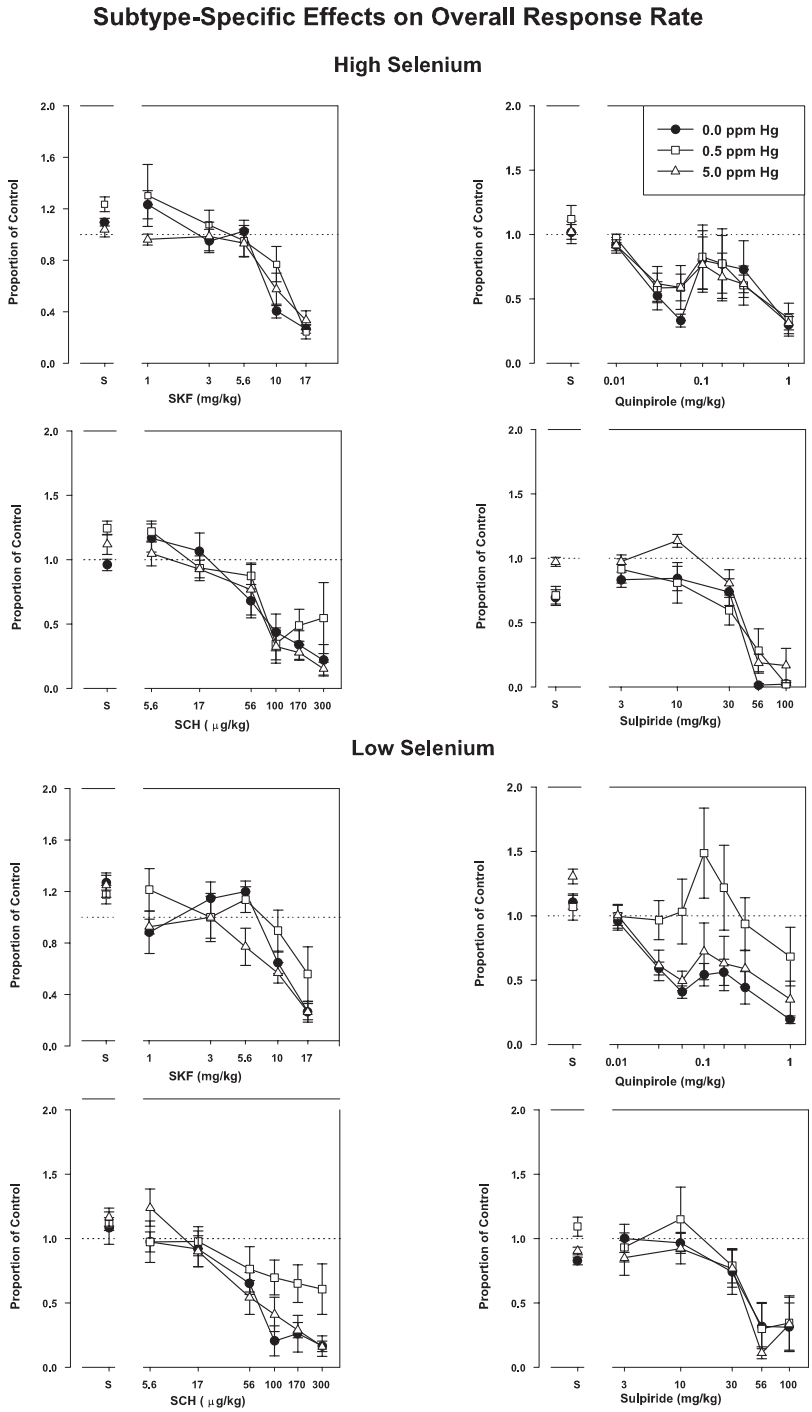

Figure 6. Overall response rate for the 0.0 ppm Hg group (filled circles), 0.5 ppm Hg group (open squares), and 5 ppm Hg group (open triangles) for the low Se (top panel) and high Se (bottom panel) across various drug doses. Error bars represent ± 1 *SEM*.

Cocaine has serotonergic activity that might be involved in the effects reported here (Fleckenstein, Gibb, & Hanson, 2000). Serotonergic drugs modulate the discriminative stimulus properties of cocaine (Kleven & Koek, 1998; McMahon & Cunningham, 2001), cocaine's effects on locomotor activity (Bubar, McMahon, De Deurwaerdère, Spampinato, & Cunningham, 2003) and its effect on behavior under an FI schedule (Spealman, 1993). Overall, the actions of SSRI's on cocaine's psychomotor stimulant effects depend on dose, the type of response studied, and baseline rates. This is broadly analogous to the

pattern seen in cocaine-MeHg interactions in Figure 2. In mice, PN MeHg exposure resulted in long-lasting alterations in serotonin activity in striatum and cortex (Wagner et al., 2007). The possibility that serotonin neurotransmitter systems are involved in the actions of cocaine reported here cannot be ruled out at present.

Dietary Se was manipulated to test hypotheses that this nutrient may ameliorate MeHg's neurotoxicity. Se binds MeHg to form insoluble complexes that diminish its neurotoxicity whereas simultaneously reducing the bioavailability of Se (Ralston et al., 2007). According to this hypothesis, when the molar levels of MeHg exceed Se, the bioavailability of Se and its protection would be diminished, possibly creating behavioral deficits. Se did not modify the effects of MeHg on cocaine sensitivity or with any other drug examined in the 5 ppm animals. This absence of an effect corresponds to other reports that Se does not modify MeHg's developmental neurotoxicity in animal studies (Faro, do Nascimento, San Jose, Alfonso, & Duran, 2000; Reed et al., 2006) or in human populations (Choi et al., 2008).

Nonetheless, some observations of the 0.5 ppm group raise the possibility of a subtle interaction that might be detectable with a larger sample. When MeHg and Se interactions were seen, they occurred in the 0.5 ppm Hg groups. Neonate siblings of the rats described here had Hg:Se molar brain ratios that exceeded 10 for all rats exposed during gestation to 5 ppm MeHg, regardless of dietary Se exposure (Newland, Reed et al., 2006). Thus, Hg levels far exceed Se levels in the 5 ppm groups, and Se protection would not be expected. For the 0.5 ppm Hg groups, however, the mean Hg:Se ratios for animals in the low and high Se groups were 1.2 and 0.45, respectively. Consequently, rats in the low Se, 0.5 ppm Hg group had higher brains levels of Hg than Se, whereas the opposite was true for animals in the high Se, 0.5 ppm Hg group. This may explain why we see an effect of Se exposure in the 0.5 ppm Hg groups following cocaine administration. This may also explain why so much variability appeared in this group because some of the littermates of the animals examined here had an Hg:Se ratio above one and some below one. It should be noted that studies of the siblings of these animals have generally failed to detect interactions between Se and MeHg, so Se's modulation of MeHg's developmental effect may be described as subtle, inconsistent or, perhaps, due to Se's interactions with DA function.

To summarize, developmental exposure to MeHg has been shown in other reports to impair the formation of the cerebral cortex and to impair behavioral tasks that tap cortical function. Here, we report, using an environmentally relevant exposure regimen (Newland, Paletz, & Reed, 2008) that this developmental neurotoxicant also selectively alters the sensitivity to a DA reuptake inhibitor. These results show a specific effect of MeHg that is important to human exposures and, more broadly, raise the possibility that low-level, developmental exposure to this neurotoxicant could model the abnormal cortical development.

References

Abdo, K. M. (1994). *NTP technical report on toxicity studies of sodium selenate and sodium selenite* (Tech. Rep. No. NIH 94–3387). Research Triangle Park, NC: U.S. Department of Health and Human Services, NIH.

Andrzejewski, M. E., Spencer, R. C., & Kelley, A. E. (2005). Instrumental learning, but not performance, requires dopamine D1-receptor activation in the amygdala. *Neuroscience, 135,* 335–345.

Atchison, W. D., Joshi, U., & Thornburg, J. E. (1986). Irreversible suppression of calcium entry into nerve terminals by methylmercury. *The Journal of Pharmacology and Experimental Therapeutics, 238,* 618–624.

Barone, S., Jr., Haykal-Coates, N., Parran, D. K., & Tilson, H. A. (1998). Gestational exposure to methylmercury alters the developmental pattern of trk-like immunoreactivity in the rat brain and results in cortical dysmorphology. *Developmental Brain Research, 109,* 13–31.

Bartolome, J., Trepanier, P., Chait, E. A., Seidler, F. J., Deskin, R., & Slotkin, T. A. (1982). Neonatal methylmercury poisoning in the rat: Effects on development of central catecholamine neurotransmitter systems. *Toxicology and Applied Pharmacology, 65,* 92–99.

Bartolome, J., Whitmore, W. L., Seidler, F. J., & Slotkin, T. (1984). Exposure to methlmercury in utero: Effects on biochemical development of catecholamine neurotransmitter systems. *Life Sciences, 35,* 657–670.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60,* 1111–1120.

Beyrouty, P., Stamler, C. J., Liu, J. N., Loua, K. M., Kubow, S., & Chan, H. M. (2006). Effects of prenatal methylmercury exposure on brain monoamine oxidase activity and neurobehaviour of rats. *Neurotoxicology and Teratology, 28,* 251–259.

Bubar, M., McMahon, L., De Deurwaerdère, P., Spampinato, U., & Cunningham, K. (2003). Selective serotonin reuptake inhibitors enhance cocaine-induced locomotor activity and dopamine release in the nucleus accumbens. *Neuropharmacology, 44,* 342–353.

Burbacher, T. M., Rodier, P. M., & Weiss, B. (1990). Methylmercury developmental neurotoxicity: A comparison of effects in humans and animals. *Neurotoxicology and Teratology, 12,* 191–202.

Cagiano, R., De Salvia, M. A., Renna, G., Tortella, E., Braghiroli, D., Parenti, C., et al. (1990). Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats. *Neurotoxicology and Teratology, 12,* 23–28.

Calaminus, C., & Hauber, W. (2008). Guidance of instrumental behavior under reversal conditions requires dopamine D1 and D2 receptor activation in the orbitofrontal cortex. *Neuroscience, 154,* 1195–1204.

Castano, A., Ayala, A., Rodriguez-Gomez, J. A., Herrera, A. J., Cano, J., & Machado, A. (1997). Low selenium diet increases the dopamine turnover in prefrontal cortex of the rat. *Neurochemistry International, 30,* 549–555.

Castano, A., Cano, J., & Machado, A. (1993). Low selenium diet affects monoamine turnover differentially in substantia nigra and striatum. *Journal of Neurochemistry, 61,* 1302–1307.

Choi, A. L., Budtz-Jørgensen, E., Jørgensen, P. J., Steuerwald, U., Debes, F., Weihe, P., et al. (2008). Selenium as a potential protective factor against mercury developmental neurotoxicity. *Environmental Research, 107,* 45–52.

Eccles, C. U., & Annau, Z. (1982). Prenatal methyl mercury exposure: II. Alterations in learning and psychotropic drug sensitivity in adult offspring. *Neurobehavioral Toxicology and Teratologyl, 4,* 377–382.

Faro, L. R., do Nascimento, J. L., San Jose, J. M., Alfonso, M., & Duran, R. (2000). Intrastriatal administration of methylmercury increases in vivo dopamine release. *Neurochemical Research, 25,* 225–229.

Fleckenstein, A. E., Gibb, J. W., & Hanson, G. R. (2000). Differential effects of stimulants on monoaminergic transporters: Pharmacological consequences and implications for neurotoxicity. *European Journal of Pharmacology, 406,* 1–13.

Floresco, S. B., Magyar, O., Ghods-Sharifi, S., Vexelman, C., & Tse, M. T. (2006). Multiple dopamine receptor subtypes in the medial prefrontal

cortex of the rat regulate set-shifting. *Neuropsychopharmacology, 31,* 297–309.

Hopf, W., Cascini, M. G., Gordon, A. S., Diamond, I., & Bonci, A. (2003). Cooperative activation of dopamine D1 and D2 receptors increases spike firing of nucleus accumbens neurons via G-protein subunits. *Journal of Neuroscience, 23,* 5079–5087.

Hughes, J. A., & Sparber, S. B. (1978). d-Amphetamine unmasks postnatal consequences of exposure to methylmercury in utero: Methods for studying behavioral teratogenesis. *Pharmacogy, Biochemistry, and Behavior, 8,* 365–375.

Kleven, M. S., & Koek, W. (1998). Discriminative stimulus properties of cocaine: Enhancement by monoamine reuptake blockers. *Journal of Pharmacology & Experimental Therapeutics, 284,* 1015–1025.

Lee, S. P., So, C. H., Rashid, A. J., Varghese, G., Cheng, R., Lança, A. J., et al. (2004). Dopamine D1 and D2 receptor co-activation generates a novel phospholipase C-mediated calcium signal. *The Journal of Biological Chemistry, 279,* 35671–35678.

McMahon, L. R., & Cunningham, K. A. (2001). Antagonism of 5-hydroxytryptamine2A receptors attenuates the behavioral effects of cocaine in rats. *Journal of Pharmacology & Experimental Therapeutics, 297,* 357–363.

National Research Council. (1995). *Nutrient requirements of laboratory animals.* Washington, DC: National Academy Press.

National Research Council. (1996). *Guide for the care and use of laboratory animals.* Washington, DC: National Academy Press.

Newland, M. C., Donlin, W. D., Paletz, E. M., & Banna, K. M. (2006). Developmental behavioral toxicity of methylmercury: Consequences, conditioning, and cortex. In E. D. Levin & J. J. Buccafusco (Eds.), *Animal models of cognitive impairment.* (pp. 101–146). Boca Raton, FL: CRC.

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008). Methylmercury and nutrition: Adult effects of fetal exposures in experimental models. *NeuroToxicology, 29,* 783–801.

Newland, M. C., Reed, M. N., LeBlanc, A., & Donlin, W. D. (2006). Brain and blood mercury and selenium after chronic and developmental exposure to methylmercury. *NeuroToxicology, 27,* 710–720.

Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicological Sciences, 50,* 106–116.

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicology and Teratology, 26,* 179–194.

Nishikido, N., Furuyashiki, K., Naganuma, A., Suzuki, T., & Imura, H. (1987). Maternal selenium deficiency enhances the fetolethal toxicity of methyl mercury. *Toxicology and Applied Pharmacology, 88,* 322–238.

Paletz, E. M., Craig-Schmidt, M. C., & Newland, M. C. (2006). Gestational exposure to methylmercury and n-3 fatty acids: Effects on high- and low-rate operant behavior in adulthood. *Neurotoxicology and Teratology, 28,* 59–73.

Paletz, E. M., Day, J. J., Craig-Schmidt, M. C., & Newland, M. C. (2007). Spatial and visual discrimination reversals in adult and geriatric rats exposed during gestation to methylmercury and n - 3 polyunsaturated fatty acids. *NeuroToxicology, 28,* 707–719.

Ralston, N. V., Blackwell, J. L., III, & Raymond, L. J. (2007). Importance of molar ratios in selenium-dependent protection against methylmercury toxicity. *Biology of Trace Element Research, 119,* 255–268.

Rasmussen, E. B., & Newland, M. C. (2001). Developmental exposure to methylmercury alters behavioral sensitivity to *d* amphetamine and pentobarbital in adult rats. *Neurotoxicology and Teratology, 23,* 45–55.

Reed, M. N., Banna, K. M., Donlin, W. D., & Newland, M. C. (2008). Effects of gestational exposure to methylmercury and dietary selenium on reinforcement efficacy in adulthood. *Neurotoxicology and Teratology, 30,* 29–37.

Reed, M. N., & Newland, M. C. (2007). Prenatal methylmercury exposure increases responding under clocked and unclocked fixed interval schedules of reinforcement. *Neurotoxicology and Teratology, 29,* 492–502.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: Effects on a spatial discrimination reversal in adulthood. *NeuroToxicology, 27,* 721–732.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76 A diet. *Journal of Nutrition, 127* (5 Suppl), 838S–841S.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C., Jr. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition, 123,* 1939–1951.

Robbins, T. W. (2005). Chemistry of the mind: Neurochemical modulation of prefrontal cortical function. *Journal of Comparative Neurology, 493,* 140–146.

Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Current Opinion in Neurobiology, 6,* 228–236.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia. *Cerebral Cortex, 10,* 272–284.

Seu, E., Lang, A., Rivera, R., & Jentsch, J. (2008). Inhibition of the norepinephrine transporter improves behavioral flexibility in rats and monkeys. *Psychopharmacology, 201,* 1250–1254.

Sirois, J. E., & Atchison, W. D. (1996). Effects of mercurials on ligand- and voltage-gated ion channels: A review. *NeuroToxicology, 17,* 63–84.

Sirois, J. E., & Atchison, W. D. (2000). Methylmercury affects multiple subtypes of calcium channels in rat cerebellar granule cells. *Toxicology and Applied Pharmacology, 167,* 1–11.

Spealman, R. D. (1993). Modification of behavioral effects of cocaine by selective serotonin and dopamine uptake inhibitors in squirrel monkeys. *Psychopharmacology, 112,* 93–99.

Stern, S., Cox, C., Cernichiari, E., Balys, M., & Weiss, B. (2001). Perinatal and lifetime exposure to methylmercury in the mouse: Blood and brain concentrations of mercury to 26 months of age. *NeuroToxicology, 22,* 467–477.

Vahter, M. E., Mottet, N. K., Friberg, L. T., Lind, S. B., Charleston, J. S., & Burbacher, T. M. (1995). Demethylation of methylmercury in different brain sites of *Macaca Fascicularis* monkeys during long-term subclinical methylmercury exposure. *Toxicology and Applied Pharmacology, 134,* 273–284.

Wagner, G. C., Reuhl, K. R., Ming, X., & Halladay, A. K. (2007). Behavioral and neurochemical sensitization to amphetamine following early postnatal administration of methylmercury (MeHg). *NeuroToxicology, 28,* 59–66.

Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicology and Teratology, 21,* 83–88.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience, 5,* 483–494.

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