EFFECTS OF DOPAMINE CHALLENGES ON CLOCKED FIXED-INTERVAL SCHEDULE PERFORMANCE FOR RATS PRENATALLY EXPOSED TO METHYLMERCURY AND SELENIUM

Except where reference is made to the work of others, the work described in this dissertation is my own or was done in collaboration with my advisory committee. This dissertation does not include proprietary or classified information.

_____

Miranda Nicole Reed

Certificate of Approval:

_____	_____
Christopher J. Correia Assistant Professor Psychology	M. Christopher Newland, Chair Alumni Professor Psychology
_____	_____
Martha C. Escobar Assistant Professor Psychology	D. Mark Carpenter Associate Professor Mathematics & Statistics

_____

Joe F. Pittman
Interim Dean
Graduate School

EFFECTS OF DOPAMINE CHALLENGES ON CLOCKED FIXED-INTERVAL SCHEDULE PERFORMANCE FOR RATS PRENATALLY EXPOSED TO METHYLMERCURY AND SELENIUM

Miranda Nicole Reed

A Dissertation

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Doctor of Philosophy

Auburn, Alabama
August 4, 2007

EFFECTS OF DOPAMINE CHALLENGES ON CLOCKED FIXED-INTERVAL SCHEDULE PERFORMANCE FOR RATS PRENATALLY EXPOSED TO METHYLMERCURY AND SELENIUM.

Miranda Nicole Reed

_____
Signature of Author

_____
Date of Graduation

VITA

Miranda Nicole Reed, daughter of Gary Lynn Reed and Gloria Darlene Reed, was born November 27, 1980, in Decatur, Alabama. She graduated from East Lawrence High School in 1999. She attended Auburn University in Auburn, Alabama for 3 years where she graduated summa cum laude with a Bachelor of Art degree in Psychology. She entered Graduate School at Auburn University's Department of Psychology, in September, 2002. She received her Master's degree in May of 2005.

DISSERTATION ABSTRACT

EFFECTS OF DOPAMINE CHALLENGES ON CLOCKED FIXED-INTERVAL SCHEDULE PERFORMANCE FOR RATS PRENATALLY EXPOSED TO METHYLMERCURY AND SELENIUM.

Miranda Nicole Reed

Doctor of Philosophy, August 4, 2007
(Master of Science, May 13, 2005)
(B.S., Auburn University, 2002)

124 Typed Pages

Directed by M. Christopher Newland

Fish consumption has been linked with health benefits, but fish are also a source of methylmercury (MeHg), a neurotoxicant implicated in well-documented population poisonings. To examine putative MeHg-induced alterations in the function of the dopamine (DA) neurotransmitter system, the offspring of Long-Evans rats were examined. Pregnant rats were exposed to 0, 0.5, or 5 ppm MeHg via their drinking water before mating and during gestation and lactation, as well as to a diet either high or low in selenium (Se), a nutrient hypothesized to protect against MeHg damage, creating 2x3 factorial design. Female offspring of these breeders were weaned at postnatal day 21, at which time exposure to MeHg ended, but special Se diets continued. 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 Se 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. Drug challenges were conducted with multiple doses of cocaine, desipramine, SKF-38393, quinpirole, SCH-23390, and sulpiride, drugs selected for their effects on the D_1 and D_2 receptor subtypes. Animals exposed to 5 ppm MeHg displayed an increased sensitivity to cocaine, whereas the effects of cocaine for the 0.5 ppm Hg groups depended on dietary Se exposure, producing an interaction among cocaine dose, MeHg and Se exposure. There were no other interactions with any of the dopamine direct agonists or antagonists, suggesting that co-activation of the D_1 and D_2 receptors is required to produce the MeHg interactions seen with cocaine.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Christopher Newland for his patience and guidance. She would also like to thank her committee members Drs. Chris Correia, Mark Carpenter, and Martha Escobar, for their constructive comments. She also wishes to thank Luke Barnes and her family for their support during the course of the investigation.

Style manual or journal used: Publication Manual of the American Psychological Association (5th Ed.)

Computer software used: Microsoft Word XP, Microsoft Excel XP, Rs Series for Windows, Med-PC, SigmaPlot 8.0, EndNote 7, and Systat 11

.

TABLE OF CONTENTS

LIST OF TABLES

Chapter Three

LIST OF FIGURES

Chapter Two

Chapter Three

CHAPTER 1

PRENATAL EXPOSURE TO METHYLMERCURY AND SELENIUM: EFFECTS ON DOPAMINE FUNCTIONING

Methylmercury (MeHg) was clearly recognized as a developmental neurotoxicant in the 1950s in Minamata, Japan (Smith & Smith, 1975). Inhabitants of Minamata Bay ingested fish from MeHg contaminated waters, resulting in chronic high-level exposure. Children from exposed mothers displayed signs of mental retardation, physical malformations (e.g. Marsh et al., 1987), and there were at least 30 cases of cerebral palsy. In these cases, there were minimal adverse effects reported among the mothers of these severely affected children (Harada, 1968). A later event occurred in 1972 when the Iraqi population ate seeds treated with MeHg fungicide. The seeds were intended for planting, but the labels were in English, a language that the rural Iraqis could not read. So, they baked the seed into bread. Like in Minamata, those exposed prenatally were the most affected (Amin-Zaki & Majeed, 1981). Thus, while adults can experience neurological effects from MeHg, the greatest effects result from developmental exposure. Although the placental barrier can block many toxic elements, MeHg is able to cross it, resulting in higher concentrations on the fetal side than the maternal side (Iyengar & Rapp, 2001). Methylmercury then enters the developing brain, resulting in long-term damage (Kerper, Ballatori, & Clarkson, 1992).

Although the two above-mentioned studies were of great importance for establishing the detrimental effects of MeHg, they are not characteristic of the normal dosing that occurs in the general population. The general population experiences lower doses chronically. The doses that occurred in Japan and Iraq were undoubtedly high, with maximum levels in hair reaching 705 ppm and 148 ppm (Greenwood, 1985; Marsh, 1987), respectively, and in Iraq, the exposure was much more punctate than that typically seen. This presented difficulties for those interested in identifying the hazards associated with lower exposure levels, i.e., 12 ppm or less in hair, since most cases involved higher exposures.

In an attempt to examine children exposed to environmentally relevant MeHg levels, three epidemiological studies were conducted. These included populations from the Seychelles Islands, Faroe Islands, and New Zealand. All three populations consumed large quantities of fish, thereby providing ongoing exposure to MeHg at lower levels. The New Zealand study (Kjellstrom et al., 1989; Kjellstrom, Kennedy, Wallis, & Mantell, 1986) tested prenatally exposed children at 4 and 6 years of age on tests that measure mental and motor development. Deficits emerged at both times for children exposed to 6 ppm or more, and these children displayed a 3-point decrement in full-scale IQ on the Wechsler Intelligence Scale-Revised. The Faroe Islands found deficits on tests of language, memory and attention in children whose mothers had a mean hair level of 6.8 ppm MeHg (Grandjean, Weihe, White, & Debes, 1998; Grandjean et al., 1997). MeHg exposure was also associated with delays in brainstem evoked potentials (Murata, Weihe, Budtz-Jorgensen, Jorgensen, & Grandjean, 2004) and decreased sympathetic- and parasympathetic-mediated modulation of heart rate variability (Grandjean, Murata,

Budtz-Jorgensen, & Weihe, 2004). The Seychelles study, however, found only one adverse association with MeHg despite testing 48 neurodevelopmental endpoints. MeHg exposure prolonged time to complete a grooved pegboard test with the nonpreferred hand (Myers et al., 2003). Oddly, some children in the Seychelles displayed *improved* test scores on some endpoints as either pre- or postnatal mercury levels increased (Davidson et al., 1998; Davidson et al., 1999).

There were several differences between these studies. These included the biomarker of exposure, types of cognitive tests used, age of testing, and the sources of exposure (Jacobson, 2001), the latter being the focus of the current experiment. The Seychellois consumed a steady diet of fish. The Faroees ate a steady diet of fish as well but consumed pilot whale intermittently. This is important for a few reasons. The first is that pilot whale contains 10-20 times the MeHg concentrations of most fish, so exposure was pulsitile (Grandjean & Weihe, 1993). The second reason is that whales and other marine mammals also contain polychlorinated biphenyls (PCBs), another environmental contaminant. This contamination is high in the Faroes but almost nonexistent in the Seychelles. It may be that PCBs and MeHg interact to produce greater decrements (Bemis & Seegal, 1999). The third reason is that these whales are not eaten steadily but on an irregular basis. This means that while the mean average of MeHg found in hair samples were approximately the same for the two islands, the exposure schedule varied. The Faroes experienced a larger dose of MeHg over a shorter period of time. This may be more detrimental than lower doses given over a longer period of time.

Because the Seychelles Islands did not experience the pulsed doses of MeHg caused by whale blubber, the population could be demonstrating the benefits of a diet

high in fish. Fish are known to be high in selenium (Se) and long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA). Whale blubber, however, contains almost no DHA (Uauy-Dagach, 1996), and it has been shown that both MeHg and Se levels increase in fish as they age to the point where Se levels exceed those of MeHg (Lourdes, Culvin-Aralar, & Furness, 1991). In pilot whales, however, MeHg levels begin to exceed those of Se levels with aging (Julshamn, Anderson, Ringdal, & Morkore, 1987). Therefore, while the diet of individuals living in the Seychelles Islands was largely composed of fish with relatively higher levels of Se than MeHg, the diet of Faroe Islanders periodically contained whale meat containing relatively high MeHg:Se ratios. It has been suggested that detrimental effects of MeHg are only seen when molar MeHg concentrations are greater than molar selenium concentrations and that consumption of high Se levels confers protection from MeHg-induced neurological damage, possibly explaining the differential outcomes observed in these studies (Raymond & Ralston, 2004).

A National Research Council committee reviewed the MeHg literature and was able to approximate a dose-effect curve based on these 3 epidemiological studies (National Research Council, 2000). It was concluded that MeHg, even at low-levels, could produce adverse effects, despite the negative findings of the Seychelles study, which used a smaller cohort than the Faroe Islands and had only 50% statistical power to detect MeHg effects (National Research Council, 2000). In 2001, the U.S. Environmental Protection Agency (EPA) decided to keep the reference dose (RfD) for methylmercury, a daily intake that is likely to be *without* appreciable risk of deleterious effects during a lifetime, at 0.1 micrograms/kg/day (National Research Council, 2000), which is

equivalent to eating one 6 ounce can of tuna per week for an adult. This RfD was originally based on the 1971 Iraqi poisoning incident, and the data from the Faroe Islands and New Zealand supported its continued use.

Mercury Sources and Large-Scale Exposures

Three types of Hg exposure that are of toxicological relevance to human populations include the (1) inorganic forms, elemental or metallic Hg ("mercury vapor"; Hg^0), (2) mercurous Hg (Hg^{1+}) and mercuric Hg (Hg^{2+}), both of which usually occur as inorganic mercuric salts (I-Hg), and (3) the organic forms, methylmercury (MeHg) and ethylmercury (e.g. National Research Council, 2000). Occupational groups are typically exposed to Hg^0 or I-Hg, whereas the general population is exposed to MeHg through the dietary consumption of fish.

Although Hg does exist naturally and can be released through volcanoes and other natural sources, such as the weathering of rock, approximately 70% of elemental mercury is produced by anthropogenic emissions from coal-fired power facilities, waste incineration, chloralkali production and other industrial sources (UNEP, 2002). Power plants account for approximately 41% of anthropogenic emissions. Once airborne, mercury is deposited into soil and water. By reactions facilitated in microorganisms, inorganic mercury methylates into organic mercury, such as MeHg (National Research Council, 2001). Small fish feed upon these aquatic biota, and larger fish feed upon smaller fish, and there is a bioaccumulation up the food chain. Through each step of the food web, these concentrations bioamplify reaching up to 5 orders of magnitude higher at the top than those at the bottom of the web (EPA, 1997).

In January 2003, the EPA announced a proposal that would decrease the strict controls over emissions of mercury from coal-fired power plants. This would allow mercury emissions to remain as high as 26 tons/year through 2010 as compared to the existing restrictions allowing only 5 tons/year by 2008 (EPA, 2004). It has been estimated that the economic costs of developmental MeHg toxicity attributable to American power plants would be $1.3 billion each year due to the lifelong loss of intelligence and resulting loss of productivity for these individuals (Trasande, Landrigan, & Schechter, 2005). This estimated cost does not include the cardiovascular impacts of MeHg exposure or exposure occurring in the first two years of life during which myelination occurs and the blood-brain barrier is vulnerable to MeHg penetration (Rodier, 1995).

Given the estimated increase in mercury emissions over the next few years, and the resulting increase in MeHg-contaminated fish, fish consumption by pregnant women may decrease in order to remain under the reference dose of 0.1 micrograms/kg/day, an unfortunate consequence given the link between fish and important health benefits. Because of the beneficial effects of fish consumption, the long-term goal should be a reduction of the MeHg levels in fish, not replacement of fish in the diet with other food sources. This may prove a difficult goal given the estimated increase in mercury emissions. Findings of adverse effects following developmental MeHg exposure in animal research at doses near the RfD might prompt the EPA to reinstate the once strict controls over mercury emissions that has recently been lessened. In addition, animal research examining the detrimental effects of low doses of MeHg, and the possible protective effects of nutrients co-occurring in fish, such as selenium, would assist

advisory committees in recommendations on which fish and sea mammals should be consumed, and which should not, based on the relative MeHg to selenium ratios found in those fish. This would allow seafood to remain in the diet of pregnant women.

The following paper uses a MeHg dose that is near the RfD (~1.5 times the RfD, after adjusting for differences in mercury binding to rat and human red blood cells), as well as a dose above the RfD (~ 15 times the RfD), to examine the interactions between MeHg and selenium on dopamine functioning and behavior under a fixed interval schedule of reinforcement. The remainder of the paper provides the rationale for these endpoints by reviewing (1) the effects of MeHg on operant behavior, dopamine and cortical functioning, (2) the use of a fixed interval schedule and drug challenges to uncover the neurochemical interactions of MeHg on behavior, (3) possible attenuation of MeHg's toxic effects by selenium, and (4) selenium's alterations of dopamine functioning.

Effects of MeHg on the Response-Reinforcer Relationship

Operant behavior is behavior that is guided by or is sensitive to its consequences; that is, operant conditioning is the alteration of behavior due to changes in the consequences that follow the behavior. These responses and consequences take place within a context, and the stimuli present during a specific situation provide cues as to the likely consequences of a response. Discrimination is the extent to which the response occurs only within the presence of a particular context or stimulus, referred to as the discriminative stimulus. Thus, each behavioral act can be examined in terms of three parts: the discriminative stimulus (preceding event or context), the operant response, and

the consequence (reinforcer or punisher). These constitute the three-term contingency. It is impossible to eliminate any portion of the three-term contingency, but in experimental arrangements, the relationship between the discriminative stimulus (Sd) and the response can be emphasized, or emphasis can be placed on the relationship between the response and the reinforcer (Newland, Donlin, Paletz, & Banna, 2006; Newland & Paletz, 2000). Studies of memory involve presentation and removal of the context before a response is able to occur and therefore emphasize contextual control over behavior.

Alterations of the stimulus-response relationship allow for examination of sensory function, memory, generalization, or discrimination. Alternatively, the reinforcing efficacy of a consequence or the acquisition of a response on a particular task, specifically tasks minimizing the role of short-term remembering, can be examined by altering the response-reinforcer relationship. For example, fixed- or progressive-ratio schedules alter the number of responses required to produce a reinforcer, and thereby emphasize the response-reinfocer relationship, but there is some involvement of discrimination or memorial processes. On the other hand, procedures such as delayed-matching-to-sample, radial arm mazes, and delayed discrimination procedures alter discrimination or memory while only minimally altering the response-reinforcer relationship. It has been proposed that MeHg's developmental effects are primarily on the response-reinforcer relationship with discrimination, and possibly memory, only minimally affected (Newland & Paletz, 2000). Most approaches in behavioral toxicology, however, emphasize discrimination or memory (Miller & Eckerman, 1986). These studies, as well as what research has been done with MeHg and the

response-reinforcer relationships, are reviewed below with emphasize given to effects that are associated with developmental MeHg exposure but observed in juveniles and adults.

MeHg & Memory

There are few studies examining memory and MeHg, and their results are disparate. Studies using a spatial delayed alternation task revealed no deficits in memory following 0, 50, 70, or 90 μg/kg/day MeHg exposure (Gilbert, Burbacher, & Rice, 1993). A spatial delayed alternation task involves an animal alternating between responses while various delays are inserted between trials. The delays require the animal to remember, a procedure said to tap working-memory, where it went on the last trial following some delay and make the alternative response on the present trial. The response-reinforcer relationship remains the same, but the context changes on each trial. Monkeys exposed to MeHg *in utero* were tested on a spatial delayed alternation task at 7 to 9 years of age. No differences occurred between treated and control monkeys on the variable delay schedule performance. In fact, MeHg exposure *facilitated* performance as indicated by more correct trials and fewer incorrect responses than controls.

Other tasks believed to examine memory found MeHg-related deficits. However, these tasks may tap other systems besides memory. For example, Gunderson, Grant-Webster, Burbacher, and Mottet (1988) report that MeHg hinders visual recognition memory performance in infant Macaca fascicularis monkeys exposed prenatally. In this task, young monkeys are shown pictures of other monkeys, and the time spent looking at either picture is monitored (similar to the Fagan task used with children). Monkeys and children typically spend more time looking at the novel slide, presumably because it is

unfamiliar. The MeHg-exposed group showed random attention to the novel slide, indicating impairment for visual recognition memory performance, while the control group spent more time on the novel picture (Gunderson et al., 1988; Gunderson, Grant, Burbacher, Fagan, & Mottet, 1986). The interpretation was that the MeHg group spent equal time on both slides because there was no recognition of the previously presented slide. This impairment of visual recognition memory was believed to be the underlying cause. As noted in a recent review (Newland & Paletz, 2000), these results could instead indicate an agnosia, or a loss of ability to recognize objects, persons, sounds, shapes or smells while the specific sense is not defective nor is there any significant memory loss or even prosopagnosia (an inability to recognized faces). This is in-line with MeHg cortical damage to areas involved in high-order visual processing (Newland & Paletz, 2000).

In rodent studies, mice exposed up to 8 mg/kg/day (more than 10 times higher than the doses used in the present study) did not show effects of MeHg on a spatial delayed alternation task (Dore et al., 2001; Goulet, Dore, & Mirault, 2003). In a modification of this procedure, mice exposed to 8 mg/kg/day were required to first choose between two parallel paths, distinguished by visual cues (Goulet et al., 2003). The colored path chosen on the sample run must then be chosen on the test run. This latter portion of the test is believed to test reference memory. At the end of the colored path, the animal then had a second choice between two arms based solely on spatial location, and the correct arm was the arm not chosen during the sample run (i.e. an alternation task). This portion is believed to measure working memory. Reference memory was unaffected by MeHg in this task, similar to studies finding no MeHg-related alterations in

the long-term retention of a visual cue in a Y maze task (Dore et al., 2001) or Morris water-maze tasks employing long-term or reference memory (Markowski et al., 1998). However, MeHg-treated females, but not males, did show deficits in the working memory task (the alternation portion). The reasons for sex-related differences are not known, but brain levels of MeHg treated-females, but not treated-males, were higher than controls in this study. Thus, females may retain more MeHg than males, making them more vulnerable. The reasons for alterations in working memory on the second portion of the modified maze task, but not the typical spatial delayed alternation tasks described earlier, may be related to task acquisition. The spatial alternation task, which did not find MeHg-related differences, was first learned without any delays and then delays, or the working-memory component, were added. On the modified task, the reference memory component (colored pathways) and working memory component (alternation) were presented and had to be acquired simultaneously, possibly making the task more taxing. This leaves open the possibility that the difficulty was not with the spatial alternation, or working-memory component, but with the acquisition or learning of the task, an explanation consistent with MeHg-related difficulties in learning the response-reinforcer relationship.

MeHg & Discrimination

The picture of MeHg's effects on discrimination is clearer than that of memory. Developmental exposure to MeHg has not been reported to result in discrimination deficits. As mentioned previously, discrimination involves the occurrence of a response but only in the associated context, a demonstration of the three-term contingency (Newland & Paletz, 2000). Monkeys exposed gestationally to 0, 10, 35 or 50 μg/kg/day

were tested on a nonspatial discrimination-reversal task as adults. In this procedure, geometric stimuli served as the discriminative cues that indicated which lever to press. There were no differences among the groups (Rice, 1992). In another study, rats exposed gestationally to 4 mg/kg/day of MeHg and tested on a visual discrimination-reversal task at two months of age did not differ on accuracy when compared to controls (Schreiner, Ulbrich, & Bass, 1986). Moreover, no effects of prenatal MeHg exposure were detected in olfactory discrimination (Buelke-Sam et al., 1985).

Rats exposed developmentally to 0.5 ppm MeHg displayed a small but significant increase in errors on delayed and nondelayed spatial alternation tasks, but the MeHg effects were not related to the delay, thereby eliminating memory processes as an explanation (Widholm, Villareal, Seegal, & Schantz, 2004). The pattern of errors was not random, however, and was consistent with perseveration on the lever that did not produce a reinforcer. This study implies a diminished sensitivity to consequences for MeHg-exposed animals, consistent with the explanation of an alteration of the response-reinforcer relationship. This concept is expanded on below.

MeHg & Response Acquisition

Newland and colleagues (Newland, Reile, & Langston, 2004; Newland, Yezhou, Logdberg, & Berlin, 1994; Reed, Paletz, & Newland, 2006) have shown that MeHg exposure alters the sensitivity to reinforcement, an expression of the response-reinforcer relationship (Newland & Paletz, 2000). This is reflected in several ways, including the acquisition of choice and of large ratio responding. For example, gestational MeHg exposure has been shown to retard transitioning in concurrent schedules for young monkeys (Newland et al., 1994) and rats (Newland et al., 2004). In the typical concurrent

schedule procedure, random or variable interval reinforcement schedules operate independently on two levers with the reinforcement densities varying for each lever. Behavior is usually allowed to reach a steady-state before new response-reinforcer contingencies are implemented. When the relative reinforcement on a lever changed, MeHg-exposed animals shifted their behavior in accordance with the change in reinforcement rates more slowly than controls (Newland et al., 2004; Newland et al., 1994). Once these animals reached steady-state, however, there were no longer differences between exposed and control animals.

In a similar study, rats exposed gestationally to 0.5 and 5 ppm MeHg showed impaired acquisition and an increased number of errors on the first and third reversals of a spatial discrimination reversal procedure (Reed et al., 2006). In this procedure, left lever-pressing was reinforced under a FR1. After reaching 85% correct responding for three consecutive days, the location of reinforcement shifted to the right-lever. The pattern of errors indicated that MeHg-exposed rats perseverated, or continued to respond, on the left-lever, which was associated with reinforcement during the original discrimination and second reversal, instead of transitioning behavior to the right-lever during the first and third reversals.

These deficits could arise because of reduced discriminative control by the spatial location of reinforcement, or alternatively, because of diminished sensitivity to differences in reinforcement contingencies, evident as perseveration. As discussed above, discriminative control seems an unlikely candidate. The more likely explanation for these deficits in transitioning is a diminished sensitivity to changing reinforcement contingencies.

This suggestion is in accordance with findings of increased and persistent responding by gestationally MeHg-exposed rats on fixed ratio (FR) and progressive ratio (PR) tasks relative to controls, which exhibit decreased and erratic response patterns, consistent with extinction (Paletz, Craig-Schmidt, & Newland, 2006). This persistent responding suggests MeHg-exposed animals are insensitive to reductions in reinforcement rate, producing perseverative responding, and is consistent with findings of diminished sensitivity to changes in relative reinforcement rates in concurrent schedules (Newland et al., 2004; Newland et al., 1994) and spatial discrimination procedures (Reed et al., 2006). It should be noted that these findings of perseveration could also be explained in terms of an increase in the efficacy of the reinforcer for MeHg animals, an account that is difficult to disentangle from perseveration.

Role of Dopamine in the Response-Reinforcer Relationship

Dopamine is a member of the catecholamine family and is a precursor to epinephrine and norepinephrine. There are two major dopamine subtypes that can be distinguished molecularly (Huntley, Morrison, Prikhozhan, & Sealfon, 1992), behaviorally (Terry, Gilbert, & Cooper, 1995), and pharmacologically (Hall, Farde, & Sedvall, 1988). The D_1-like (D_1 and D_5) receptors result in activation of adenylyl cyclase and phospholipase C following ligand binding. The second class is the D_2-like (D_2, D_3, and D_4) receptors. Upon ligand binding of these receptors, there is inhibition of adenylyl cyclase, inhibition of calcium channels, and stimulation of potassium flow. Postsynaptic receptors can be either D_1-like or D_2-like. Autoreceptors, which are located on the dopamine-secreting neuron and regulate the synthesis and release of dopamine by the

neuron, are typically D_2-like receptors. Psychomotorstimulants, such as cocaine, block the reuptake of dopamine by blocking the dopamine transporter (DAT), which is involved in terminating the dopamine signal by removing dopamine from the synapse and returning it to the releasing neuron. This blockade of DAT results in more dopamine in the synapse. Thus, the psychostimulants are not specific for either D_1-like or D_2-like receptors and activate both. There are, however, drugs that are specific for the dopamine receptor subtypes, allowing questions regarding the individual contributions of these receptors to be examined (Cooper, Bloom, & Roth, 2003).

The analyses of these two receptor types can be important for several reasons. One is the location of the receptors. As already mentioned, D_2-like receptors can be autoreceptors located on the presynaptic neuron. D_1-like receptors are typically located on the periphery of the postsynaptic receptor. Following dopamine release, D_1 receptors in the nearby vicinity, but not necessarily those on the periphery of the synapse, would be activated, whereas D_2 autoreceptors would be saturated (Schultz, 1998). As the synaptic dopamine levels decreased, D_2 receptors would remain activated for longer than D_1 receptors, which may explain the different behavioral consequences of altering the activity of these two receptor families. In general, D_1-like receptor activation produces patterns of general search and behavioral activation, whereas D_2-like receptor activation produces behavior that is more highly focused on the task at hand, prolonging and maintaining appetitive behaviors, as well as producing perseverative responding. For example, SKF38393, a D_1-like agonist, increases the amount of time spent grooming (Bratcher, Farmer-Dougan, Dougan, Heidenreich, & Garris, 2005; Eilam, Talangbayan, Canaran, & Szechtman, 1992; Watchel, Brooderson, & White, 1992) and searching and

sniffing at the back of an operant chamber. In a choice task, this D1 agonist results in undermatching during concurrent schedules (Bratcher et al., 2005). Quinpirole, a D_2-like agonist, produces stereotypy in locomotor and oral behaviors (Szechtman, Talangbayan, Canaran, Dai, & Eilam, 1994). With operant tasks, these stereotypies are focused on the response lever (i.e. chewing of the lever) and where the food is delivered (i.e. constant "checking") (Bratcher et al., 2005). In addition, quinpirole produces effects that might be interpreted as perseverative or oversensitivity to reinforcement. For example, following quinpirole administration, rats exhibit increased and prolonged time in areas of reinforcement delivery and are more resistant to extinction (Kurylo, 2004; Kurylo & Tanguay, 2003).

Dopamine is involved in several pathways in the central nervous system. These paths originate with dopamine cell bodies in the midbrain that send fibers to several cortical and subcortical areas, including the nucleus accumbens, striatum, and frontal cortex. These latter three pathways are reviewed here, and their potential involvement with the response-reinforcer relationship is examined. The nigrostriatal pathway, which originates with cell bodies in the substantia nigra (SN) and terminates in the striatum is involved in the orchestration of movement. D_1 receptors are located on the striato-nigral axons in the substantia nigra (Ariano et al., 1989), whereas D_2 (Akaoka, Charlety, Saunier, Buda, & Chouvet, 1992) and D_3 (Bouthenet et al., 1991) receptors are located presynaptically on the SN dendrites. Striatal neurons develop specific firing patterns but only after several pairings of the response and reinforcer, suggesting its involvement in steady-state behavior (Schultz, Tremblay, & Hollerman, 1998). However, steady-state

behavior, such as that in concurrent schedules, is typically not altered by MeHg at low doses.

The mesolimbic pathway connects the ventral tegmentum of the midbrain to the nucleus accumbens of the limbic system. Both D_1 and D_2 receptors are abundantly expressed as postsynaptic receptors in the nucleus accumbens (Mengod, Martinez-Mir, Vilaro, & JM, 1989). Dopamine neurons in this pathway are typically involved in differentiating the magnitude of reinforcers presented. These neurons respond preferentially after presentation of different reinforcers, such as cocaine versus food (Carelli, 2002; Salamone, Correa, Mingote, & Weber, 2003). Although dopamine in this area was originally believed to be released following any pleasurable event, it is now known that it is released after aversive events as well (Ungless, Magill, & Bolam, 2004). Because dopamine fires less when the magnitude of the current reinforcer is less than the magnitude previously experienced, and fires more when the magnitude is greater than that previously experienced, new theories suggest these dopamine neurons are involved in prediction, decision-making and in the detection of changes in reinforcement contingencies (Montague, Hyman, & Cohen, 2004). Under this explanation, dopamine is needed for evaluation of rewards and the sequence of actions needed to obtain these rewards. It remains to be shown that MeHg directly alters this pathway.

The last dopamine pathway considered is the mesocortical pathway that connects the ventral tegmentum to the cortex. D_1 receptors are located throughout most of the cerebral cortex (Mengod et al., 1989), whereas D_4 receptors are specific for the frontal cortex (Van Tol et al., 1991). Cortical dopamine neurons discriminate between two reinforcers, regardless of the stimuli associated with those reinforcers, and these neurons

reflect the animals' preference in a choice setting, suggesting these neurons process the relative value of reinforcement (Tremblay & Schultz, 1999a, 1999b). Frontal cortical regions are also involved in perseveration, impulsivity, and progressive ratio performance (Evenden, 1999; Kheramin et al., 2005; Kheramin et al., 2002; Mobini et al., 2002; Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000). Perseveration on a discrimination reversal task has been seen in rats following excitotoxic damage to the orbital prefrontal cortex (Chudasama & Robbins, 2003) and in marmosets after lesions to the frontal lobes (Ridley, Clark, Durnford, & Baker, 1993). Humans with age-related decreases in cortical size perseverated on tasks such as the Wisconsin Card Sorting Task (Head, Raz, Gunning-Dixon, Williamson, & Acker, 2002; Raz, Gunning-Dixon, Head, Dupuis, & Acker, 1998). Thus, the behavioral effects of MeHg may be related to alterations in the cortex and/or the dopamine neurons located there.

The MeHg profile of altered sensitivity to reinforcement changes and perseverative responding is consistent with the sensitivity of the cortex to developmental MeHg. Developmental MeHg exposure has been shown to produce altered morphology of cortical neurons, reduced widths of cortical lamina (Barone, Haykal-Coates, Parran, & Tilson, 1998; Berlin, Grant, Hellberg, Hellstrom, & Schultz, 1975) and morphological aberrations in mitochondria in cortical neuron fibers (O'Kusky, 1983). In addition, MeHg alters the functioning of dopamine, which , as mentioned above, is involved in reinforcement processes underlying voluntary behavior (Salamone, Arizzi, Sandoval, Cervone, & Aberman, 2002; Spanagel & Weiss, 1999; Wise, 2004).

Synaptosomal uptake of dopamine (DA) is altered in rodents exposed developmentally to MeHg. Initially, there is a decreased uptake of DA until

approximately postnatal days 10-13, which is followed by an increased uptake that continues throughout adulthood (Bartolome et al., 1982). Dopamine transmitters and turnover are increased in the first week of life as well (Bartolome et al., 1982; Bartolome, Whitmore, Seidler, & Slotkin, 1984). The increase in uptake that occurs near day 10 may not be directly related to MeHg. Instead, the increase in uptake may be a reaction to the increase in neurotransmitter levels, a regulatory mechanism that compensates for the increase in DA and norepinephrine transmitters and turnover.

In addition, adult rats exposed gestationally to MeHg showed enhanced sensitivity to amphetamine, a dopaminenergic and noradrenergic agonist, but not haloperidol, a D_2 inverse agonist (Rasmussen & Newland, 2001). The haloperidol results suggest that MeHg does not alter post-synaptic dopamine receptors, at least not D_2, but may alter the generation, release, or reuptake of dopamine. Amphetamine blocks the reuptake of dopamine via the dopamine transporter (DAT) (Pilf, Drobny, Reither, Hornykiewicz, & Singer, 1995), which is involved in terminating the dopamine signal by removing dopamine from the synapse and returning it to the releasing neuron. In addition, amphetamine causes vesicles filled with dopamine to fuse to the cell membrane and release dopamine into the synapse (Sulzer et al., 1995). Thus, amphetamine alters both the release and the reuptake of dopamine. It also has similar effects in noradrenergic synapses. Cocaine, however, alters the reuptake of dopamine by blocking the dopamine transporter but has no effect on the release of dopamine (Tsukada, Harada, Nishiyama, Ohba, & Kakiuchi, 2000). Because of its specificity, cocaine should be a more specific drug challenge. The use of cocaine, rather than amphetamine, may help to determine

whether MeHg's effects are on the release or the reuptake of dopamine and whether they are specific to dopamine

Gestational exposure to MeHg has also been shown to reduce monoamine oxidase (MAO) activity, an enzyme that catalyzes the oxidation of dopamine, in the developing embryo and brainstem of offspring (Beyrouty et al., 2006). This may explain the MeHg-enhanced sensitivity to dopamine agonists. If MAO activity is reduced, there would be less dopamine catalyzed after the administration of a dopamine agonist, and thus, more dopamine remaining in the synapse.

Overall, developmental MeHg exposure appears to adversely affect cortical developmental and the dopamenergic neurotransmitter system, altering the sensitivity of behavior to reinforcing consequences.

Fixed Interval Schedules & Pharmacological Challenges

A commonly used method to measure behavioral changes produced by exposure to toxicants is the fixed interval (FI) schedule of reinforcement, which requires one response after some fixed amount of time in order to earn a reinforcer. The response pattern in an FI is usually one of a pause after the delivery of a reinforcer with progressive increases in response rate until the end of the interval, producing the FI scallop. Therefore, both low- and high-rates of responding can be examined in the first and last portion of the interval, respectively. Another benefit of this schedule is that the change in usual response patterns produced by toxicants is typically observed across species (Rice, 1988).

Fixed interval schedules are sometimes seen as useful in measuring temporal control or discrimination. Unlike typical discrimination procedures in which the stimulus is externally available, time is considered an internal stimulus. Quarterlife, or the portion of the interval at which one-fourth of the total responses has occurred, is a measure of an animal's temporal discrimination. Decreased quarterlife values indicate that an animal responded earlier during the interval and has poorer temporal discrimination. Monkeys exposed both pre- and postnatally to MeHg at doses of 25 and 50 micrograms/kg/day exhibited shorter pause times and decreased quarterlife values on a fixed interval schedule of reinforcement when tested during infancy (Rice, 1992). Similarly, female monkeys exposed prenatally to 50, 70, or 90 micrograms/kg/day displayed decreased quarterlife values when tested during adulthood (Gilbert, Rice, & Burbacher, 1996). Thus, it is possible that MeHg exposure alters internal discrimination, or temporal discrimination, without affecting discrimination of external stimuli.

Alternatively, or perhaps synergistically with the possible temporal discrimination alterations, MeHg could have altered the efficacy of the reinforcer. Response rates on FI schedules are positively related to reinforcement magnitude of food pellets (Meltzer & Brahlek, 1968; Meltzer & Brahlek, 1970), sucrose solution (Stebbins, Mead, & Martin, 1959), and cocaine (Balster & Schuster, 1973). Although none of these studies reported quarterlife values directly, they all reported an accelerated increase in response rate when the reinforcer magnitude was increased, which would most likely appear as a decrease in quarterlife. Thus, the decrease in quarterlife values for MeHg-exposed animals (e.g. Gilbert et al., 1996; Rice, 1992) is consistent with alterations of the response-reinforcer relationship. As mentioned previously, MeHg-exposed rats tolerate higher fixed- and

progressive-ratio values than controls (Paletz et al., 2006), which typically indicates the reinforcer is considered more efficacious. If MeHg does in fact increase a reinforcer's efficacy, one would expect accelerated increases in responding under the FI schedule for MeHg-exposed rats similar to those effects described above (e.g. Gilbert et al., 1996; Rice, 1992).

Pharmacological Challenges

One method of studying the neurochemical effects of MeHg is through behavioral tests with drug challenges. A particularly beneficial schedule for this is the FI schedule because behavior occurring at both low- and high-rates can be examined. This is an important consideration when psychostimulants, such as cocaine, are to be used. Dopamine agonists increase low-rate behavior, such as that early in the interval, and decrease high-rate behavior, such as that late in the interval with a variety of reinforcers, such as food (Barrett, Dworkin, & Zuccarelli, 1977), water (Hearst, 1961), heat (Weiss & Laties, 1963), light onset (Gomer & Jakubczak, 1974), and electrical brain stimulation (Carey & Goodall, 1973). This is commonly referred to as the rate-dependency effect of stimulants. By using an FI schedule, MeHg's effects on low- and high-rate behavior, as well as the possible differential interaction of these behaviors with dopamenergic drugs, can be examined.

One exception to the rate-dependent effects of dopamine agonists is seen in behavior that is strongly controlled by external stimuli. For example, Laties and Weiss (1966) maintained one group of pigeons under a FI 5 min schedule of reinforcement and another group under a clocked FI (CFI) 5 min schedule of reinforcement. In the CFI 5 min component, 5 different visual stimuli were associated with each minute of the

interval, which was referred to as adding a "clock". Following amphetamine administration, the pattern of responding for the FI group was disrupted with low-rate behavior early in the FI increasing and high-rate behavior at the end of the FI decreasing, but the temporal pattern of responding for the CFI group was minimally affected. Laties and Weiss (1966) accounted for this difference by suggesting that supplementing temporal control with an external clock blocked the rate-increasing effects of amphetamine. This phenomenon has since been replicated several times with numerous schedules in pigeons (Carey & Goodall, 1973; Leander & McMillan, 1974; Odum & Schall, 1999).

The use of a multiple FI clock FI (CFI) schedule of reinforcement with dopamenergic drug challenges may help to further elucidate some of MeHg's toxicity. Under baseline conditions, MeHg animals should come under stimulus control to a similar degree as controls, even if overall response rates differ, since MeHg does not appear to alter stimulus control. Conversely, given the enhanced sensitivity of MeHg-exposed rats to amphetamine (Rasmussen & Newland, 2001), MeHg-exposed animals should be more sensitive to the rate-dependent effects of stimulants in the FI component. Likewise, a left-ward shift in the dose-effect curve for the CFI component might be expected, since at high doses of amphetamine, rate-dependent effects are seen even when behavior is under strong stimulus control (Laties, 1972). The doses required to produce rate-dependent effects in the CFI may be less for MeHg-exposed animals than controls. The current study sought to explore this hypothesis by employing a *Mult* FI 120" CFI 120" schedule of reinforcement with dopamenergic drug challenges. Drug challenges focused on cocaine, a dopamenergic and adrenergic agonist, desipramine, an adrenergic

agonist, and D_1-like and D_2-like agonists and antagonists in an attempt to disentangle MeHg's effects on the dopamenergic system. The drug challenges were conducted so as to determine specificity of a neurotransmitter system, as well as whether a single receptor subtype is involved in MeHg's effects. If there are effects of cocaine, but not desipramine, this suggests that dopamine, but not norepinephrine, is altered by MeHg. The use of D_1 and D_2 agonists and antagonists allow for dopamine receptor subtype differentiation.

Interactions between MeHg and Selenium in Animal Studies

Once in the nervous system, MeHg appears to affect it in a variety of ways. MeHg can cause alterations of neurotransmitter systems (Dave, Mullaney, Goderie, Kimelberg, & Aschner, 1994), changes in cell permeability to Ca^{2+} (Atchison & Hare, 1994), alterations in the conduction of axonal action potentials (Traxinger & Atchison, 1987), disruption of microtubules in neuronal cytoskeleton (Miura & Imura, 1987) and mitochondria abnormalities (Atchison, 1987). Additionally, MeHg forms a Selenium-Hg (inorganic) complex in the brain and diverts selenium (Se) from selenoprotein synthesis, potentially inducing a deficiency of Se in the brain (Vahter et al., 1995). MeHg also suppresses the activity of glutathione peroxidase, a selenoenzyme, without altering the levels of Se in the liver (Nishikido, Furuyashiki, Naganuma, Suzuki, & Imura, 1987), suggesting the bioavailability of Se was decreased. Thus, MeHg may compromise Se functioning without decreasing its tissue levels. This has led to the suggestion that part of the neurobehavioral toxicity of MeHg may result from MeHg binding to Se and

decreasing its bioavailability in the brain (Watanabe, Yin, Kasanuma, & Satoh, 1999). This possibility is examined below.

Of the 22 amino acids, two possess selenium: selenomethionine and selenocysteine. Selenomethionine is believed to be an unregulated storage compartment for selenium and is structurally similar to methionine. On the other hand, selenocysteine is tightly regulated and needed in many essential biological functions, such as prevention of cellular damage from free radicals, normal thyroid functioning, and a role in the immune system (Raymond & Ralston, 2004). This tight regulation of Se content can be seen in studies attempting to deplete Se levels in certain regions. For example, it is particularly difficult to deplete Se levels in the brain, pituitary, and thyroid through dietary manipulations in a single generation or even when a selenium deficient diet is experienced by multiple generations. In one study, selenium content in the brain decreased only to slightly less than 60% of normal levels after 16 generations of mice on a selenium-deficient diet, whereas Se concentrations in the liver, skeletal muscle and blood can be drastically decreased to less than 1% of normal levels after only six generations (Behne, Pfeifer, Rothlein, & Kyriakopoulos, 2000). This tight control over brain content implies that this nutrient is very important for neural function, but its precise role is not yet understood.

Selenoprotein P knockout mice fed a diet containing less than 0.1 ppm Se had brain Se levels that were reduced to 43%, the lowest levels yet achieved, and this caused reduced body weight, poor motor coordination, and reduced fertility in males (Hill et al., 2003). These disruptions were reversed quickly by diet supplementation with 2 ppm Se. When the selenocysteinyl-tRNA gene is removed in mice, there is total disruption of

selenoprotein synthesis that results in early embryonal lethality (Bosl, Takaku, Oshima, Nishimura, & Taketo, 1997), again signifying the importance of Se to biological functions. Thus, any toxicant or substance capable of binding and interfering with the functioning of Se and production of selenoproteins could reduce the pool of selenium that is biologically available, leading to unfavorable effects.

This could be important ot an understanding of MeHg's neurotoxicity. MeHg has a high affinity constant for selenocysteine's Se ($\sim 10^{-22}$) and an even higher affinity constant for the free selenide produced during selenocysteine synthesis ($\sim 10^{-45}$) (Dyrssen & Wedborg, 1991). These mercury selenide complexes are thought to be metabolically inert given the low solubility (10^{-58} to 10^{-65}) of the precipitates (Nuttall, 1987).

Interactions between Hg and Se were first noted in rats concurrently given sodium selenite (SS) and mercuric chloride ($HgCl_2$), which alleviated the lethal toxicity of $HgCl_2$ (Parizek & Ostadalova, 1967). For a while, the interaction of Se, usually as SS, and mercuric salts, usually as $HgCl_2$, was the most studied. Recently, attention has shifted to the interactions between SS and MeHg, an interaction that is more relevant to human health (Watanabe, 2002). Nevertheless, both interactions deserve review, since once inside the body, some of the MeHg undergoes demethylation to form inorganic mercuric salts as suggested by the presence of high concentrations of mercuric salts in monkeys chronically fed MeHg (Bjorkman et al., 1995). The exact mechanism of demethylation of MeHg is not known, however.

Most studies examining the interaction between SS and $HgCl_2$ have focused on effects in the peripheral nervous system, but similar results might be expected in the central nervous system. Studies examining the interaction between concurrently

administered intravenous injections of mercuric salts and SS suggest that the depression of mercury toxicity occurs because either (1) a high molecular weight complex is formed between Se, Hg and a plasma protein, recently discovered to be selenoprotein P (Yoneda & Suzuki, 1997), that reduces the mercury accumulation in the kidneys because the complex is barely filtered through the glomerulus, (2) an inert high molecular weight complex is formed in the erythrocytes and remains there, and (3) non-diffusible, stable complexes of Hg and Se are formed within organs, such as the kidneys and liver (Imura & Naganuma, 1991). Glutathione appears to be essential for the formation of high molecular weight complexes between plasma proteins, Hg, and Se, most likely because it reduces selenite to selenide, which more easily reacts with Hg and plasma proteins (Naganuma & Imura, 1983). The high molecular weight Hg and Se complexes within the liver after concurrent administration were found to be hardly separable from each other: the ratio of Hg:Se almost always equaled one (Imura & Naganuma, 1991). Any protection that does occur requires that Se be administered concurrently with Hg. If the $HgCl_2$ and Se are not administered within a very short time of one another, as short as an hour, the lethality and toxicity of Hg can be enhanced rather than prevented by Se (Naganuma, Ishii, & Imura, 1984). Furthermore, both chemicals must be given at equimolar intravenous doses. When the powdered livers of rats administered SS were used instead of SS itself, the formation of the Hg-Se complex was limited and renal Hg was not reduced (Magos, Clarkson, & Hudson, 1984). These observations suggest that the benefits of Se may be limited.

More recently, focus has turned to the interactions between MeHg and Se. In *adult-onset exposure*, a diet low in Se was found to enhance MeHg's neurotoxicity as

compared to mice fed a Se-sufficient diet (Imura, 1986). MeHg's weight gain suppression and mortality effects were reduced following SS supplementation (H. Ganther, C. Goudie, M. Sunde, M. Kopecky, & P. Wagner, 1972). Furthermore, selenite added to a mixture of protein-bound MeHg caused the release of the MeHg (Sumino, Yamamoto, & Kitamura, 1977). Following chromatography, elemental and mass spectral analyses, the compound formed was identified as bis(methylmercuric) selenide (BMS) (Naganuma & Imura, 1980). The selenide was most likely the result of glutathione-mediated reduction of selenite to selenide, as suggested by i*n vitro* studies that found BMS from MeHg and selenite in the presence of glutathione (Naganuma & Imura, 1980). Unfortunately, this BMS complex does not account for the ability of selenite to depress MeHg toxicity. BMS was found to be unstable and degraded quickly in blood *in vitro* (Naganuma, Kojima, & Imura, 1980). Besides for this BMS complex, kinetic interactions between MeHg and Se have not been widely reported. One recent report suggests that selenide possibly demethylates MeHg. In this study, S-adenosyl methionine (SAM), the common methyl donor used for methylating selenide to the volatile and exhalable form, is required at greater concentrations in a SS-only treated group than in a SS+MeHg treated group, suggesting MeHg is acting as the methyl donor instead of SAM (Gregus, Gyurasics, Csanaky, & Pinter, 2001). If this is true, then the newly formed mercuric salts might interact with other selenide molecules, possibly further reducing the bioavailability of Se.

As noted above, MeHg is particularly detrimental to the developing fetus. With *developmental exposure*, MeHg reduces the activity of the selenoprotein glutathione peroxidase in the fetal brain (El-Demerdash, 2001), and perinatal selenium deficiencies

disrupt the thyroid hormone economy (Watanabe, 2001). MeHg disrupts the activity of iodothyronine deiodinases (DIs), a class of selenoproteins, that could lead to a harmful excess of thyroid hormones in the fetal brain (Watanabe, Yoshida, Kasanuma, Kun, & Satoh, 1999). Since MeHg can cross the blood-brain barrier and has such a high affinity for Se, sequestering of Se by MeHg may be the cause of MeHg's developmental pathophysiology. Studies have shown that fetotoxicity, neurotoxicity and developmental toxicity are alleviated to some extent when SS is co-administered with MeHg. For example, MeHg's fetolethality was increased in Se-deficient mice compared to Se-sufficient mice (Nishikido et al., 1987).

Developmental neurobehavioral protection has been demonstrated as well, but the protective effects are only short-term. Mice on a Se-deficient diet displayed a greater degree of MeHg impairment in the development of walking and open-field activity as compared to mice on a Se-sufficient diet (Watanabe, Yin et al., 1999). Thus, Se may confer some protection during development against MeHg's detrimental effects. However, aside from SS's alleviation of MeHg-induced hypoactivity of mice tested at two months of age (Fredriksson et al., 1993), these protective effects were reported to disappear before adulthood (Watanabe, Yin et al., 1999). Furthermore, potential interactions between developmental MeHg exposure and selenium have not been examined well into adulthood nor have they been examined using behavioral tasks and drug challenges believed to tap different neurotransmitter systems. Little behavioral work has been down with selenium, but selenium's effects on neurotransmitter systems, particularly dopamine, have been characterized. These effects are reviewed below.

Selenium and Dopamine

Monoamine oxidases (MAOs) terminate the action of dopamine, serotonin, and norepinephrine in the synapse by metabolizing it to an inactive form. This enzme catalyzes the oxidative removal of an amine group from the monoamines, which include dopamine, epineneprhine, norepinenephrine, and serotonin. MAO deaminates dopamine to form aldehyde, ammonia, and hydrogen peroxide. If glutathione peroxidase (GPx) is present, the hydrogen peroxide is reduced to water. If GPx levels are reduced, and a catalyst, such as iron, is present, the hydrogen peroxide is broken down into hydroxide ion radicals, a highly reactive and toxic species that will react with any oxidizable compound in vicinity, including cell membranes. In recent decades, the biological damage produced by catecholamine metabolism, and the resultant pool of hydrogen peroxide, has been noted in a number of neurodegenerative diseases, including Parkinson's disease (Olanow, 1990). Because selenium is part of the active site of GPx, the antioxidant protection of glutathione peroxidase depends heavily on the presence of selenium. Nutritional selenium deficits result in a decrease in GPx activity (Combs & Combs, 1984), as well as a variety of alterations in dopamine functioning in numerous brain regions.

In a series of experiments, female rats were fed a diet of either 0.001 ppm or 0.2 ppm Se for 15 days. When the hippocampus was examined, those on the low Se diet had a 40% increase in dopamine (DA) content, a 4-fold increase in DA turnover, and a 22% increase in the DA metabolite, dihydroxyphenylacetic acid or DOPAC, which indicates an increase in DA synthesis and catabolism (Castano et al., 1995). In addition, there was a two-fold increase in the enzyme tyrosine hydroxylase, which is the rate-limiting step in

the production of catecholamines, and a 76% increase in tyrosine hydroxylase activity. These increases may be due to the decrease in GPx activity (-29%).

In the substania nigra (SN), there was a 34% increase in DA levels, 63% increase in DA turnover, and 55% increase in 3-methoxytyramine turnover (3-MT), which is an indicator of the amount of DA released into the synaptic cleft (Castano, Cano, & Machado, 1993). In the SN, however, there was no change in DOPAC or homovanillic acid (HVA), indicating there was no increase in DA catabolism. The authors suggest that the lack of changes in DOPAC and HVA may be the result of oxidation of the free DA into a neurotoxic substance that cannot be catabolized, particularly with reduced GPx activity (-20%). Previous studies have shown that neurotransmitters and free radicals can interact to produce endogenous neurotoxins (Fishman, Rubins, Chen, Dickey, & Volicer, 1991).

In the prefrontal cortex, basal DA levels did not differ between selenium groups, but there was a 40% increase in the turnover rate, a 59.5% increase in DOPAC and a 47.1% decrease in HVA levels for the low Se group (Castano et al., 1997). The prefrontal cortex is unique in that HVA typically exceeds DOPAC (Sharp, Zetterstorm, & Ungerstedt, 1986). The co-occurrence of an increase in DA turnover and increases in the DOPAC/HVA ratio is indicative of the degeneration seen during aging (Vereno, Machado, & Cano, 1990), suggesting that a diet low in Se may induce degeneration in as short as 15 days. In addition, the GPx levels in the prefrontal cortex were reduced, as well as the basal concentrations and turnover of 3-MT. There was an increase in tyrosine hydroxylase but no change in the actual amount of the enzyme, suggesting that the prefrontal cortex and hippocampus have different long-term regulation of this enzyme.

Interestingly, in the striatum, there were no changes in DA levels, turnover or metabolites (Castano et al., 1993), which could be due to the low levels of antioxidants, such as glutathione or ubiquinone Q10, normally present in this structure (Argentiero & Tavolato, 1980). As mentioned previously, striatal neurons are believed to be involved in steady-state behavior (Schultz et al., 1998), such as that in concurrent schedules, which is typically not altered by MeHg at low doses. The lack of alterations in the striatum following Se deficiency further supports the idea of MeHg's neurotoxicity involving both selenium and dopamine. Those areas with altered dopamine functioning due to selenium deficiency, such as the prefrontal cortex, are also the areas implicated in MeHg's neurotoxicity.

The present experiment was designed to examine the interactions of MeHg and selenium on assorted components of a Multiple FI 120", Clocked FI (CFI) 120" schedule of reinforcement, as well as the effects of a battery of drugs. The experiments were conducted using a 2 (chronic Se) x 3 (gestational MeHg) full factorial design, which allows for the direct examination of the main effects of either element, as well as the interactions between MeHg and selenium. Pregnant female rats were exposed to 0 ppm, 0.5 ppm, or 5 ppm MeHg via their drinking water, and a diet that was either marginal (0.06 ppm) or rich (0.6 ppm) in selenium. The present study examined their female offspring who were exposed only during gestation to MeHg but continued the selenium diet fed to their mothers. The MeHg concentrations chosen produce levels relevant to human exposure spanning the low to moderate range (Burbacher, Rodier, & Weiss, 1990; Newland & Reile, 1999). Likewise, the selenium diets were at the low and high end of recommended intakes. The 0.06 ppm selenium concentration is lowest possible with a

casein-based diet but is still a nutritionally adequate level for rodents (National Research Council, 1995; Reeves, Nielsen, & Fahey, 1993). 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 selenium (Reeves, 1997; Reeves et al., 1993), but is below that thought to be toxic (Abdo, 1994).

Upon reaching adulthood, the offspring were trained to respond under a *Mult* FI 120", CFI 120" schedule of reinforcement. Drug challenges commenced after behavior stabilized. The drugs chosen include: cocaine, desipramine, SKF-38393, quinpirole, SCH-23390 and sulpiride. Cocaine, a dopamine agonist, with some noradrenergic activity, allows for determination of catecholaminergic involvement (Harris & Baldessarini, 1973). Desipramine, a metabolite of imipramine, is primarily a noradrenergic agonist and variations between MeHg exposed groups and controls would indicate alterations of norepinephrine neurotransmission. SKF-38393, a D_1 agonist, and SCH-23390, a D_1 antagonist (or inverse agonist), were used to examine alterations of the D1-like receptor subtype, whereas quinpirole, a D_2 agonist, and sulpiride, a D_2 antagonist, were used to access variations in the D2-like receptor subtype.

REFERENCES

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

Akaoka, H., Charlety, P., Saunier, C. F., Buda, M., & Chouvet, G. (1992). Inhibition of nigral dopamine neurons by systemic and local apomorphine: possible contribution of dendritic autoreceptors. *Neuroscience, 49*, 879-891.

Amin-Zaki, L., & Majeed, M. A. (1981). Methylmercury poisoning in the Iraqi suckling infant: A longitudinal study over five years. *Journal of Applied Toxicology, 1*, 210-214.

Argentiero, V., & Tavolato, B. (1980). Dopamine (DA) and serotonin metabolic levels in the cerebrospinal fluid (CSF) in Alzheimers's presenile dementia under basic conditions and after stimulation with cerebral cortex phospholipids (BC_PL). *J Neurol, 224*, 53-58.

Ariano, M. A., Monsma, F. J. J., Barton, A. C., Kang, H. C., Haugland, R. P., & Sibley, D. R. (1989). Direct visualization and cellular localization of D1 and D2 dopamine receptors in rat forebrain by use of fluorescent ligands. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 8570-8574.

Atchison, W. D. (1987). Effects of activation of sodium and calcium entry on spontaneous release of acetylcholine induced by methylmercury. *Journal of Pharmacology & Experimental Therapeutics, 241*(1), 131-139.

Atchison, W. D., & Hare, M. F. (1994). Mechanisms of methylmercury-induced neurotoxicity. *FASEB Journal, 8*(9), 622-629.

Balster, R. L., & Schuster, C. R. (1973). Fixed-interval schedule of cocaine reinforcement: Effect of dose and infusion duration. *Journal of the Experimental Analysis of Behavior, 20*, 119-129.

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. *Dev Brain Res, 109*(1), 13-31.

Barrett, J. E., Dworkin, S. I., & Zuccarelli, R. R. (1977). Effects of d-amphetamine, chlordiazepoxide, and promazine on responding of squirrel monkeys maintained under fixed-interval schedules of food presentation and stimulus-shock termination. *Pharmacology Biochemistry and Behavior, 7*, 529–535.

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. *Toxicol Appl Pharm, 65*(1), 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 Sci., 35*, 657-670.

Behne, D., Pfeifer, H., Rothlein, D., & Kyriakopoulos, A. (2000). Cellular and subcellular distribution of selenium and selenium-containing proteins in the rat. In A. M. Roussel, A. E. Favier & R. A. Anderson (Eds.), *Trace Elements in Man and Animals 10.* (pp. 29-34). New York:: Kluwer Academic/Plenum Publishers.

Bemis, J. C., & Seegal, R. F. (1999). Polychlorinated biphenyls and methylmercury act synergistically to reduce rat brain dopamine content in vitro. *Environmental Health Perspectives, 107*(11), 879-885.

Berlin, M., Grant, C. A., Hellberg, J., Hellstrom, J., & Schultz, A. (1975). Neurotoxicity of methylmercury in squirrel monkeys. Cerebral cortical pathology, interference with scotopic vision, and changes in operant behavior. *Arch Environ Health, 30*(7), 340-348.

Beyrouty, P., & Chan, H. M. (2006). Co-consumption of selenium and vitamin E altered the reproductive and developmental toxicity of methylmercury in rats. *Neurotoxicology & Teratology, 28*(1), 49-58.

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 neurobehavior of rats. *Neurotoxicology and Teratology, in press*.

Bjorkman, L., Mottet, K., Nylander, M., Vahter, M., Lind, B., & Friberg, L. (1995). Selenium concentrations in brain after exposure to methylmercury: relations between the inorganic mercury fraction and selenium. *Archives of Toxicology, 69*(4), 228-234.

Bosl, M. R., Takaku, K., Oshima, M., Nishimura, S., & Taketo, M. M. (1997). Early embryonic lethality caused by targeted disruption of the mouse Selenocysteine tRNA gene. *Proc Natl Acad Sci USA, 94*, 5531-5534.

Bouthenet, M. L., Souil, E., Martres, M. P., Sokoloff, P., Giros, B., & Schwartz, J. C. (1991). Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. *Brain Research, 564*, 203-219.

Bratcher, N. A., Farmer-Dougan, V., Dougan, J. D., Heidenreich, B. A., & Garris, P. A. (2005). The role of dopamine in reinforcement: Change in reinforcement sensitivity induced by D1-type, D2-type, and nonselective dopamine receptor agonists. *Journal of the Experimental Analysis of Behavior, 84*, 371-399.

Buelke-Sam, J., Kimmel, C. A., Adams, J., Nelson, C. J., Vorhees, C. V., Wright, D. C., et al. (1985). Collaborative Behavioral Teratology Study: results. *Neurobehavioral Toxicology & Teratology, 7*(6), 591-624.

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.

Bushnell, P. J. (1989). Behavioral effects of acute p-xylene inhalation in rats: autoshaping, motor activity, and reversal learning. *Neurotoxicol Teratol, 10*, 569-577.

Carelli, R. M. (2002). Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. 'natural' reinforcement. *Physiology & Behavior, 76*(3), 379-387.

Carey, R. J., & Goodall, E. B. (1973). A comparison of the effects of amphetamine on fixed interval performance maintained by electrical stimulation of the brain versus food reinforcement. *Pharmacology Biochemistry and Behavior, 1*, 237–239.

Castano, A., Ayala, A., Rodriguez-Gomez, J. A., de la Cruz, C. P., Revilla, E., Cano, J., et al. (1995). Increase in dopamine turnover and tyrosine hydroxylase enzyme in hippocampus of rats fed on low selenium diet. *Journal of Neuroscience Research, 42*(5), 684-691.

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*(6), 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*(4), 1302-1307.

Chudasama, Y., & Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *J Neurosci, 23*(25), 8771-8780.

Combs, G. F., & Combs, S. B. (1984). The nutritional biochemistry of selenium. *Annu Rev Nutr, 4*, 257-280.

Cooper, J. R., Bloom, F. E., & Roth, R. H. (2003). *The Biochemical Basis of Neuropharmacology*. New York: Oxford.

Dave, V., Mullaney, K. J., Goderie, S., Kimelberg, H. K., & Aschner, M. (1994). Astrocytes as mediators of methylmercury neurotoxicity: effects on D-aspartate and serotonin uptake. *Developmental Neuroscience, 16*(3-4), 222-231.

Davidson, P. W., Myers, G. J., Cox, C., Axtell, C., Shamlaye, C., Sloane-Reeves, J., et al. (1998). Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study [see comments]. *JAMA, 280*(8), 701-707.

Davidson, P. W., Myers, G. J., Shamlaye, C., Cox, C., Gao, P., Axtell, C., et al. (1999). Association between prenatal exposure to methylmercury and developmental outcomes in Seychellois children: effect modification by social and environmental factors. *Neurotoxicology, 20*, 833-842.

Dore, F. Y., Goulet, S., Gallagher, A., Harvey, P. O., Cantin, J. F., D'Aigle, T., et al. (2001). Neurobehavioral changes in mice treated with methylmercury at two different stages of fetal development. *Neurotoxicology & Teratology, 23*(5), 463-472.

Dyrssen, D., & Wedborg, M. (1991). The sulfur-mercury (II) system in natural waters. *Water, Air, and Soil Pollution, 56*(507-19).

Eilam, D., Talangbayan, H., Canaran, G., & Szechtman, H. (1992). Dopaminergic control of locomotion, mouthing, snout contact, and grooming: Opposing roles of D-sub-1 and D-sub-2 receptors. *Pharmacology, 106*(447-454).

El-Demerdash, F. (2001). Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver, and blood of rats. *Journal of Environmental Science and Health, 36*(4), 489-499.

EPA. (1997). *Mercury Report To Congress: Health Effects of Mercury and Mercury Compounds* (No. EPA-425/R-97-007). Washington, DC: United States Environmental Protection Agency.

EPA. (2004). EPA Proposes Options for Significantly Reducing Mercury Emissions from Electric Utilities., from http:www.epa.gov/air/mercuryrule/hg_factsheet1_29_04.pdf [accessed 3 February 2006]

Evenden, J. (1999). The pharmacology of impulsive behavior in rats V: the effects of drugs on responding under a discrimination task using unreliable visual stimuli. *Psychopharmacology, 143*, 111-122.

Fishman, J., Rubins, J., Chen, J., Dickey, B., & Volicer, L. (1991). Modification of brain guanine nucleotide-binding regulatory proteins by tryptamine-4,5-dione, a neurotoxic derivative from serotonin. *J Neurochem, 56*, 1851-1854.

Fredriksson, A., Gardlund, A., Bergman, K., Oskarsson, A., Ohlin, B., Danielsson, B., et al. (1993). Effects of maternal dietary supplementation with selenite on the postnatal development of rat offspring exposed to methyl mercury in utero. *Pharmacology & Toxicology, 72*(6), 377-382.

Ganther, H., Goudie, C., Sunde, M., Kopecky, M., & Wagner, P. (1972). Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. . *Science, 175*(26), 1122-1124.

Ganther, H. E., Goudie, C., Sunde, M. L., Kopecky, M. J., & Wagner, P. (1972). Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science, 175*(26), 1122-1124.

Gilbert, S. G., Burbacher, T. M., & Rice, D. C. (1993). Effects of in utero methylmercury exposure on a spatial delayed alternation task in monkeys. *Toxicology & Applied Pharmacology, 123*(1), 130-136.

Gilbert, S. G., Rice, D. C., & Burbacher, T. M. (1996). Fixed interval/fixed ratio performance in adult monkeys exposed in utero to methylmercury. *Neurotoxicology & Teratology, 18*(5), 539-546.

Gomer, F. E., & Jakubczak, L. F. (1974). Dose-dependent selective facilitation of response-contingent light-onset behavior by d-amphetamine. *Psychopharmacologia, 34*, 199–208.

Goulet, S., Dore, F., & Mirault, M. (2003). Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. *Neurotoxicology & Teratology, 25*(3), 335-347.

Grandjean, P., Murata, K., Budtz-Jorgensen, E., & Weihe, P. (2004). Cardiac autonomic activity in methylmercury neurotoxicity: 14-year follow-up of Faroese birth cohort. *J Pediatr, 144*(2), 417-428.

Grandjean, P., & Weihe, P. (1993). Neurobehavioral effects of intrauterine mercury exposure: potential sources of bias. *Environmental Research, 63*(1), 176-183.

Grandjean, P., Weihe, P., White, R. F., & Debes, F. (1998). Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. *Environmental Research, 77*(2), 165-172.

Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S., Yokoyama, K., et al. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology & Teratology, 19*(6), 417-428.

Greenwood, M. R. (1985). Methylmercury poisoning in Iraq. An epidemiological study of th 1971-1972 outbreak. *Journal of Applied Toxicology, 5*, 148-159.

Gregus, Z., Gyurasics, A., Csanaky, I., & Pinter, Z. (2001). Effects of methylmercury and organic acid mercurials on the disposition of exogenous selenium in rats. *Toxicol. Appl. Pharmacol., 174*, 177-187.

Gunderson, V. M., Grant-Webster, K. S., Burbacher, T. M., & Mottet, N. K. (1988). Visual recognition memory deficits in methylmercury-exposed Macaca fascicularis infants. *Neurotoxicology & Teratology, 10*(4), 373-379.

Gunderson, V. M., Grant, K. S., Burbacher, T. M., Fagan, J. F. d., & Mottet, N. K. (1986). The effect of low-level prenatal methylmercury exposure on visual recognition memory in infant crab-eating macaques. *Child Development, 57*(4), 1076-1083.

Hall, H., Farde, L., & Sedvall, G. (1988). Human dopamine receptor subtypes--in vitro binding analysis using 3H-SCH 23390 and 3H-raclopride. *Journal of neural transmission., 73*, 7-21.

Harada, Y. (1968). Congential (or fetal) Minamata disease. In *Minamata Disease (Study Group of Minamata Disease, eds.)*. Kumamato, Japan: Kumamato University.

Head, D., Raz, N., Gunning-Dixon, F., Williamson, A., & Acker, J. D. (2002). Age-related differences in the course of cognitive skill acquisition: the role of regional cortical shrinkage and cognitive resources. *Psychol Aging*, 72-84.

Hearst, E. (1961). Effects of d-amphetamine on behavior reinforced by food and water. *Psychological Reports, 8*, 301–309.

Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., et al. (2003). Deletion of Selenoprotein P alters distribution of selenium in the mouse. *J Biolog Chem, 278*(16), 1360-1366.

Huntley, G. W., Morrison, J. H., Prikhozhan, A., & Sealfon, S. C. (1992). Localization of multiple dopamine receptor subtype mRNAs in human and monkey motor cortex and striatum. *Brain research. Molecular brain research., 15*(3-4), 181-188.

Imura, N. (1986). The role of micronutrient, selenium, in the manifestation of toxicity of heavy metals. In P. Chambers, P. Gehring & F. Sakai (Eds.), *New concepts and developments in toxicology* (pp. 115-123): Elsevier.

Imura, N., & Naganuma, A. (1991). Possible mechanisms of detoxifying effect of selenium on the toxicity of mercury compounds. In T. Suzuki, N. Imura & T. Clarkson (Eds.), *Advances in mercury toxicity* (pp. 490). New York: Plenum Press.

Iyengar, G. V., & Rapp, A. S. O. (2001). Human placenta as a "Duel" biomarker for monitoring fetal and maternal environmental with special reference to potentially toxic trace elements. Part 3: toxic trace elements in placenta and placenta as a

biomarker for these elements. *Science of the Total Environment, 280*((1-3)), 221-238.

Julshamn, K., Anderson, A., Ringdal, O., & Morkore, J. (1987). Trace elements intake in the Faroe Islands. I. Element levels in edible parts of pilot whales (Globicephalus Meleanus). *Sci Total Environ, 65*, 53-62.

Kerper, L. E., Ballatori, N., & Clarkson, T. W. (1992). Methylmercury transport across the blood-brain barrier by an amino acid carrier. *American Journal of Physiology, 267*, 761-765.

Kheramin, S., Body, S., Herrera, F. M., Bradshaw, C. M., Szabadi, E., Deakin, J. F., et al. (2005). The effect of orbital prefrontal cortex lesions on performance on a progressive ratio schedule: implications for models of inter-temporal choice. *Behav Brain Res, 156*(1), 145-152.

Kheramin, S., Body, S., Mobini, S., Ho, M. Y., Velazquez-Martinez, D. N., Bradshaw, C. M., et al. (2002). Effects of quinolinic acid-induced lesions of the orbital prefrontal cortex on inter-temporal choice: a quantitative analysis. *Psychopharmacology, 165*(1), 9-17.

Kjellstrom, T., Kennedy, P., Wallis, S., Stewart, A., Friberg, L., & Lind, B. (1989). *Physical and mental development of children with prenatal exposure to mercury from fish. Stage II: Interviews and psychological tests at age 6.* Paper presented at the National Swedish Environmental Protection Board Report 3642, Solna, Sweden.

Kjellstrom, T., Kennedy, S., Wallis, & Mantell. (1986). *Physical and mental development of children with prenatal exposure to mercury from fish. Stage I: Preliminary test at age 4.* Paper presented at the National Swedish Environmental Protection Board Report 3642, Solna, Sweden.

Kurylo, D. D. (2004). Effects of quinpirole on operant conditioning: perseveration of behavioral components. *Behavioural Brain Research, 2004*, 117-124.

Kurylo, D. D., & Tanguay, S. (2003). Effects of quinpirole on behavioral extinction. *Physiol Behav, 80*, 1-7.

Laties, V. G. (1972). The modification of drug effects on behavior by external discriminative stimuli. *J Pharmacol Exp Therap, 183*, 1-13.

Laties, V. G. (1975). The role of discriminative stimuli in modulating drug action. *Federation Proceedings, 34*(9), 1880-1888.

Laties, V. G., & Evans, H. L. (1980). Methylmercury-induced changes in operant discrimination by the pigeon. *Journal of Pharmacology & Experimental Therapeutics, 214*(3), 620-628.

Laties, V. G., & Weiss, B. (1966). Influence of drugs on behavior controlled by internal and external stimuli. *J Pharmacol Exp Therap, 152*, 388-396.

Leander, J. D., & McMillan, D. E. (1974). Rate-dependent effects of drugs. I. Comparisons of d-Amphetamine, pentobarbital, and chlorpromazine on multiple and mixed schedules. *J Pharmacol Exp Therap, 188*, 726-739.

Lieving, L. M., Odum, A. L., & Schaal, D. W. (2002). The effects of morphine on chained and clocked fixed-interval schedule performance in pigeons. *Behavioural Pharmacology, 13*, 221-228.

Lourdes, M. A., Culvin-Aralar, A., & Furness, R. W. (1991). Mercury and selenium interaction: a review. *Ecotoxicol Environ Safety, 21*, 348-364.

Magos, L. (1991). Overview on the protection given by selenium against mercurials. In T. Suzuki, I. Nobumasa & T. W. Clarkson (Eds.), *Advances in Mercury Toxicology* (pp. 289-298). New York: Plenum.

Magos, L., Clarkson, C. W., & Hudson, A. R. (1984). Differences in the effects of selenite and biological selenium on the chemical form and distribution of mercury after the simultaneous administration of $HgCl_2$ and selenium to rats. *J Pharmacol Exp Ther, 228*, 478-483.

Markowski, V. P., Flaugher, C. B., Baggs, R. B., Rawleigh, R. C., Cox, C., & Weiss, B. (1998). Prenatal and lactational exposure to methylmercury affects select parameters of mouse cerebellar development. *Neurotoxicology, 19*(6), 879-892.

Marsh, D. O. (1987). Dose-response relationships in humans: Methyl Mercury epidemics in Japan and Iraq. In *The Toxicity of Methyl Mercury* (pp. 45-53). Baltimore: Johns Hopkins University Press.

Marsh, D. O., Clarkson, T. W., Cox, C., Myers, G. J., Amin-Zaki, L., & Al-Tikriti, S. (1987). Fetal methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. *Archives of Neurology, 44*(10), 1017-1022.

Meltzer, D., & Brahlek, J. A. (1968). Quantity of reinforcement and fixed-interval performance. *Psychonomic Science, 12*, 207-208.

Meltzer, D., & Brahlek, J. A. (1970). Quantity of reinforcement and fixed-interval performance: Within-subject effects. *Psychonomic Science, 20*, 30-31.

Mengod, G., Martinez-Mir, M., Vilaro, M., & JM, P. (1989). Localization of the mRNA for the dopamine D2 receptor in the rat brain by in situ hybridization histochemistry. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 8560-8564.

Miller, D. B., & Eckerman, D. A. (1986). Learning and memory measures. In Z. Annau (Ed.), *Neurobehavioral Toxicology* (pp. 94-149). Baltimore: Johns Hopkins University Press.

Miura, K., & Imura, N. (1987). Mechanism of methylmercury cytotoxicity. *Crit. Rev. Toxicol., 18*(3), 161-188.

Mobini, S., Body, S., Ho, M. Y., Bradshaw, C. M., Szabadi, E., Deakin, J. F., et al. (2002). Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology, 160*(3), 290-298.

Mobini, S., Chiang, T. J., Ho, M. Y., Bradshaw, C. M., & Szabadi, E. (2000). Comparison of the effects of clozapine, haloperidol, chlorpromazine and d-amphetamine on performance on a time-constrained progressive ratio schedule and on locomotor behaviour in the rat. *Psychopharmacology, 152*(1), 47-54.

Moller-Madsen, B., & Danscher, G. (1991). Localization of mercury in CNS of the rat. IV. The effect of selenium on orally administered organic and inorganic mercury. *Toxicol Appl Pharm, 108*(3), 457-473.

Montague, P. R., Hyman, S. E., & Cohen, J. D. (2004). Computational roles for dopamine in behavioural control. *Nature, 431*, 760-767.

Murata, K., Weihe, P., Budtz-Jorgensen, E., Jorgensen, P. J., & Grandjean, P. (2004). Delayed brainstem auditory evoked potential latencies in 14-uear-old children exposed tomethylmercury. *J Pediatr, 144*(2), 177-183.

Myers, G. J., Davison, P., Cox, C., Shamlaye, C., Palumbo, D., & Cernichiari, E. (2003). Prenatal methylmercury exposure from the ocean fish consumption in the Seychelles child development study. *Lancet, 361*, 1686-1692.

Naganuma, A., & Imura, N. (1980). Bis(methylmercuric) selenide as a reaction product from methylmercury and selenite in rabbit blood. *Commun. Chem. Pathol. Pharmacol., 27*(163-73).

Naganuma, A., & Imura, N. (1983). Mode of interaction mercuric mercury with selenite to form high-molecular weight substance in rabbit blood in vitro. *Chem Biol Interact, 43*, 271.

Naganuma, A., Ishii, Y., & Imura, N. (1984). Effect of administration sequence of mercuric chloride and sodium seleite on their fates and toxicities in mice. *Exotoxicol Environ Saf, 8*(572-577.).

Naganuma, A., Kojima, Y., & Imura, N. (1980). Interaction of methylmercury and selenium in mouse: Formation and decomposition of bis(metylmercuric) selenide. *Res Commun Chem Pathol Pharmacol, 30*, 301-316.

National Research Council. (1995). *Nutrient Requirements of Laboratory Animals*. Washington, D.C.: National Academy Press.

National Research Council. (2000). *Toxicological Effects of Methylmercury*. Washington, D.C.: National Academy Press.

National Research Council. (2001). *Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders*. Washington, D.C.: 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): CRC Press.

Newland, M. C., & Paletz, E. M. (2000). Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology, 21*(6), 1003-1027.

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*(5), 710-720.

Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicol Sci, 50*(1), 106-116.

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicol Teratol, 26*(2), 179-194.

Newland, M. C., Yezhou, S., Logdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicol Appl Pharm, 126*(1), 6-15.

Nigrosh, B. J., Slotnick, B. M., & Nevin, J. A. (1975). Sensory modality and stimulus control in the pigeon : Cross-species generality of single-incentive selective-association effects. *Journal of Comparative and Physiological Psychology, 89*(4), 285-294.

Nishikido, N., Furuyashiki, K., Naganuma, A., Suzuki, T., & Imura, H. (1987). Maternal selenium deficiency enhances the fetolethal toxicity of methyl mercury. *Toxicol. Appl. Pharmacol., 88*, 322-238.

Nuttall, K. L. (1987). A model for metal selenide formation under biological conditions. *Med Hypoth, 24*, 217-221.

O'Kusky, J. (1983). Methylmercury poisoning of the developing nervous system: morphological changes in neuronal mitochondria. *Acta Neuropathologica, 61*(2), 116-122.

Odum, A. L., & Schaal, D. W. (1999). The effects of morphine on clocked fixed-interval performance: stimulus function or strength of stimulus control? *Behav Pharmacol, 10*(3), 243-255.

Odum, A. L., & Schall, D. W. (1999). The effects of morphine on fixed-interval patterning and temporal discrimination. *Behav Pharmacol, 10*, 243-255.

Olanow, C. W. (1990). Oxidation reactions in Parkinson's disease. *Neurology, 40*(3`), 32-37.

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. *Neurotoxicol Teratol, 28*, 59-73.

Parizek, J., & Ostadalova, I. (1967). The protective effect of small amounts of selenite in sublimate intoxication. *Experientia, 23*(142-143).

Paule, M. G., Meck, W. H., McMillan, D. E., McClure, G. Y., Bateson, M., Popke, E. J., et al. (1999). The use of timing behaviors in animals and humans to detect drug and/or toxicant effects. *Neurotoxicology & Teratology, 21*(5), 491-502.

Pilf, C., Drobny, H., Reither, H., Hornykiewicz, O., & Singer, E. A. (1995). Mechanism of the dopamine-releasing actions of amphetamine and cocaine: plasmalemmal dopamine transporter versus vesicular monoamine transporter. *Mol Pharmacol, 47*, 368-373.

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

Raymond, L. J., & Ralston, N. V. C. (2004). Mercury:selenium interactions and health implications. *Seychelles Medical and Dental Journal (SMDJ), 7*(1), 72-77.

Raz, N., Gunning-Dixon, F. M., Head, D., Dupuis, J. H., & Acker, J. D. (1998). Neuroanatomical correlates of cognitive aging: evidence from structural magnetic resonance imaging. *Neuropsychology*, 95-114.

Reed, M. N., Banna, K. M., Donlin, W. D., & Newland, M. C. (under review). Effects of gestational exposure to methylmercury and dietary selenium on reinforcement efficacy in adulthood *Behavioral Neuroscience*.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: effects on spatial discrimination reversal in adulthood. *NeuroToxicology, 27*(5), 721-732.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr, 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. *J Nutr, 123*(11), 1939-1951.
Rice, D. C. (1988). Quantification of operant behavior. *Toxicol. Letters, 43*, 361-379.
Rice, D. C. (1992). Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance. *Neurotoxicology, 13*(2), 443-452.
Rice, D. C., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from human and animal models. *Environmental Health Perspectives, 108*(3), 511-533.
Ridley, R. M., Clark, B. A., Durnford, L. J., & Baker, H. F. (1993). Stimulus-bound perseveration after frontal ablations in marmosets. *Neuroscience*, 595-604.
Rodier, P. M. (1995). Developing brain as a target of toxicity. *Environmental Health Perspectives, 103*(suppl 6), S73-S76.
Salamone, J. D., Arizzi, M. N., Sandoval, M. D., Cervone, K. M., & Aberman, J. E. (2002). Dopamine antagonists alter response allocation but do not suppress appetite for food in rats: contrast between the effects of SKF 83566, raclopride, and fenfluramine on a concurrent choice task. *Psychopharmacology, 160*(4), 371-380.
Salamone, J. D., Correa, M., Mingote, S., & Weber, S. M. (2003). Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry, and drug abuse. *Journal of Pharmacology & Experimental Therapeutics, 305*(1), 1-8.
Schreiner, G., Ulbrich, B., & Bass, R. (1986). Testing strategies in behavioral teratology: II. Discrimination learning. *Neurobehavioral Toxicology & Teratology, 8*(5), 567-572.
Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, 80*(1).
Schultz, W., Tremblay, L., & Hollerman, J. R. (1998). Reward prediction in primate basal ganglia and frontal cortex. [Review] [30 refs]. *Neuropharmacology, 37*(4-5), 421-429.
Sharp, T., Zetterstorm, T., & Ungerstedt, U. (1986). An *in vivo* study of dopamine release and metabolism in rat brain regions using intracerebral dialysis. *J. Neurochem, 47*, 113-122.
Smith, W. E., & Smith, A. M. (1975). *Minamata*. New York: Holt, Rinehart, and Winston.
Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: past and current status. *Trends in Neurosciences, 22*(11), 521-527.
Stebbins, W. C., Mead, P. B., & Martin, J. M. (1959). The relation of amount of reinforcement to performance under a fixed-interval schedule. *Journal of the Experimental Analysis of Behavior, 2*, 351-355.

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*(4), 467-477.

Steuerwald, U., Weihe, P., Jorgensen, P. J., Bjerve, K., Brock, J., Heinzow, B., et al. (2000). Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. [see comments]. *Journal of Pediatrics, 136*(5), 599-605.

Sulzer, D., Chen, T., Lau, Y., Kristensen, H., Rayport, S., & Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J Neuroscience, 15*, 4102-4108.

Sumino, K., Yamamoto, R., & Kitamura, S. (1977). A role of selenium against methylmercury toxicity. *Nature, 268*, 73-74.

Szechtman, H., Talangbayan, H., Canaran, G., Dai, H., & Eilam, D. (1994). Dynamics of behavioral sensitization induced by the dopamie agonist quinpirole and a proposed central energy control mechanism. *Psychopharmacology, 115*, 95-104.

Terry, P., Gilbert, D. B., & Cooper, S. J. (1995). Dopamine receptor subtype agonists and feeding behavior. *Obesity Research 3*, 515S-523S

Trasande, L., Landrigan, P. J., & Schechter, C. (2005). Public health and economic consequences of methyl mercury toxicity to the developing brain. *Environmental Health Perspectives, 113*, 590-596.

Traxinger, D. L., & Atchison, W. D. (1987). Comparative effects of divalent cations on the methylmercury-induced alterations of acetylcholine release. *Journal of Pharmacology & Experimental Therapeutics, 240*(2), 451-459.

Tremblay, L., & Schultz, W. (1999a). Relative reward preference in primate orbitofrontal cortex.[comment]. *Nature, 398*(6729), 704-708.

Tremblay, L., & Schultz, W. (1999b). Relative reward preference in primate orbitofrontal cortex.[see comment]. *Nature, 398*(6729), 704-708.

Tsukada, H., Harada, N., Nishiyama, S., Ohba, H., & Kakiuchi, T. (2000). Dose-response and duration effects of acute administrations of cocaine and GBR12909 on dopamine synthesis and transporter in the conscious monkey brain: PET studies combined with microdialysis. *Brain Research, 31*, 141-148.

Uauy-Dagach, R., Valenzuela, A. (1996). Marine oils: the health benefits of n-3 fatty acids. [*Nutrition Reviews, 54*(11 PT 2), S102-108.

UNEP. (2002). Global Mercury Assessment Report. [Retrieved 2006], from http://www.chem.unep.ch/mercury/Report/GMA-report-TOC.htm

Ungless, M. A., Magill, P. J., & Bolam, J. P. (2004). Uniform iInhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science, 26*, 2040-2042.

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. *TAP, 134*, 273-284.

Van Tol, H. H. M., Bunzow, J. R., H., G., Sunahara, R. K., Seeman, P., Niznik, H. B., et al. (1991). Cloning of a human dopamine D4 receptor gene with high affinity for the antipsychotic clozapine. *Nature, 350*, 610-614.

Vereno, J. L., Machado, A., & Cano, J. (1990). Determination of levels of biogenic amines and their metabolites and both forms of monoamine oxidase in prefrontal cortex of aged rats. *Mechanisms of Ageing and Development, 56*, 253-263.

Watanabe, C. (2001). Selenium deficiency and brain functions: the significance for methylmercury toxicity. *Nippon Eiseigaku Zasshi - Japanese Journal of Hygiene, 55*(4), 581-589.

Watanabe, C. (2002). Modification of mercury toxicity by selenium: practical importance?. *Tohoku Journal of Experimental Medicine, 196*(2), 71-77.

Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicol Teratol, 21*(1), 83-88.

Watanabe, C., Yoshida, K., Kasanuma, Y., Kun, Y., & Satoh, H. (1999). In utero methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. *Environmental Research, 80*(3), 208-214.

Watchel, S. R., Brooderson, R. J., & White, F. J. (1992). Parametric and pharmacological analyses oenhanced grooming response elicited by the D1 receptor agonist SKF38393 in the rat. *Psychopharmacology, 109*, 41-48.

Weiss, B., & Laties, V. L. (1963). Effects of amphetamine, chlorpromazine, and pentobarbital on behavioral thermoregulation. *Journal of Pharmacology and Experimental Therapeutics, 140*, 1-7.

West, J. R. (1987). Fetal alcohol-induced brain damage and the problem of determining temporal vulnerability: A review. *Alcohol Drug Res, 7*, 423–441.

Whanger, P. (1992). Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. [Review]. *Journal of Trace Elements & Electrolytes in Health & Disease, 6*(4), 209-221.

Widholm, J. J., Villareal, S., Seegal, R. F., & Schantz, S. L. (2004). Spatial alternation deficits following developmental exposure to Aroclor 1254 and/or methylmercury in rats. *Toxicol Sci, 82*(2), 577-589.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience, 5*(6), 483-494.

Yoneda, S., & Suzuki, K. (1997). Equimolar Hg-Se complex binds to selenoprotein P. *Biochemical and Biophysical Research Communications, 231*, 7-11.

CHAPTER 2

PRENATAL METHYLMERCURY EXPOSURE INCREASES RESPONDING UNDER CLOCKED AND UNCLOCKED FIXED INTERVAL SCHEDULES OF REINFORCEMENT

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) x 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.

Introduction

Methylmercury (MeHg) is a known developmental neurotoxicant found in fish and marine mammals. Fish, however, are also an important nutrient rich in selenium and long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA). In rodent studies examining adult-onset MeHg exposure, selenium (Se) ameliorated some of the effects of chronic, high-level MeHg exposure (H. E. Ganther, C. Goudie, M. L. Sunde, M. J. Kopecky, & P. Wagner, 1972; Imura, 1986; Moller-Madsen & Danscher, 1991). 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 (Nishikido et al., 1987), 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 (Watanabe, Yin et al., 1999). In contrast, a Se-sufficient diet has not attenuated MeHg's neurobehavioral toxicity (Beyrouty & Chan, 2006; Reed et al., 2006) with the exception of one study (Watanabe, Yin et al., 1999). In this one study, Se excess reduced 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 (Fredriksson et al., 1993). 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 (Reed et al., 2006). 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 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 pattern produced by MeHg-exposure could be viewed as a reduced sensitivity to changing reinforcement contingencies or an increased reinforcer efficacy (Newland, Donlin et al., 2006). 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 (Newland et al., 2004; Newland et al., 1994), resulted in more rapid acquisition of lever-pressing and lack of ratio strain under large fixed-ratio schedules of reinforcement (Paletz

et al., 2006), and a tolerance for higher ratios under a progressive ratio procedure (Paletz et al., 2006; Reed, Banna, Donlin, & Newland, under review).

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 (Meltzer & Brahlek, 1968; Meltzer & Brahlek, 1970), sucrose solution (Stebbins et al., 1959), and cocaine (Balster & Schuster, 1973). 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. Buelke-Sam et al., 1985; Rice, 1992; Schreiner et al., 1986), 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) (Laties & Weiss, 1966; Odum & Schaal, 1999), 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 (Buelke-Sam et al., 1985; Newland & Paletz, 2000; Rice, 1992; Schreiner et al., 1986), 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 (Magos, 1991; Raymond & Ralston, 2004; Steuerwald et al., 2000; Watanabe, 2002; Whanger, 1992). The experiments were conducted using a 2 (chronic Se) x 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 (Burbacher et al., 1990; Newland & Reile, 1999), as determined by brain mercury (Newland, Reed, LeBlanc, & Donlin, 2006). 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 (National Research Council, 1995; Reeves et al., 1993). 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 (Reeves, 1997; Reeves et al., 1993), but is below that thought to be toxic (Abdo, 1994).

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.

Methods

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) x 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 postnatal 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 x 45.7 cm x 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 gm/day so as to maintain their body weight at 250 grams. 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. They were 11 ± 1 months of age at the beginning of the present experiment and 21 ± 1 months of age when the omission procedure began.

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 Exposures; see Figure 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.

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.

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 Figure 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, 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 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 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 (Stern, Cox, Cernichiari, Balys, & Weiss, 2001), 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 (Newland & Reile, 1999). Fluid consumption reported in the earlier paper (Newland & Reile, 1999) 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 (Newland & Reile, 1999; Stern et al., 2001). Throughout the remainder of life, all F_1 rats received plain tap water to drink. Male breeders received exposure to plain tap water only.

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”L x 9 ½”W x 11 ½”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.

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.

Training. Upon reaching adulthood, rats were trained to lever-press on the right lever using autoshaping (Bushnell, 1989; Newland et al., 2004). 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 and a 0.5 sec, 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 hours 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".

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.

Reinforcement Omission Trials. At 21 months of age, an intervention resembling the "peak interval" procedure (Paule et al., 1999) 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”.

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 posthoc tests.

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:

1) *Overall response rate* – the total number of responses throughout the interval divided by 120”
2) *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.

3) *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.

4) *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 quarter-life 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.

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.

Results

FI CFI Condition: 13 Months

There was a within-subject effect of the clock on all response-rate measures. As seen in Figure 2, overall response rates were slightly greater 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 [$F(1,36)=7.943, P=.008$].

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 Figure 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 [$F(1,36)=9.386, P=.004$; Figure 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 [$F(2,36)=4.182, P=.023$; Figure 3, left panel]. For both the FI and CFI components, the High Se controls had lower quarterlife values than all other exposure groups (Ps<0.05), except the Low Se, 0.5 ppm Hg group (Ps>0.1 for both components).

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

For quarterlife, there was interaction between age and Se for the CFI component [$F(1,31)=4.317, P=.046$; Figure 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 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 (*P*s>0.1), and no other statistically significant within-subjects effects of age or interactions with age were observed (*P*s>0.1).

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 [$F(1,30)=144.4, P<.001$; Figure 4].

There was a between-subjects effect of MeHg [$F(2,30)=3.6, P=.04$; Figure 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 Hg groups (*P*s<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 (*P*s>0.1)

Discussion

Prenatal exposure to MeHg and a lifelong diet that was either marginal or rich in Se were manipulated in a 2 (Se) x 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 (Laties, 1972; Laties, 1975) or adult-onset, chronic MeHg exposure (Laties & Evans, 1980). 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.

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 work with clocked FIs and pigeons (e.g. Laties & Weiss, 1966; Lieving, Odum, & Schaal, 2002). 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 (Nigrosh, Slotnick, & Nevin, 1975).

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.

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 (Buelke-Sam et al., 1985; Newland & Paletz, 2000; Rice, 1992; Schreiner et al., 1986). 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 postnatal exposure to MeHg in monkeys (Gilbert et al., 1996; Rice, 1992). 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 (West, 1987). The monkeys in the previous studies (Gilbert et al., 1996; Rice, 1992) were exposed during the third trimester. Since neurogenesis of the cerebellum and hippocampus dentate granule cells occurs primarily during the third trimester (Rice & Barone, 2000), 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 (Gilbert et al., 1996; Newland et al., 2004; Newland et al., 1994; Paletz et al., 2006; Reed et al., 2006; Rice, 1992) 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 (Gilbert et al., 1996; Newland et al., 2004; Newland et al., 1994; Paletz et al., 2006; Reed et al., 2006; Rice, 1992). 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 (Buelke-Sam et al., 1985; Newland & Paletz, 2000; Rice, 1992; Schreiner et al., 1986) 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 (Laties & Evans, 1980).

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 (Reed et al., 2006) 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.

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 (Raymond & Ralston, 2004). 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

(Newland, Reed et al., 2006), 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 (Newland, Reed et al., 2006). 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 (Behne et al., 2000; Hill et al., 2003) than the 10-fold range used here (Newland, Reed et al., 2006).

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 (Fredriksson et al., 1993) that disappear in older animals or that are not apparent in fixed-interval responding or reinforcement omission procedures.

REFRENCES

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

Balster, R. L., & Schuster, C. R. (1973). Fixed-interval schedule of cocaine reinforcement: Effect of dose and infusion duration. *Journal of the Experimental Analysis of Behavior, 20*, 119-129.

Behne, D., Pfeifer, H., Rothlein, D., & Kyriakopoulos, A. (2000). Cellular and subcellular distribution of selenium and selenium-containing proteins in the rat. In A. M. Roussel, A. E. Favier & R. A. Anderson (Eds.), *Trace Elements in Man and Animals 10.* (pp. 29-34). New York:: Kluwer Academic/Plenum Publishers.

Beyrouty, P., & Chan, H. M. (2006). Co-consumption of selenium and vitamin E altered the reproductive and developmental toxicity of methylmercury in rats. *Neurotoxicology & Teratology, 28*(1), 49-58.

Buelke-Sam, J., Kimmel, C. A., Adams, J., Nelson, C. J., Vorhees, C. V., Wright, D. C., et al. (1985). Collaborative Behavioral Teratology Study: results. *Neurobehavioral Toxicology & Teratology, 7*(6), 591-624.

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.

Bushnell, P. J. (1989). Behavioral effects of acute p-xylene inhalation in rats: autoshaping, motor activity, and reversal learning. *Neurotoxicol Teratol, 10*, 569-577.

Fredriksson, A., Gardlund, A., Bergman, K., Oskarsson, A., Ohlin, B., Danielsson, B., et al. (1993). Effects of maternal dietary supplementation with selenite on the postnatal development of rat offspring exposed to methyl mercury in utero. *Pharmacology & Toxicology, 72*(6), 377-382.

Ganther, H. E., Goudie, C., Sunde, M. L., Kopecky, M. J., & Wagner, P. (1972). Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science, 175*(26), 1122-1124.

Gilbert, S. G., Rice, D. C., & Burbacher, T. M. (1996). Fixed interval/fixed ratio performance in adult monkeys exposed in utero to methylmercury. *Neurotoxicology & Teratology, 18*(5), 539-546.

Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., et al. (2003). Deletion of Selenoprotein P alters distribution of selenium in the mouse. *J Biolog Chem, 278*(16), 1360-1366.

Imura, N. (1986). The role of micronutrient, selenium, in the manifestation of toxicity of heavy metals. In P. Chambers, P. Gehring & F. Sakai (Eds.), *New concepts and developments in toxicology* (pp. 115-123): Elsevier.

Laties, V. G. (1972). The modification of drug effects on behavior by external discriminative stimuli. *J Pharmacol Exp Therap, 183*, 1-13.
Laties, V. G. (1975). The role of discriminative stimuli in modulating drug action. *Federation Proceedings, 34*(9), 1880-1888.
Laties, V. G., & Evans, H. L. (1980). Methylmercury-induced changes in operant discrimination by the pigeon. *Journal of Pharmacology & Experimental Therapeutics, 214*(3), 620-628.
Laties, V. G., & Weiss, B. (1966). Influence of drugs on behavior controlled by internal and external stimuli. *J Pharmacol Exp Therap, 152*, 388-396.
Lieving, L. M., Odum, A. L., & Schaal, D. W. (2002). The effects of morphine on chained and clocked fixed-interval schedule performance in pigeons. *Behavioural Pharmacology, 13*, 221-228.
Magos, L. (1991). Overview on the protection given by selenium against mercurials. In T. Suzuki, I. Nobumasa & T. W. Clarkson (Eds.), *Advances in Mercury Toxicology* (pp. 289-298). New York: Plenum.
Meltzer, D., & Brahlek, J. A. (1968). Quantity of reinforcement and fixed-interval performance. *Psychonomic Science, 12*, 207-208.
Meltzer, D., & Brahlek, J. A. (1970). Quantity of reinforcement and fixed-interval performance: Within-subject effects. *Psychonomic Science, 20*, 30-31.
Moller-Madsen, B., & Danscher, G. (1991). Localization of mercury in CNS of the rat. IV. The effect of selenium on orally administered organic and inorganic mercury. *Toxicol Appl Pharm, 108*(3), 457-473.
National Research Council. (1995). *Nutrient Requirements of Laboratory Animals*. Washington, D.C.: 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): CRC Press.
Newland, M. C., & Paletz, E. M. (2000). Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology, 21*(6), 1003-1027.
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*(5), 710-720.
Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicol Sci, 50*(1), 106-116.
Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicol Teratol, 26*(2), 179-194.
Newland, M. C., Yezhou, S., Logdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicol Appl Pharm, 126*(1), 6-15.
Nigrosh, B. J., Slotnick, B. M., & Nevin, J. A. (1975). Sensory modality and stimulus control in the pigeon : Cross-species generality of single-incentive

selective-association effects. *Journal of Comparative and Physiological Psychology, 89*(4), 285-294.

Nishikido, N., Furuyashiki, K., Naganuma, A., Suzuki, T., & Imura, H. (1987). Maternal selenium deficiency enhances the fetolethal toxicity of methyl mercury. *Toxicol. Appl. Pharmacol., 88*, 322-238.

Odum, A. L., & Schaal, D. W. (1999). The effects of morphine on clocked fixed-interval performance: stimulus function or strength of stimulus control? *Behav Pharmacol, 10*(3), 243-255.

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. *Neurotoxicol Teratol, 28*, 59-73.

Paule, M. G., Meck, W. H., McMillan, D. E., McClure, G. Y., Bateson, M., Popke, E. J., et al. (1999). The use of timing behaviors in animals and humans to detect drug and/or toxicant effects. *Neurotoxicology & Teratology, 21*(5), 491-502.

Raymond, L. J., & Ralston, N. V. C. (2004). Mercury:selenium interactions and health implications. *Seychelles Medical and Dental Journal (SMDJ), 7*(1), 72-77.

Reed, M. N., Banna, K. M., Donlin, W. D., & Newland, M. C. (under review). Effects of gestational exposure to methylmercury and dietary selenium on reinforcement efficacy in adulthood *Behavioral Neuroscience*.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: effects on spatial discrimination reversal in adulthood. *NeuroToxicology, 27*(5), 721-732.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr, 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. *J Nutr, 123*(11), 1939-1951.

Rice, D. C. (1992). Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance. *Neurotoxicology, 13*(2), 443-452.

Rice, D. C., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from human and animal models. *Environmental Health Perspectives, 108*(3), 511-533.

Schreiner, G., Ulbrich, B., & Bass, R. (1986). Testing strategies in behavioral teratology: II. Discrimination learning. *Neurobehavioral Toxicology & Teratology, 8*(5), 567-572.

Stebbins, W. C., Mead, P. B., & Martin, J. M. (1959). The relation of amount of reinforcement to performance under a fixed-interval schedule. *Journal of the Experimental Analysis of Behavior, 2*, 351-355.

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*(4), 467-477.

Steuerwald, U., Weihe, P., Jorgensen, P. J., Bjerve, K., Brock, J., Heinzow, B., et al. (2000). Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. [see comments]. *Journal of Pediatrics, 136*(5), 599-605.

Watanabe, C. (2002). Modification of mercury toxicity by selenium: practical importance?. *Tohoku Journal of Experimental Medicine, 196*(2), 71-77.

Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicol Teratol, 21*(1), 83-88.

West, J. R. (1987). Fetal alcohol-induced brain damage and the problem of determining temporal vulnerability: A review. *Alcohol Drug Res, 7*, 423–441.

Whanger, P. (1992). Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. [Review]. *Journal of Trace Elements & Electrolytes in Health & Disease, 6*(4), 209-221.

FIGURE CAPTIONS

Figure 2.1. 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.

Figure 2.2. 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. Error bars represent ± 1 SEM.

Figure 2.3. 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 (right) months of age. Error bars represent ± 1 SEM.

Figure 2.4. 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.

Figure 2.5. 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.

FIGURE 2.1

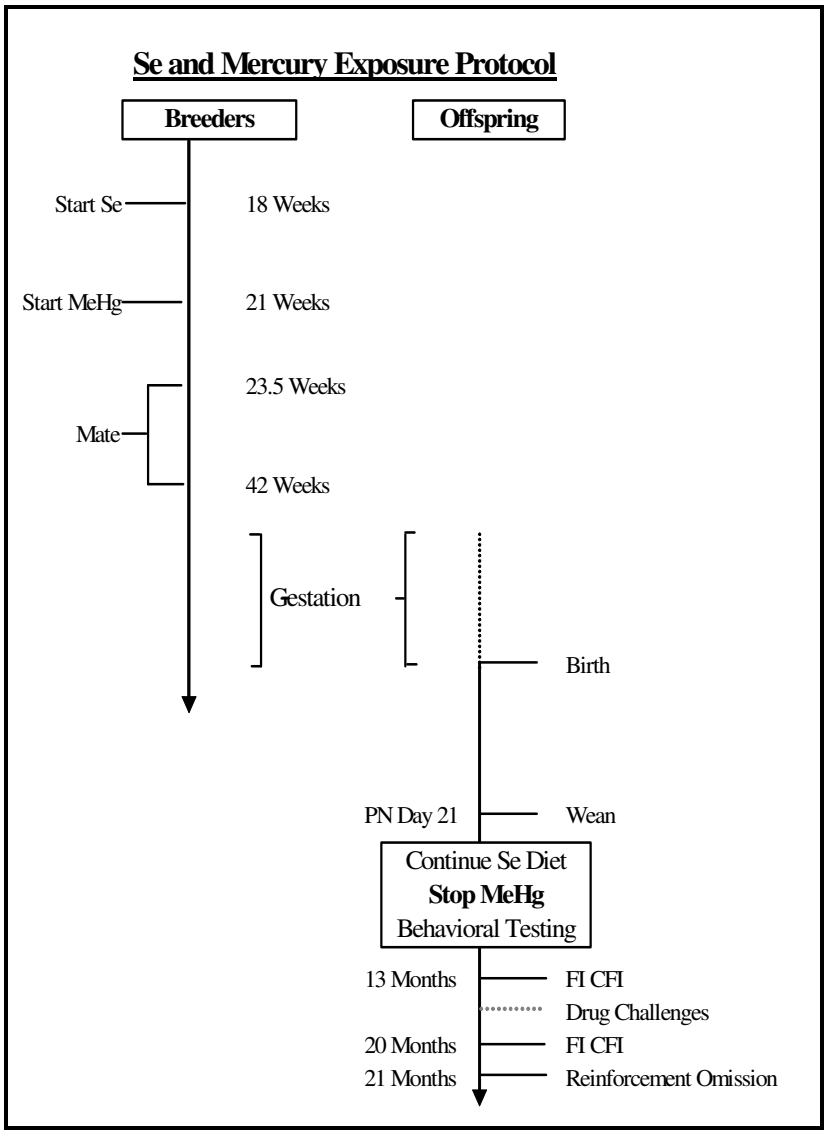

Se and Mercury Exposure Protocol
Breeders
Offspring
Start Se
18 Weeks
Start MeHg
21 Weeks
23.5 Weeks
Mate
42 Weeks
Gestation
Birth
PN Day 21
Wean
Continue Se Diet
Stop MeHg
Behavioral Testing
13 Months
FI CFI
Drug Challenges
20 Months
FI CFI
21 Months
Reinforcement Omission

FIGURE 2.2

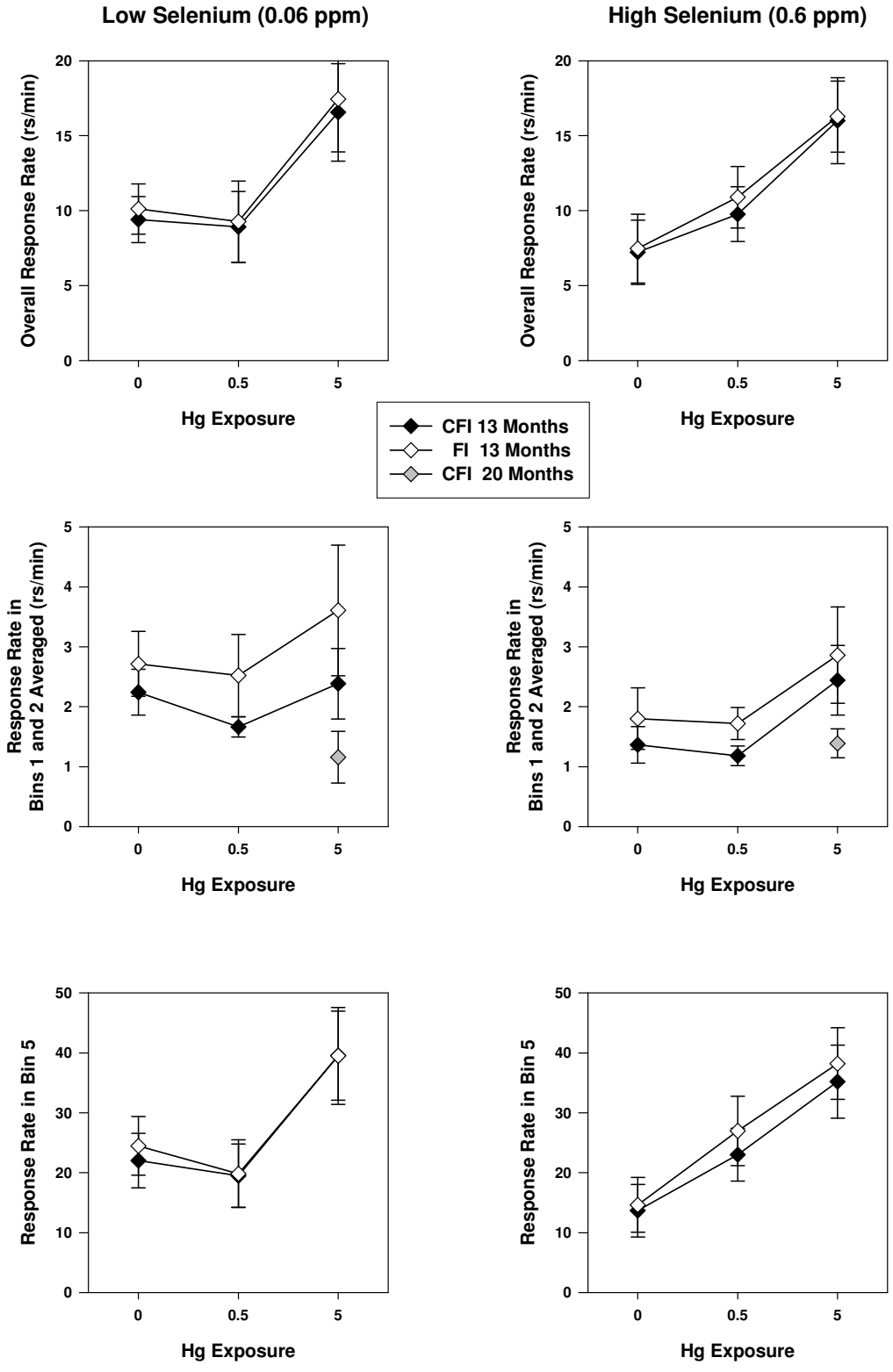

Low Selenium (0.06 ppm)
High Selenium (0.6 ppm)
Overall Response Rate (rs/min)
Response Rate in Bins 1 and 2 Averaged (rs/min)
Response Rate in Bin 5
Hg Exposure
0
0.5
5
CFI 13 Months
FI 13 Months
CFI 20 Months

FIGURE 2.3

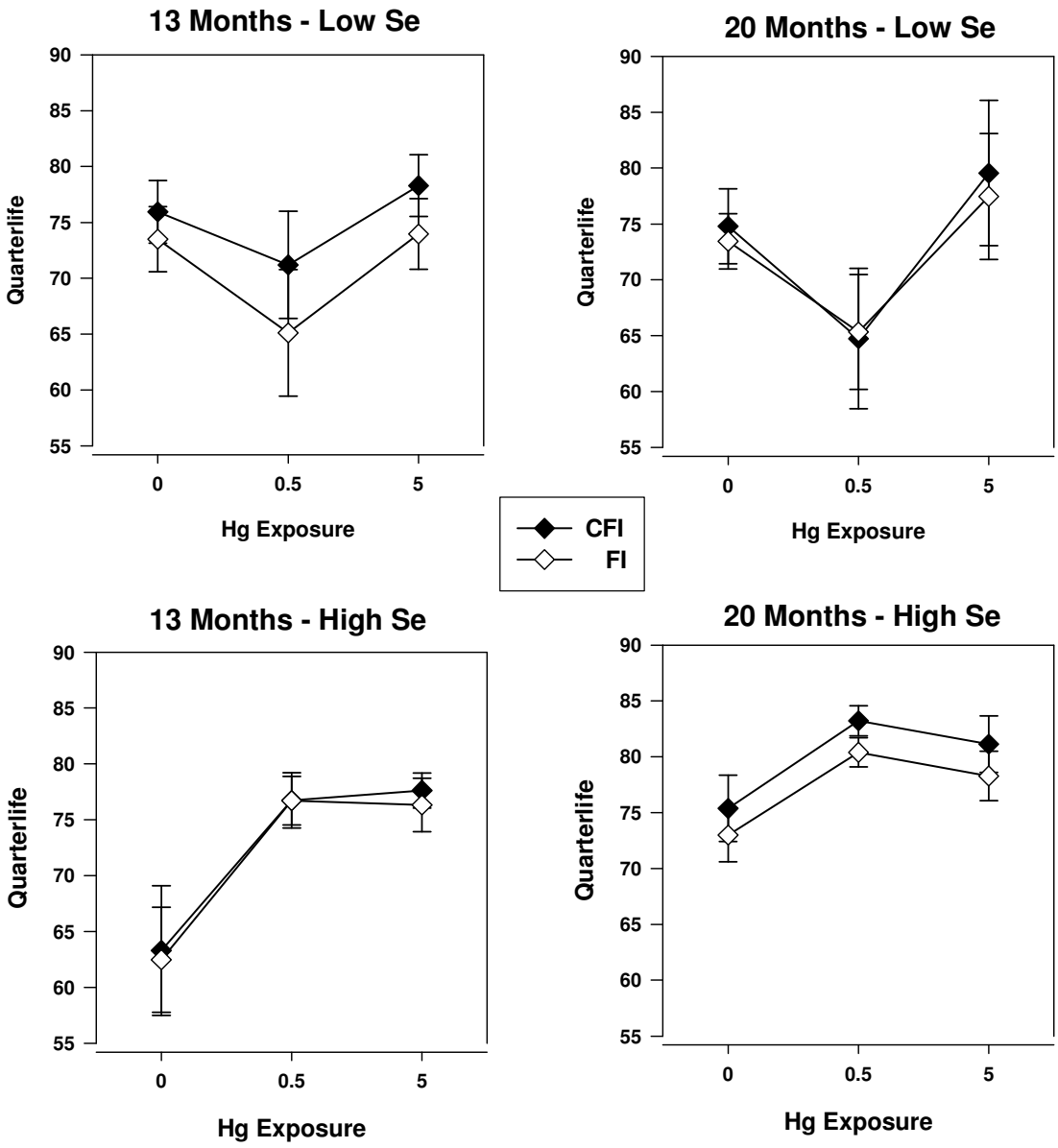

13 Months - Low Se
20 Months - Low Se
13 Months - High Se
20 Months - High Se
Quarterlife
Hg Exposure
90
85
80
75
70
65
60
55
0
0.5
5
CFI
FI

FIGURE 2.4

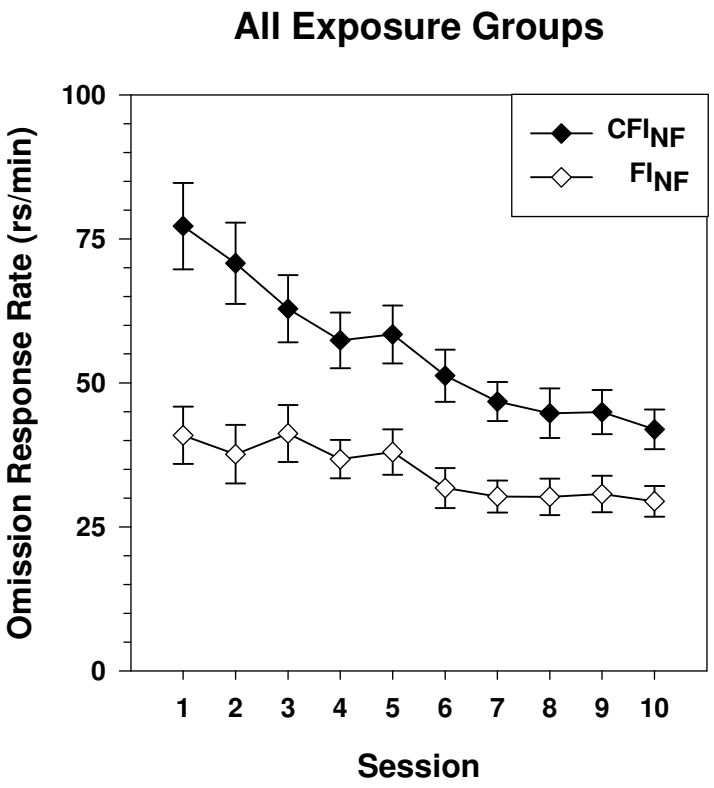
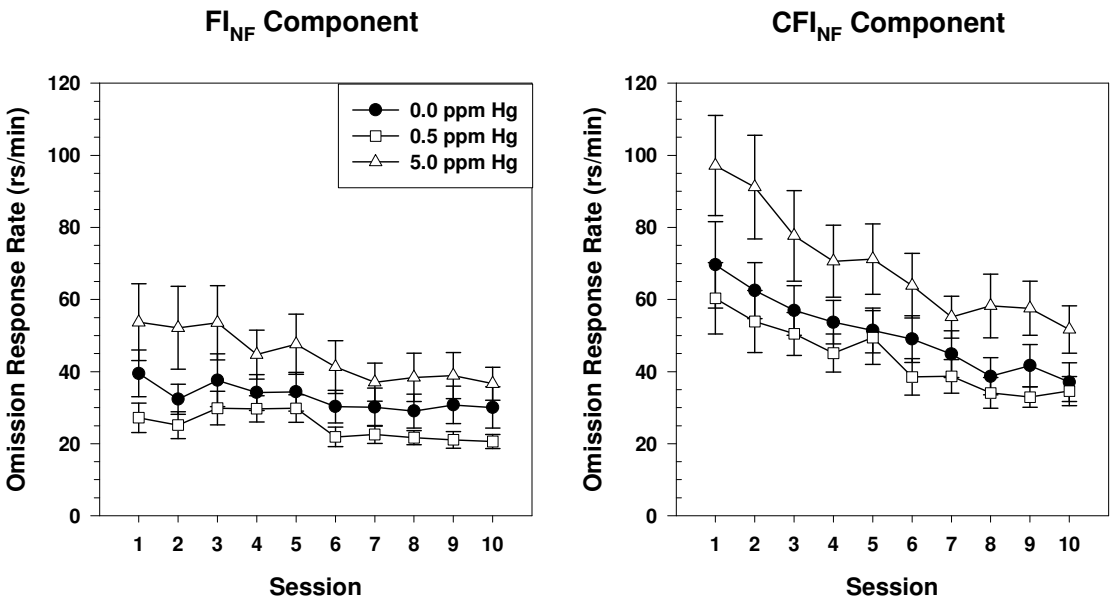

All Exposure Groups
Omission Response Rate (rs/min)
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
Session
CFI NF
FI NF

FIGURE 2.5


FI$_{NF}$ Component
CFI$_{NF}$ Component
Omission Response Rate (rs/min)
Session
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
0
20
40
60
80
100
120
1
2
3
4
5
6
7
8
9
10

CHAPTER 3

GESTATIONAL METHYLMERCURY EXPOSURE ALTERS SENSITIVITY TO COCAINE: OPERANT BEHAVIOR UNDER A CLOCKED FIXED INERVAL SCHEDULE

Previous studies have shown that developmental methylmercury (MeHg) exposure results in increased sensitivity to *d* amphetamine, a dopamine (DA) agonist. This effect may be due to the reduced bioavailability of selenium (Se) following MeHg exposure, which in turn may result in increased DA levels and turnover. To test this hypothesis, female rats were exposed *in utero* to 0, 0.5, or 5 ppm MeHg via drinking water and received a diet that was either marginal or rich in Se. At eleven months of age, a multiple schedule consisting of alternating fixed interval (FI) and clocked FI (CFI) components was arranged. Drug challenges were conducted with multiple doses of cocaine, desipramine, SKF-38393, quinpirole, SCH-23390, and sulpiride, drugs selected for their effects on the D_1 and D_2 receptor subtypes. Animals exposed to 5 ppm MeHg displayed an increased sensitivity to cocaine, whereas the effects of cocaine for the 0.5 ppm Hg groups depended on dietary Se exposure, producing an interaction among cocaine dose, MeHg and Se exposure. There were no other interactions with any of the DA direct agonists or antagonists, suggesting that co-activation of the D_1 and D_2 receptors is required to produce the MeHg interactions seen with cocaine.

Introduction

Acute administration of *d* amphetamine to behaving animals has revealed methylmercury-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) and general locomotor activity (Cagiano et al., 1990), suggesting disruption of dopamine neurotransmission following exposure to developmental methylmercury (MeHg). Dopamine projections play a critical role in both choice behavior (Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006; Ridley, Haystead, & Baker, 1981) and reinforcement processes (Beninger & Hahn, 1983; Robbins et al., 1986; Robbins & Everitt, 1996; Schultz, 1998; Schultz, Dayan, & Montague, 1997; Schultz, Tremblay, & Hollerman, 1998, 2000, 2003; Wise & Rompre, 1989). These same processes are modified by developmental MeHg exposure (Newland, Donlin, Paletz, & Banna, 2006). Thus, MeHg-induced disruption of dopamine function may underlie such behavioral effects as slowed transitions during a choice-in-transition procedure (Newland, Reile, & Langston, 2004; Newland, Yezhou, Logdberg, & Berlin, 1994), more rapid acquisition of lever-pressing and lack of ratio strain under large fixed-ratio schedules of reinforcement (Paletz, Craig-Schmidt, & Newland, 2006), and a tolerance for higher ratios under a progressive ratio procedure

(Paletz et al., 2006; Reed, Banna, Donlin, & Newland, under review). All of these tasks involve choice behavior and/or reinforcement processes.

MeHg may also alter selenium (Se) function in the central nervous system (Raymond & Ralston, 2004). MeHg forms a Se-Hg (inorganic mercury) complex in the brain and 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 dopamine (DA) levels, DA turnover, and various DA metabolites, such as 3-methoxytyramine turnover (3-MT), which is an indicator of the amount of DA released into the synaptic cleft (Castano et al., 1995; Castano et al., 1997; Castano, Cano, & Machado, 1993). Thus, 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.

The present experiment was designed to (1) examine the interactions of MeHg and Se on low- and high-rate operant behavior using a fixed interval (FI) schedule of reinforcement (2) determine whether these effects can be ameliorated by stimuli correlated with the passage of time, (3) extend previous findings of MeHg interactions with other dopamine agonists (Cagiano et al., 1990; Eccles & Annau, 1982; Hughes & Sparber, 1978; Rasmussen & Newland, 2001), and (4) determine whether a particular dopamine receptor subtype is responsible for such interactions. Pregnant rats consumed drinking water containing 0, 0.5, or 5 ppm of mercury (Hg) as methylmercuric chloride, and a diet that was either marginal (0.06 ppm) or rich (0.6 ppm) in Se. Their female offspring, who were exposed to MeHg only during gestation but continued their

respective Se diet, were examined. The MeHg concentrations chosen produce levels relevant to human exposure spanning the low to moderate range (Burbacher, Rodier, & Weiss, 1990; Newland & Reile, 1999). Likewise, 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 *Mult* FI 120", Clocked FI (CFI) 120" schedule of reinforcement. A fixed interval 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. Exteroceptive stimuli were correlated with the passage of time in the "clocked" FI (CFI) (Laties & Weiss, 1966; Odum & Schaal, 1999) in order to examine MeHg's interaction with a potential "behavioral prosthesis" that sometimes blunts the disruptive action of drugs, especially psychomotor stimulants (Laties, 1972; Laties, 1975). Drug challenges with cocaine, desipramine, SKF-38393, quinpirole, SCH-23390, and sulpiride commenced after behavior stabilized. Cocaine is a dopamine reuptake inhibitor with greater specificity for dopamine than amphetamine (Harris & Baldessarini, 1973). Desipramine, primarily a noradrenergic reuptake inhibitor, was used because both cocaine and amphetamine (used in previous studies) have some noradrenergic activity, and a lack of group differences following desipramine administration would allow us to exclude cocaine's noradrenergic activity. 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, whereas quinpirole, a D_2 agonist, and sulpiride, a D_2 antagonist, were used to access variations in the D_2-like receptor subtype.

Methods

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-hour light-dark cycle (lights on at 7:00 a.m.). Females were used in order to facilitate comparisons with adult-onset MeHg exposures conducted with the dams after parturition. 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 throughout life (detailed below) forming a 2 (chronic Se) x 3 (developmental MeHg) factorial design. There were five to eight 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 x 45.7 cm x 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 gm/day so

as to maintain their body weight at 250 grams. Rats that shared a home cage also received the same Se diet (see Exposures), so that 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 ± 1 months of age at the beginning of the present experiment.

Selenium 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 (Time line in 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. Selenium content of the diets was analyzed with each shipment using inductively coupled plasma mass spectrometry (ICP-MS). Analyses revealed that 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 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 three 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. Methylmercury 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; hereinafter 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 (Newland & Reile, 1999) 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 postnatal day 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 received exposure to plain tap water only.

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 5:00 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, 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 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”L x 9 ½”W x 11 ½”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.

Behavioral Methods

At the beginning of the study and throughout experimental testing, body weights did not differ among any of the exposure groups. Each of the three squads of subjects, consisting of 16 animals, was 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 insure they were placed in the appropriate chamber and home cage.

Training. Upon reaching adulthood, rats were trained to lever-press on the right lever using autoshaping (Newland et al., 2004). After the lever was pressed 10 times, the autoshaping procedure ended, and a Fixed Ratio 1 schedule (every response was

reinforced) was in effect. A single lever-press resulted in the delivery of a 45 mg sucrose pellet as a 0.5 sec, 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 hours elapsed, whichever occurred first. Then, right lever presses were placed on a Fixed-Interval (FI) 5" schedule of food reinforcement in which the first response after 5" was reinforced. The FI parameter increased every session to FI 15", FI 30" FI 90" and finally FI 120".

Mult FI CFI Condition. A multiple schedule consisting of alternating FI 120" and Clocked FI (CFI) 120" components was then arranged. When the FI 120" schedule was in effect, the first lever-press after 120" produced food. When the CFI 120" 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, but they did not bring behavior under adequate stimulus control, 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 (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.

Drug Challenges. After animals reached stable performance on measures of response rate, which required approximately two months, acute dose-effect curves were determined in the following order: cocaine (1-40 mg/kg), desipramine (0.3-17 mg/kg),

SKF-38393 (1-17 mg/kg), quinpirole (0.03-1 mg/kg), SCH-23390 (5.6-100 μg/kg), and sulpiride (3-100 mg/kg). Cocaine, desipramine, SKF-38393, quinpirole, and SCH-23390 were dissolved in saline and administered *i.p.* in a 1 ml/kg volume, except SKF, which was administered in a 2 ml/kg volume. Sulpiride was dissolved in a 0.1 N HCl and saline solution and administered *i.p*. in a 1 ml/kg volume.

After injections, rats were placed into the experimental chamber. Ten minutes elapsed between an injection and the onset of the session. Cocaine, desipramine, SKF, quinpirole and low doses of the dopamine antagonists, SCH and sulpiride, were administered Tuesdays and Fridays. Higher doses of the dopamine antagonists were administered once weekly. Sometimes intermediate doses were repeated after the dose-effect curve was determined. Mondays and Wednesdays were non-injection control days. Saline or 0.1 N HCl 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. After the dose-effect determination ended for one drug, the rats had one week of non-drug sessions before the next drug was administered. The rats were tested in age-matched squads, so they were between 13-14 months of age at the beginning of drug challenges and 18-19 months of age by the end of the experiment.

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 tests and Tukey *post hoc* comparisons. 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 reported with an emphasis on interactions between MeHg or Se exposure and drug dose. For subsequent drugs, analyzes were conducted to detect effects similar or opposite to those seen with cocaine, since 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 which to emphasize graphically.

Each segment of the CFI, and the corresponding FI, component was divided equally into five bins. Response count 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, three dependent variables were analyzed:

5) *Overall response rate* – the total number of responses throughout the interval divided by 120”
6) *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.
7) *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.

Log transforms were performed on some dependent measures in order to equate variability across groups. For analysis of each drug, data for the three dependent variables were averaged across four or five control sessions for each round of drug. 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, In Press). This allowed sensitivity to drug dose to be evaluated while adjusting for baseline response rate differences.

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 – 8 rats per cell. Drug dose and component (FI vs. CFI) 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, Huyn-Feldt or Greenhouse-Geiser adjustments to degrees of freedom were used to account for lack of sphericity in the dataset.

Results

Baseline

Baseline effects have been described in another paper (Reed & Newland, In Press) and will only be reviewed 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 and CFI components (Ps<0.05). Response rates did not differ among the groups during bins 1 and

2 averaged [$F(1,36)=6.943, P=.012$]. 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 [$F(2,36)=0.12, P=.887$].

There was a within-subject effect of the clock on all response-rate measures. Overall response rates were slightly greater in the FI component than in the CFI 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 FI bin 5 rates [$F(1,36)=7.943, P=.008$].

Cocaine

For all dependent measures, there was an effect of cocaine dose (Ps<0.001). For overall rate (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 5.6-17 mg/kg of cocaine (Ps<0.05), producing an inverted U-shaped dose-effect curve. 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<0.01). The elevated responding and variability in the high Se, 0 ppm Hg group was driven mainly by one animal, although others in this group displayed rates increases as well. This animal was not a statistical outlier and has been included in the figure and analysis. The 5 ppm Hg groups did not differ from each other (Ps>0.1). There was no effect of the clock and no interaction of clock with cocaine (Ps>0.1), so only data from the FI (no-clock) component are shown.

For measures of low-rate behavior (Figure 3), there was an interaction between cocaine dose and MeHg [$F(12,210)=2.388, P=.048$]. There was a substantial cocaine-induced rate increase, sometimes as high as 10 to 15-fold, for all groups except the 5 ppm

Hg, particularly at 17 mg/kg for both components (*P*s<.05 compared to controls and 0.5 ppm Hg rats) and 30 mg/kg cocaine in the CFI component (*P*s<.05 compared to controls and 0.5 ppm Hg rats). It should be noted that one high Se, 5 ppm Hg animal was removed from the analysis as an outlier due to its exceptionally high response rate, 75-90 times its baseline rate, in the CFI component following administration of 5.6 and 10 mg/kg cocaine. Including this animal created an interaction among cocaine dose, the clock stimuli, and MeHg [$F(12,216)=1.989$,$P=.029$]. Figure 3 does not include this animal, and illustrates that there was no interaction among cocaine dose, clock and MeHg without the outlier [$F(12,210)=1.861$,$P=.097$].

With high-rate behavior in the last bin (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 (*P*s<.05).

Desipramine

Desipramine decreased overall rate, rate in bins 1 & 2 averaged, and bin 5 rate (*P*s<.001). For both overall rate and measures of low-rate behavior (not shown), there were no effects of MeHg nor were there any interactions between MeHg and desipramine dose (*P*s>0.1).

For high rate responding in Bin 5, there was an interaction among the clock, MeHg, and Se [$F(2,36)=6.659$,$P=.003$], but there was no interactions between any measure and desipramine (*P*s>0.1; Figure 5).

Specific Agonists

There was an effect of SKF dose, a D_1 agonist, (*P*s<0.001) for all measures, except low-rate behavior [$F(5,180)=1.946$,$P=.092$]. For overall rate and measures of low-

and high-rate behavior, there were no interactions between MeHg and SKF dose, Se and SKF, or an interaction among MeHg, Se, and SKF dose (*P*s>0.1; Figure 6).

There was an effect of quinpirole dose, a D_2 agonist, for all measures (*P*s<.001). Quinpirole showed a W-shaped dose-effect relationship in all exposure groups. For overall rate, there appears to be elevated responding in the low Se, 0.5 ppm Hg group compared to the other groups (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 3-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 dose, Se and SKF, or an interaction among MeHg, Se, and SKF dose (*P*s>0.1) for any of the three measures.

Specific Antagonists

For all measures, there were rate-decreasing effects of SCH dose, a D_1 antagonist (*P*s<.001), but for overall rate and measures of low- and high-rate behavior, there were no interactions between MeHg and SKF dose, Se and SKF, or an interaction among MeHg, Se, and SKF dose (*P*s>0.1, Figure 6). On all three measures, sulpiride, a D_2 antagonist, caused a dose-related decrease in responding (*P*s<.001), but on no measure

was there an interaction between MeHg and SKF dose, Se and SKF, or an interaction among MeHg, Se, and SKF dose (*P*s>0.1, Figure 6).

Discussion

Gestational exposure to MeHg and a lifelong diet that was either marginal or rich in Se were manipulated in a 2 (Se) x 3 (MeHg) factorial design, which allowed for assessment of the main effects of these elements, as well as their potential interaction with a variety of DA agonist and antagonists. The present investigation extends the findings of previous studies in which gestational exposure to MeHg altered sensitivity to *d* amphetamine, a DA reuptake inhibitor (Cagiano et al., 1990; Eccles & Annau, 1982; Hughes & Sparber, 1978; Rasmussen & Newland, 2001). The current study used cocaine, rather than amphetamine, because amphetamine has a high affinity for both the DA and norepinephrine (NE) transporter, whereas cocaine is more specific for the DA transporter (Harris & Baldessarini, 1973). Cocaine, however, does have a weak affinity for the NE transporter, so desipramine was administered to exclude cocaine's action on NE as a potential reason for MeHg-rats' sensitivity to cocaine. There were no differences among the groups following desipramine administration, suggesting that sensitivity following cocaine administration was due to its dopaminergic, not noradrenergic, action.

No MeHg- or Se- related sensitivity to the specific D_1 and D_2 agonists or antagonists was seen. The reason for this is unknown, but some possibilities can be noted. First, MeHg's alterations of DA functioning may involve presynaptic mechanisms. Cocaine is an indirect agonist that acts on the presynaptic DA transporter, whereas the D_1 and D_2 specific drugs were all direct agonists or antagonists acting postsynaptically on

specific receptors. MeHg alters Ca^{2+} influx and storage (Atchison, Joshi, & Thornburg, 1986; Sirois & Atchison, 2000; Sirois & Atchison, 1996) such that there may be elevated Ca^{2+} in the terminal boutons of the presynaptic neuron. Ca^{2+} promotes neurotransmitter release, so the combination of MeHg-induced Ca^{2+} activity could amplify the attenuation of reuptake caused by cocaine, and amphetamine in previous studies (Rasmussen & Newland, 2001). Evidence against this hypothesis comes from the absence of an interaction with desipramine, a NE reuptake inhibitor, which is an indirect presynaptic agonist, like cocaine. Alterations in Ca^{2+} stores or influx should produce enhanced sensitivity to all reuptake inhibitors, but the effects are specific to cocaine and DA.

A second possibility is that co-activation of the D_1 and D_2 receptors is required. Co-activation of D_1 and D_2 receptors in the basal ganglia and the nucleus accumbens result in an increase of postsynaptic intracellular Ca^{2+} 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, Cascini, Gordon, Diamond, & Bonci, 2003). Thus, cocaine may produce differences among MeHg groups because it is an indirect agonist, causing activation of both D_1 and D_2 receptors and pathways that neither receptor subtype agonist can activate alone.

Clock Effects

In previous studies (Laties, 1972; Laties, 1975), exteroceptive stimuli correlated with the passage of time have blunted the disruptive effects of various drugs, including psychomotor stimulants. In the current study, the clock stimuli afforded no protection against the acute drug effects, and there were no interactions of MeHg or Se with the

clock. The reason for the discrepancy between the earlier studies and the current one are not clear at present. In the present experiment, the effect of the clock stimuli was quite small in magnitude and was statistically significant only because comparisons were conducted as a repeated measure (Reed & Newland, in print). Every animal showed higher rates during the unclocked FI component than during the clocked component. Thus, while the clock had an effect on behavior, the small magnitude could have been overwhelmed by the larger effects of other interventions, such as drug administrations.

MeHg & Se Interactions

One hypothesis regarding MeHg toxicity proposes that toxicity is seen when the molar levels of MeHg exceed that of Se (Raymond & Ralston, 2004). MeHg binds Se, forming insoluble complexes that diminish MeHg's neurotoxic effects while simultaneously diminishing the bioavailability of Se. Thus, 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. In the present study, when MeHg and Se interactions were seen, they occurred in the 0.5 ppm Hg groups but not the 5 ppm Hg groups. The 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 exposure (Newland, Reed, LeBlanc, & Donlin, 2006). Thus, Hg levels far exceed Se levels, so Se protection would not be expected. For the 0.5 ppm Hg groups, 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 we see such variability in this group, since some had an Hg:Se ratio above one and some below one. Also, it should be noted that studies of the siblings of these animals (Reed & Newland, in print; Reed, Paletz, & Newland, 2006) have generally failed to detect interactions between Se and MeHg, so the effect can best be described as subtle and erratic or, perhaps, due to Se's interactions with dopamine function.

Previous studies have shown that when Se levels are decreased there is an increase in DA levels, DA turnover, and various DA metabolites (Castano et al., 1995; Castano et al., 1997; Castano et al., 1993), which may explain the enhanced sensitivity to cocaine in MeHg-exposed animals. Interestingly, the pattern of responding for the low Se, 0.5 ppm Hg group and the two 5 ppm Hg groups is different. One might expect the response patterns of these groups to be similar since all three groups have a molar ratio of Hg:Se exceeding one, although for the low Se, 0.5 ppm Hg group, the molar ratio was very close to 1.0. The reason for these differences is not currently understood. It is possible that for the two 5 ppm Hg groups the response pattern is due primarily to MeHg toxicity, which produces more effects than merely reducing the bioavailability of Se levels, whereas the response pattern of the low Se, 0.5 ppm Hg group is due to decreases in bioavailability of Se without the other pervasive MeHg effects.

Conclusions

The present study provides a replication and extension of earlier reports of MeHg-induced sensitivity to DA agonists. This effect appears to require simultaneous activation of both D_1 and D_2 receptors and occurs regardless of whether exteroceptive stimulus control is provided by the clock. This latter effect is consistent with other reports

(Newland & Paletz, 2000) that MeHg's effects on learning appear to be related to altered reinforcement function while discrimination or memorial processes appear to be relatively spared, at least at very low-level exposures; the current exposure level produced low micromolar concentrations of Hg in whole brain (Newland, Reed et al., 2006). It can also be noted that the MeHg exposure occurred only during gestation, so these effects reflect long-lasting, perhaps irreversible changes in DA function. While speculative, this raises possibilities that other reports that developmental MeHg exposure accelerates age-related declines on the performance of high rate behavior (Newland & Rasmussen, 2000) might reflect MeHg-related alterations in DA pathways, since many motor pathways involve DA.

REFERENCES

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

Atchison, W. D., Joshi, U., & Thornburg, J. E. (1986). Irreversible suppression of calcium entry into nerve terminals by methylmercury. *journal of Pharmacology & Experimental Therapeutics 238*(2), 618-624.

Beninger, R. J., & Hahn, B. L. (1983). Pimozide blocks establishment but not expression of amphetamine-produced environment-specific conditioning. *Science, 220*, 1304-1306.

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 & Teratology, 12*(1), 23-28.

Castano, A., Ayala, A., Rodriguez-Gomez, J. A., de la Cruz, C. P., Revilla, E., Cano, J., et al. (1995). Increase in dopamine turnover and tyrosine hydroxylase enzyme in hippocampus of rats fed on low selenium diet. *Journal of Neuroscience Research, 42*(5), 684-691.

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*(6), 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*(4), 1302-1307.

Eccles, C. U., & Annau, Z. (1982). Prenatal methyl mercury exposure: II. Alterations in learning and psychotropic drug sensitivity in adult offspring. *Neurobehav Toxicol Teratol, 4*(3), 377-382.

Harris, J. E., & Baldessarini, R. J. (1973). Uptake of (3H)-catecholamines by homogenates of rat corpus striatum and cerebral cortex: effects of amphetamine analogues. *Neuropharmacology, 12*(7), 669-679.

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*(12), 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. *Pharmacol Biochem Be, 8*(4), 365-375.

Laties, V. G. (1972). The modification of drug effects on behavior by external discriminative stimuli. *J Pharmacol Exp Therap, 183*, 1-13.

Laties, V. G. (1975). The role of discriminative stimuli in modulating drug action. *Federation Proceedings, 34*(9), 1880-1888.

Laties, V. G., & Weiss, B. (1966). Influence of drugs on behavior controlled by internal and external stimuli. *J Pharmacol Exp Therap, 152*, 388-396.

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*(34), 35671-35678.

National Research Council. (1995). *Nutrient Requirements of Laboratory Animals*. Washington, D.C.: 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): CRC Press.

Newland, M. C., & Paletz, E. M. (2000). Animal studies of methylmercury and PCBs: what do they tell us about expected effects in humans? *Neurotoxicology, 21*(6), 1003-1027.

Newland, M. C., & Rasmussen, E. B. (2000). Aging unmasks adverse effects of gestational exposure to methylmercury in rats. *Neurotoxicology & Teratology, 22*(6), 819-828.

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*(5), 710-720.

Newland, M. C., & Reile, P. A. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicol Sci, 50*(1), 106-116.

Newland, M. C., Reile, P. A., & Langston, J. L. (2004). Gestational exposure to methylmercury retards choice in transition in aging rats. *Neurotoxicol Teratol, 26*(2), 179-194.

Newland, M. C., Yezhou, S., Logdberg, B., & Berlin, M. (1994). Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. *Toxicol Appl Pharm, 126*(1), 6-15.

Nishikido, N., Furuyashiki, K., Naganuma, A., Suzuki, T., & Imura, H. (1987). Maternal selenium deficiency enhances the fetolethal toxicity of methyl mercury. *Toxicol. Appl. Pharmacol., 88*, 322-238.

Odum, A. L., & Schaal, D. W. (1999). The effects of morphine on clocked fixed-interval performance: stimulus function or strength of stimulus control? *Behav Pharmacol, 10*(3), 243-255.

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. *Neurotoxicol Teratol, 28*, 59-73.

Pessiglione, M., Seymour, B., Flandin, G., Dolan, R. J., & Frith, C. D. (2006). Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature, 442*, 1042-1045.

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

Raymond, L. J., & Ralston, N. V. C. (2004). Mercury:selenium interactions and health implications. *Seychelles Medical and Dental Journal (SMDJ), 7*(1), 72-77.

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

Reed, M. N., & Newland, M. C. (In Press). Prenatal methylmercury exposure increases responding under clocked and unclocked fixed-interval schedules of reinforcement. *Neurotoxicology & Teratology*.

Reed, M. N., & Newland, M. C. (in print). Prenatal methylmercury exposure increases responding under clocked and unclocked fixed-interval schedules of reinforcement. *Neurotoxicology & Teratology*.

Reed, M. N., Paletz, E. M., & Newland, M. C. (2006). Gestational exposure to methylmercury and selenium: effects on spatial discrimination reversal in adulthood. *NeuroToxicology, 27*(5), 721-732.

Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr, 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. *J Nutr, 123*(11), 1939-1951.

Ridley, R. M., Haystead, T. A. J., & Baker, H. F. (1981). An involvement of dopamine in higher order choice mechanisms in the monkey. *Psychopharmacology, 72*, 173-177.

Robbins, T. W., Evenden, J. L., Ksir, C., Reading, P., Wood, S., & Carli, M. (1986). The effects of d-amphetamine, alpha-flupenthixol, and mesolimbic dopamine depletion on a test of attentional switching in the rat. *Psychopharmacology, 90*(1), 72-78.

Robbins, T. W., & Everitt, B. J. (1996). Neurobehavioural mechanisms of reward and motivation. *Curr. Opin. Neurobiol, 6*, 228-236.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, 80*(1).

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science, 275*(5306), 1593-1599.

Schultz, W., Tremblay, L., & Hollerman, J. R. (1998). Reward prediction in primate basal ganglia and frontal cortex. [Review] [30 refs]. *Neuropharmacology, 37*(4-5), 421-429.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia. *Cerebral Cortex, 10*(3), 272-284.
Schultz, W., Tremblay, L., & Hollerman, J. R. (2003). Changes in behavior-related neuronal activity in the straitum during learning. *Trends in Neurosciences, 26*(6), 321-328.
Sirois, J. E., & Atchison, D. K. (2000). Methylmercury affects multiple subtypes of calcium channels in rat cerebellar granule cells. *Toxicology and Applied Pharmacology 15*(1), 1-11.
Sirois, J. E., & Atchison, W. D. (1996). Effects of mercurials on ligand- and voltage-gated ion channels: a review. *Neurotoxicology, 17*(1), 63-84.
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. *TAP, 134*, 273-284.
Watanabe, C., Yin, K., Kasanuma, Y., & Satoh, H. (1999). In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. *Neurotoxicol Teratol, 21*(1), 83-88.
Wise, R. A., & Rompre, P. P. (1989). Brain dopamine and reward. *Annu. Rev. Psychol., 40*, 191-225.

		Overall Rate		Bins 1 & 2		Bin 5	
	MeHg	FI	CFI	FI	CFI	FI	CFI
Low Se	0.0 ppm	13.11±1.69	12.41±1.54	2.71±0.54	2.24±0.38	29.46±4.89	28.04±4.55
	0.5 ppm	9.26±2.72	8.91±2.38	2.52±0.69	1.66±0.55	19.84±5.64	19.50±5.27
	5.0 ppm	17.44±3.52	16.57±3.25	3.61±1.09	2.38±0.59	39.50±8.09	39.55±7.43
High Se	0.0 ppm	7.46±2.29	7.22±2.16	1.80±0.51	1.36±0.31	14.64±4.57	13.66±4.39
	0.5 ppm	10.89±2.06	9.77±1.83	1.72±0.27	1.18±0.16	26.96±5.79	23.01±4.43
	5.0 ppm	16.28±2.37	16.0±2.87	2.86±0.80	2.44±0.58	38.22±5.98	35.20±6.09

Table 1. Baseline response rates. Mean ± SEM.

Drug	Mechanism of Action	Drug*MeHg*Se Interaction on Overall Rate
Cocaine	Dopamine reuptake inhibitor	Yes
d amphetamine	Dopamine and noradrenergic reuptake inhibitor.	Yes [1]
Desipramine	Norepinephrine reuptake inhibitor	No
SKF 38393	Direct D_1 agonist	No
Quinpirole	Direct D_2 agonist	No
SCH 23390	D_1 antagonist	No
Sulpiride	D_2 antagonist	No

[1] From Rasmussen and Newland, MeHg*amphetamine interaction

Table 2. Drug mechanisms and their effects on overall response rates.

FIGURE CAPTIONS

Figure 3.1. Timeline for breeding and exposure for F_0 breeders and F_1 offspring. Note that maternal exposure to methylmercury ended at 16 days and was reinstated after weaning. Functionally, exposure to the F1 rats ended at birth [Newland and reile, stern et al]. Breeders were not included in the present experiment.

Figure 3.2. Overall response rate in the FI component 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.

Figure 3.3. Low-rate behavior in the CFI (left) and FI (right) component 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.

Figure 3.4. High-rate behavior in the FI component 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.

Figure 3.5. High-rate behavior in the FI component 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 3.6. Overall response rate in the FI component 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.

FIGURE 3.1

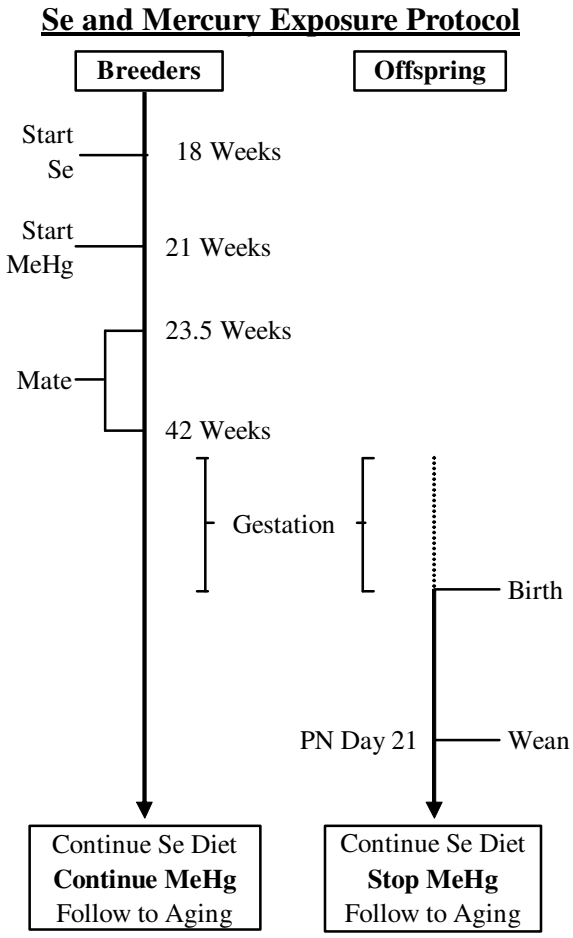

Se and Mercury Exposure Protocol
Breeders
Offspring
Start Se
18 Weeks
Start MeHg
21 Weeks
23.5 Weeks
Mate
42 Weeks
Gestation
Birth
PN Day 21
Wean
Continue Se Diet
Continue MeHg
Follow to Aging
Continue Se Diet
Stop MeHg
Follow to Aging

FIGURE 3.2

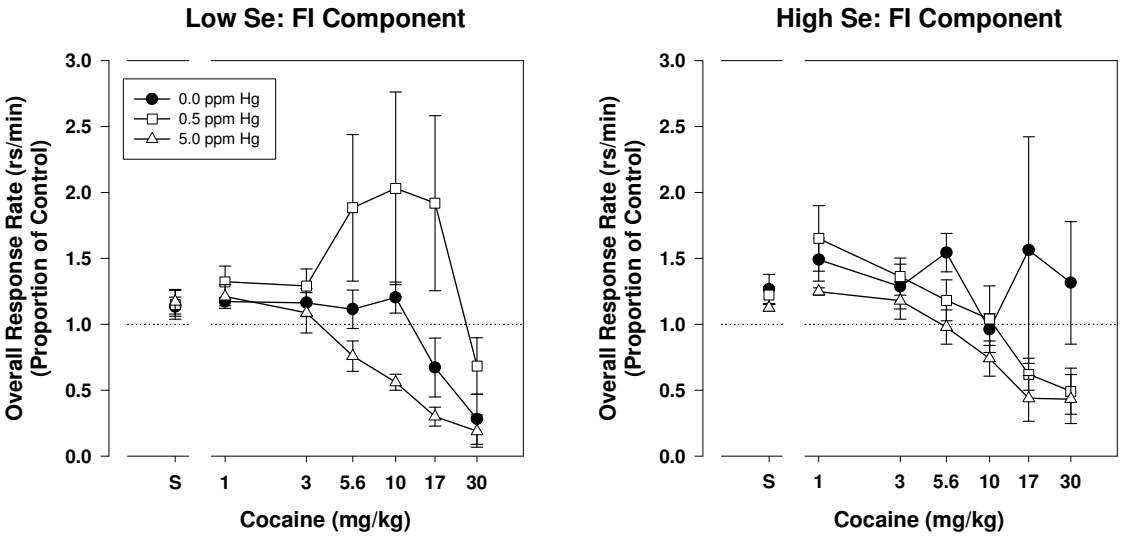

Low Se: FI Component
High Se: FI Component
Overall Response Rate (rs/min)
(Proportion of Control)
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
3.0
2.5
2.0
1.5
1.0
0.5
0.0
S
1
3
5.6
10
17
30
Cocaine (mg/kg)

FIGURE 3.3

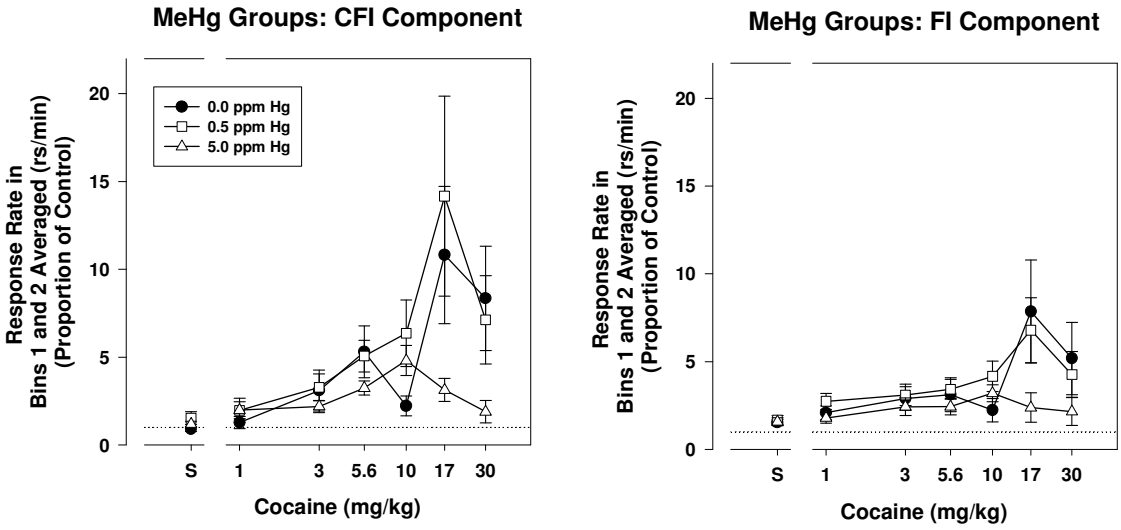

MeHg Groups: CFI Component
MeHg Groups: FI Component
Response Rate in
Bins 1 and 2 Averaged (rs/min)
(Proportion of Control)
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
0
5
10
15
20
S
1
3
5.6
10
17
30
Cocaine (mg/kg)

FIGURE 3.4

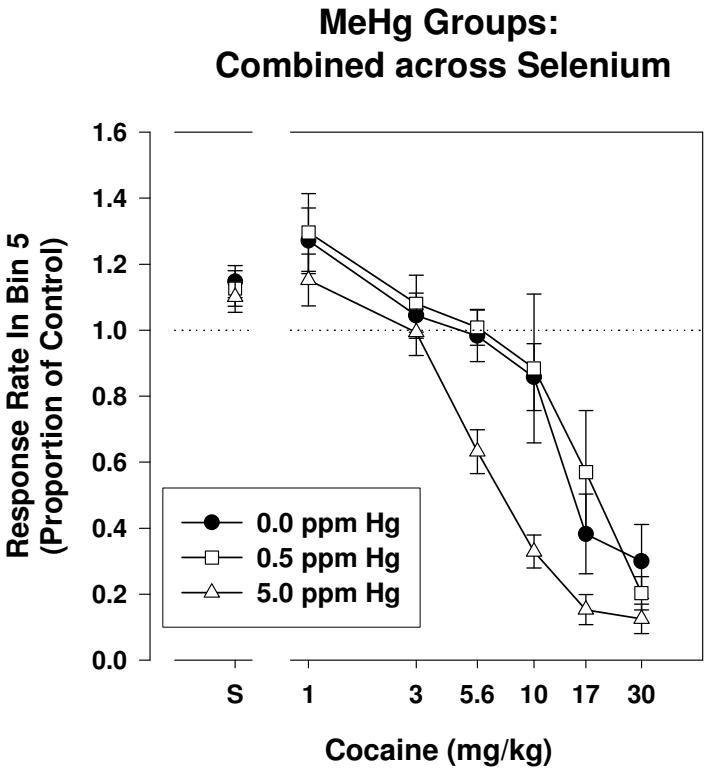

MeHg Groups:
Combined across Selenium
Response Rate In Bin 5
(Proportion of Control)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
S
1
3
5.6
10
17
30
Cocaine (mg/kg)

FIGURE 3.5

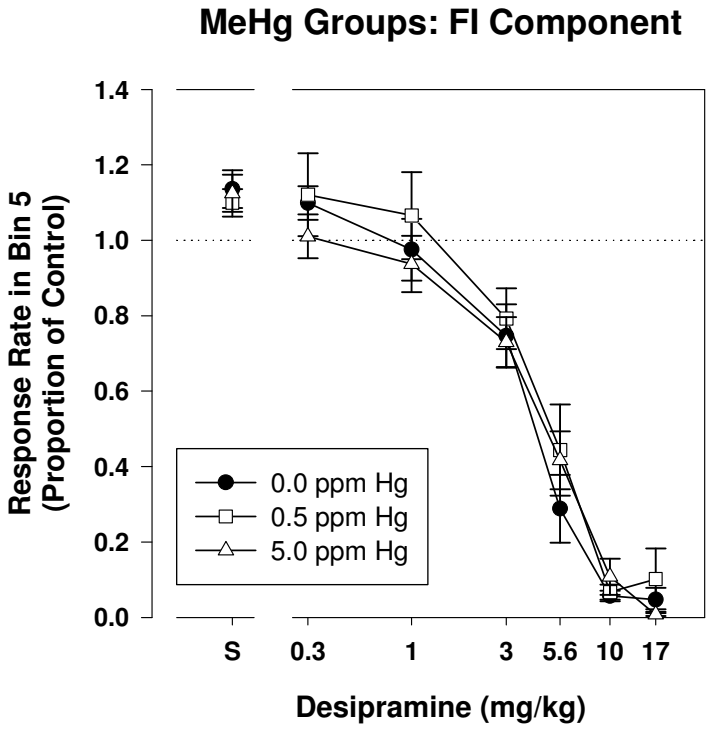
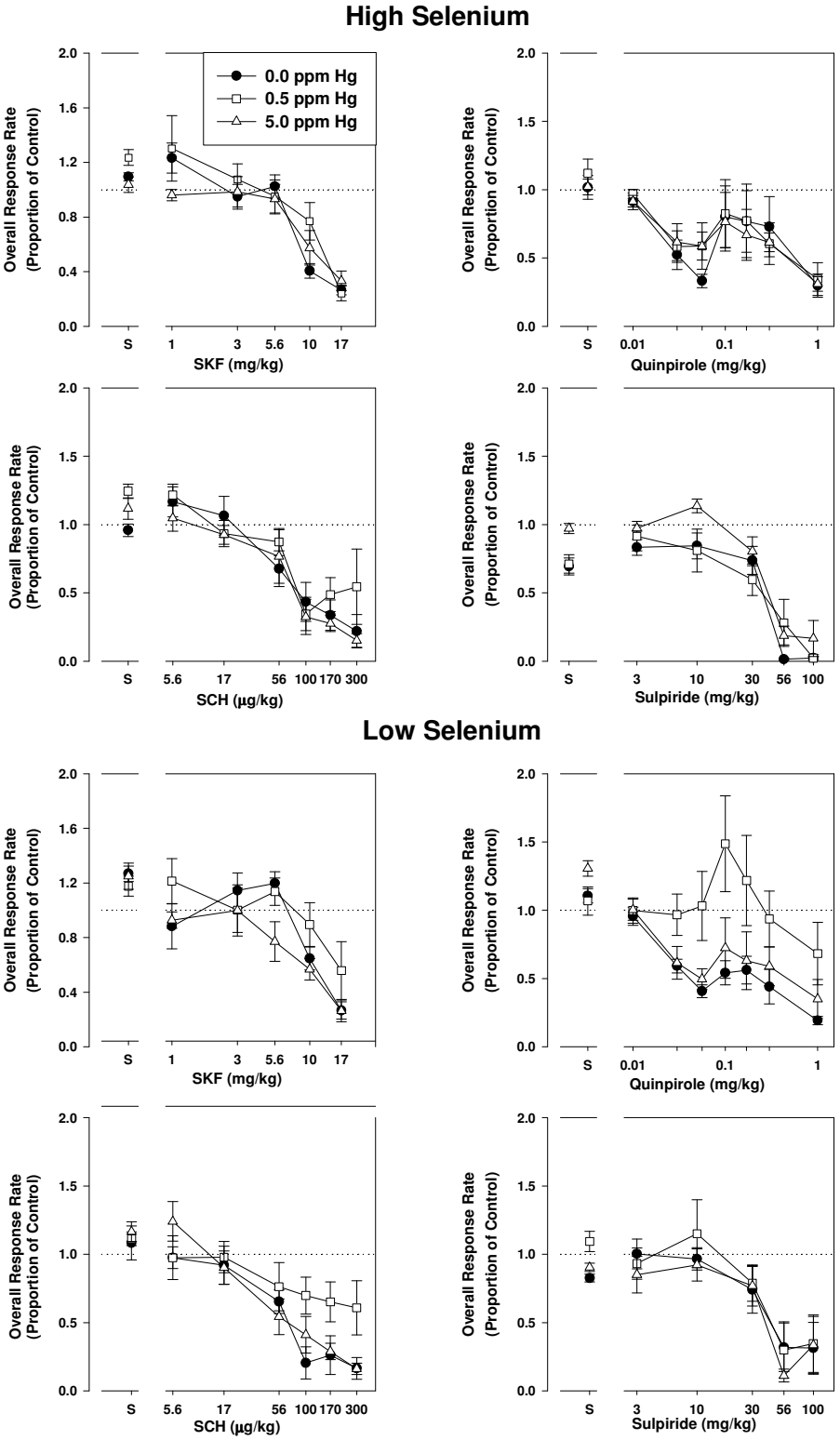

MeHg Groups: FI Component
Response Rate in Bin 5
(Proportion of Control)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
S
0.3
1
3
5.6
10
17
Desipramine (mg/kg)

FIGURE 3.6


High Selenium
0.0 ppm Hg
0.5 ppm Hg
5.0 ppm Hg
Overall Response Rate (Proportion of Control)
SKF (mg/kg)
Quinpirole (mg/kg)
SCH (µg/kg)
Sulpiride (mg/kg)
Low Selenium