Visual Signal Detection as a Measure for Sustained Attention and Short-Term Remembering in Mice Exposed to Methylmercury during Adolescence

by

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Abstract

Developmental methylmercury (MeHg) exposure is known to alter dopamine-mediated behavior associated with the prefrontal cortex. Exposure may be associated with symptoms of ADHD, such as inattention and impaired memory, which are also mediated by dopamine pathways in the prefrontal cortex. In order to assess this interaction, mice were exposed to 0, 0.3, or 3 ppm MeHg during adolescence and tested in a hybrid attention/memory task in adulthood in which mice detected a brief, unpredictable visual stimulus. Behavior was challenged by shortening the duration of a visual stimulus and introducing a novel distractor. MeHg did not alter behavior in this task. Attention was impaired at short stimulus durations and disrupted by the novel distractor, but this was not related to MeHg exposure. The lack of MeHg-related alteration in behavior in this procedure could be attributed to either differences in MeHg sensitivity during adolescence or to variations in the procedures used to assess attention/memory across species.

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List of Abbreviations

MeHg	Methylmercury
PUFA	Polyunsaturated fatty acids
GD	Gestation Day
Pb	Lead
DAT	Dopamine Transporter
ADHD	Attention-deficit/Hyperactivity Disorder
DR	Dopamine Receptor
5-CSRT	Five Choice Serial Reaction Time Task
VSD	Visual Signal Detection
PND	Postnatal Day
TO	Timeout
ITI	Inter-trial Interval
FA	False Alarm
CR	Correct Rejection
FR	Fixed Ratio
I-T	Information Theoretic Approach
AICc	Akaike information criterion
p(Hit)	Hit Rate
p(FA)	False Alarm Rate

Chapter 1

Literature Review

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Methylmercury: Neurobiology and Behavior

Methylmercury (MeHg) is an environmental contaminant introduced into human populations via consumption of long-lived predatory fish, including swordfish, tilefish, king mackerel, shark, and tuna (Afonso et al., 2015; Clarkson, 1992; Driscoll et al., 2013; EPA, 2013; Groth, 2010). Long-term developmental neurotoxicity of *in utero* MeHg exposure arises following maternal consumption of contaminated fish. Exposure to MeHg has also been shown to be related to dietary habits adopted by the family, providing exposure during late childhood and adolescence (Castaño et al., 2015; Muckle et al., 2001; Nielsen et al., 2014). Exposure to MeHg due to consumption of contaminated fish affects children's motor and neurobehavioral function at several developmental stages with the severity and breadth of these effects being dose-dependent, but disentangling the impact of fetal exposure from exposure during childhood and adolescence is impossible in epidemiological studies and this issue has only recently been addressed in experimental animal studies.

Neuromotor Effects of MeHg Exposure

Early observations of MeHg toxicity after exposure disasters revealed severe motor deficits in children. In one episode, occurring in Minamata Japan in the 1950s, numerous children showed symptoms akin to cerebral palsy. It was later discovered that these motor deficits were correlated with high concentrations of MeHg in the umbilical cord, at above 1.0 ppm, following maternal consumption of contaminated fish and concomitant damage to the cerebral cortex (Harada, 1978). Such motor impairments have been linked to altered muscle development. Wu et al., (2014) observed differences in muscle tone development in 3 day old neonates following maternal exposure to Hg during gestation. They found an association

between cord blood total Hg levels, with average cord blood concentrations of 7.92 µg/L, and reduced development of muscle tone in these children (Wu et al., 2014).

Similar motor deficits have been observed in children exposed to MeHg during postnatal development. An early study in Iraq showed that children exposed to MeHg postnatally via consumption of contaminated wheat displayed gross motor and visual impairments. Blood concentrations of total Hg in these children ranged from 7 to 25 µmol/L at the beginning of the study (Amin-zaki et al., 1978). However, these severe motor deficits have not been observed across the board with many studies in humans noting minor if no significant alterations in motor function following exposure to MeHg due to maternal fish consumption (Grandjean et al., 1997; Myers et al., 2003; Van Wijngaarden et al., 2013).

Efforts to unravel these conflicting results in human populations have given rise to experimental models of MeHg toxicity and these models have demonstrated neurodevelopmental effects of MeHg following exposure at several developmental time points. Experimental models of prenatal exposure to relatively low doses of MeHg, at 0.1 and 0.5 ppm in drinking water, have sometimes shown impairments in locomotor activity, as assessed in an open field (Daré et al., 2003; Giménez-Llort et al., 2001). Changes in locomotor activity have also been observed in animals exposed to much higher doses of MeHg, above 4 ppm, with animals exhibiting dampened open field exploration or worse performance on rotarod (Goulet et al., 2003; Sakamoto et al., 2002). Animal models of MeHg exposure tend to show similar alterations in motor activity, an aspect of MeHg's effects that, as noted previously, is not as clear following MeHg exposure in human populations. This lack of congruity between experimental models and epidemiological observations has been attributed to the presence of beneficial fatty acids often associated with fish consumption, the common route of human exposure. It is likely that MeHg

diminishes the beneficial effects of nutrients such as omega-3 polyunsaturated fatty acids (PUFA's) and selenium (Spiller, 2018; Strain et al., 2008). Intake of PUFA's and selenium is important, thus the balance between consumption of fish to promote higher intake of beneficial nutrients while abstaining from high degrees of MeHg toxicity is precarious. Because of this balance, doses of MeHg exposure often seen in human populations may not always be sufficient to induce motor deficits. However, manifestation of other behavioral deficits that arise following lower doses of MeHg exposure, such as alterations in impulsivity and attention, may be more readily detectible as compared to finer motor deficits (Boucher et al., 2012; Perez-Fernandez et al., 2019).

Neurobehavioral Effects of MeHg Exposure

Changes in behavior that is mediated, at least in part, by dopamine, such as behavioral flexibility, reinforcement sensitivity, response inhibition, short-term memory, and choice, have been observed in animal models of developmental (gestation and adolescence) MeHg toxicity (Boomhower & Newland, 2017, 2019; Gilbert, Burbacher, & Rice, 1993; Newland, Hoffman, Heath, & Donlin, 2013; Paletz, Day, Craig-Schmidt, & Newland, 2007; Reed, Paletz, & Newland, 2006). Behavioral rigidity in rats has been shown following exposure to 0.5 or 5 ppm MeHg during gestation; rats exposed to MeHg during gestation are less likely to stop responding on a particular lever after extinction has been imposed (Newland et al., 2013; Reed et al., 2006). Exposure to MeHg during gestation also causes deficits in the acquisition of choice in both non-human primate and rodent models (Newland, Reile, & Langston, 2004; Newland, Yezhou, Lögdberg, & Berlin, 1994). These alterations may be related to differences in reinforcement processing following MeHg exposure during other developmental periods (Boomhower & Newland, 2019).

While several studies have observed relations between developmental MeHg exposure and alterations in memory, the results are often inconsistent (Newland, Paletz, & Reed, 2008) with MeHg improving short-term memory in a task of spatial alternation in non-human primates exposed to MeHg throughout gestation (Gilbert et al., 1993) and impairing memory in a passive avoidance task following post-natal MeHg exposure in rats (Sakamoto et al., 2004). While this relation between MeHg and memory is unclear due to the differences in procedure and dosing, there is solid evidence that MeHg influences behaviors related to dopamine neurotransmission. This likely stems from MeHg's direct influence on the dopaminergic system and permanent changes in dopamine neurotransmission that arise following developmental MeHg exposure.

Neurobiology of MeHg

Long-term exposure to MeHg during sensitive developmental periods produces alterations in cell proliferation, gene expression, and neurochemistry. Low doses of MeHg throughout gestation (at 0, 0.01, 0.1, or 1 mg/kg/day) cause dose-dependent inhibition of neuronal migration in the cerebral cortex due to reduced expression of Rho family proteins during early stages of postnatal development (Guo et al., 2013). Rodent models of prenatal exposure to MeHg, at 3.9 mg/kg/day, show differences in maturation of monoamine systems with significant increases in norepinephrine in the cerebellum and minor decreases in striatal dopamine concentrations in rats (Lindström et al., 1991). Such effects on neurobiology are often time-sensitive with acute prenatal exposure to high doses of MeHg, 5-20 mg/kg, on day 11 of gestation (GD11) not altering neural cell proliferation (Lewandowski et al., 2003) while exposure to 8 mg/kg on GD15 produces elevated extracellular glutamate and decreased sensitivity to KCL induced glutamate release compared to animals exposed to the same dose on GD8 (Carratù et al., 2006).

Acute exposure to MeHg increases extracellular dopamine concentrations. Low concentrations of MeHg, between 1 and 10 µM, produce age-dependent increases in extracellular dopamine concentration in the striatum of adolescent rats (Dreiem et al., 2009). This is also true following high concentrations of MeHg, between 40 µM and 4 mM, in adult rats (Faro et al., 2000). These increases in extracellular dopamine are complimented by reduced concentrations of the dopamine metabolites, homovanillic acid and 3,4-dihydroxyphenlyacetic acid (Dreiem et al., 2009; Faro et al., 1998, 2000). Faro et al., (2002) show that this increase in extracellular dopamine concentration is likely related to inhibition of the dopamine transporter (DAT), and thus reduced reuptake of dopamine. Acute MeHg exposure increases extracellular dopamine concentrations and there is reasonable evidence that long-term exposure to MeHg also perturbs dopamine neurotransmission. This is seen in children displaying behaviors related to attention-deficit/hyperactivity disorder (ADHD), which is known to be associated with increased DAT density, following prenatal MeHg exposure (Boucher et al., 2012; Cheon et al., 2003; Cheuk & Wong, 2006). This is also seen in animal models with altered sensitivity to the dopamine agonist, *d*-amphetamine, observed in rats exposed to MeHg throughout development (Rasmussen & Newland, 2001). These associations make it necessary to observe correlates between developmental MeHg exposure and symptoms related to altered dopamine neurotransmission.

Attention-deficit/Hyperactivity Disorder: Neurobiology and Behavior

History and Symptomology

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder affecting approximately 5% of children and adolescents and 2.5% of adults (American Psychiatric Association, 2013; Polanczyk et al., 2007; Willcutt, 2012). Clinical diagnosis of ADHD requires an onset of signs and symptoms prior to 12 years of age (American Psychiatric

Association, 2013). Signs are typified by hyperactivity/impulsivity and/or inattention deleterious to normal functioning (Lahey et al., 1998; Sagvolden et al., 2005) with children diagnosed with ADHD also tending to display impairment of short-term memory (Martinussen et al., 2005). Hyperactivity and impulsivity are more commonly observed in children and adolescents, and it tends to wane with age, while inattention can persist into adulthood (Ingram et al., 1999). These behaviors have been linked to disruptions in several neurotransmitter systems, including glutamate, norepinephrine, and dopamine (Biederman & Spencer, 1999; Johansen, Aase, Meyer, & Sagvolden, 2002; Sagvolden et al., 2005). It is not surprising, then, that animal models have shown connections between modulators of these neurotransmitter systems and alterations in behavior related to ADHD.

Neurobiology underlying ADHD Onset

Twin studies performed by Gjone et al., (1996) and Levy et al., (1997) have shown that the manifestation of symptoms related to ADHD is largely mediated by genetic factors with these symptoms being partially mediated by the presence of polymorphisms of dopamine-related proteins (Bellgrove et al., 2005; Cook et al., 1995; Kirley et al., 2002). A variant in the 7-repeat allele of exon 3 of the gene coding for dopamine receptor D4 (DRD4) is associated with several neurodevelopmental disorders, including ADHD (Primus et al., 1997; Rowe et al., 1998). DRD4 further links to ADHD symptomology and impairments in memory as DRD4 is located largely in the hippocampus and relates to alterations in both spatial and sequential memory (Bird & Burgess, 2008; Fortin et al., 2002; Mrzljak et al., 1996; Primus et al., 1997; Tulving & Markowitsch, 1998). DRD4 polymorphisms are not the only such seen in ADHD patients. A variation in the 10-repeat variable tandem repeat (VNTR) of the allele coding for the dopamine transporter is also associated with ADHD diagnosis (Bellgrove et al., 2005; Cook et al., 1995;

Gill et al., 1997). The dopamine transporter is a major proponent underlying ADHD diagnosis as patients diagnosed with ADHD have also been shown to have increased dopamine transporter density in regions of the basal ganglia, specifically the striatum (Cheon et al., 2003; Dougherty et al., 1999).

The association between ADHD-related behavior and dopamine neurotransmission goes beyond this genetic influence as dopamine agonists are used to alleviate signs and symptoms associated with ADHD (Sagvolden & Xu, 2008; Schachar et al., 1997) by increasing the release of dopamine, blocking its re-uptake, or, in the case of drugs such as *d*-amphetamine, both (Gatley et al., 1996; Krause et al., 2000; Martinez et al., 2003; Volkow et al., 1998). Such effects have been replicated in animal models of ADHD symptomology (Bizot et al., 2011; Sagvolden & Xu, 2008; Slezak et al., 2014). Taken together, there is clear evidence that symptoms of ADHD are partially mediated by reduced dopamine neurotransmission. Such dampened dopamine activity may be a result of either 1) decreased sensitivity to dopamine due to receptor polymorphisms or 2) an overall decrease in available dopamine due to enhanced reuptake. However, the cause of such deficient dopamine activity is still only partially understood, with genetic contributions only explaining part of the onset of such signs and symptoms in humans. Another explanation relates to permanent changes in dopamine neurotransmission due to long-term exposure to drugs or toxicants known to cause such alterations—such as in the case of MeHg.

Methylmercury and Attention-Deficit/Hyperactivity Disorder

The relation between developmental exposure to MeHg and onset of symptoms related to, and diagnosis of, ADHD is unclear (Polańska et al., 2013). There is a correlation between total blood Hg levels and ADHD diagnosis, with children diagnosed with ADHD having 7

nmol/L higher blood Hg concentrations than non-diagnosed children (Cheuk & Wong, 2006). A similar relation was reported in a study of children in Québec exposed to Hg during gestation and throughout childhood. Higher cord blood Hg levels, a marker of prenatal MeHg exposure, in children was associated with elicitation of ADHD symptoms, primarily inattention (Boucher et al., 2012). A relation between developmental Hg exposure and ADHD symptomology was also observed in children living near the New Bedford harbor in Massachusetts. Notably, while hair Hg levels were associated with behaviors related to ADHD, maternal fish consumption was protective of these effects (Sagiv et al., 2012).

Not all observations of developmental Hg exposure have seen such associations. For example, a cohort of Korean children exposed to both Hg and lead (Pb) during early childhood, and likely gestation due to maternal consumption of seafood, showed no association between blood Hg levels and signs and symptoms of ADHD (Ha et al., 2009). Similar findings are reported by Kim et al., (2013) with children living near an old refinery in Omaha, Nebraska exposed to Hg and several other contaminants during childhood showing no Hg-related symptoms of ADHD. However, both Ha et al. (2009) and Kim et al. (2013) noticed an association between developmental exposure to Pb and symptoms of ADHD. This association between Pb and ADHD was also observed by Boucher et al., (2012) so it is likely that observations of ADHD symptomology in these populations is confounded by concurrent exposure to other contaminants. It is also possible that the lack of observable symptoms of ADHD are related to the presence of minerals and PUFAs that are often consumed in the diet of these fish-eating populations (Strain et al., 2008). Regardless, the unique contribution of developmental MeHg exposure on symptoms related to ADHD is poorly understood. Recent evidence in animal models of developmental MeHg exposure has potentially shed light on these

associations with mice exposed to 0.3 ppm MeHg during adolescence, a sensitive period for final maturation of dopamine neurotransmission, emitting altered impulsivity (Boomhower & Newland, 2016).

Adolescence as a Sensitive Developmental Period

Risk Assessment and Neurodevelopment

Onset of many neurodevelopmental disorders, including schizophrenia, anxiety disorder, and bipolar disorder, as well as increased impulsivity and occurrence of risk-taking behavior occurs during the adolescent period (Casey et al., 2008; Schneider, 2013; Sham et al., 1994; Spear, 2000; Walker, 2002). Adolescence is also marked by increased susceptibility to drug use (Adriani & Laviola, 2004; Adriani et al., 2003) which is linked to the noted increases in impulsivity and risk-taking behavior. The occurrence of these behaviors during this developmental period are likely linked to the large degree of cortical change occurring throughout adolescence. These changes include heightened interconnectivity between brain regions, as noted by the increase in density of white matter tracks through the basal ganglia and into the frontal, parietal, and temporal lobes (Barnea-Goraly et al., 2005; Giedd et al., 1999; Paus et al., 1999), as well as widespread maturation of cortical systems, especially within the hippocampus and striatum, occurring during this developmental period (Ben Abdallah, Slomianka, Vyssotski, & Lipp, 2010; Giedd et al., 1996; He & Crews, 2007; Larsen & Luna, 2015; McPherson, Aoyama, & Harry, 2011). Unsurprisingly, part of this change relates to the maturation of the primary neurotransmitter systems, especially dopamine which, as stated previously, is associated with impulsive behavior (Laviola et al., 1999; Schneider, 2013; Teicher et al., 1995).

Maturation of dopamine neurotransmission is a hallmark of the adolescent period. Proliferation of dopamine receptors, primarily DRD1 and DRD2, occurs in regions of the basal ganglia and frontal cortex in rat models. DRD1 and DRD2 increase in density after postnatal day (PND) 21 before stabilizing after PND30-40 (Tarazi & Baldessarini, 2000; Teicher et al., 1995). While densities of DRD1 and DRD2 tend to spike early in adolescence, the same is not true for DRD4 which remains relatively constant (Tarazi & Baldessarini, 2000). Similar to the increase in DRD1 and DRD2 density, there is also a spike in density of DAT in the basal ganglia after PND 21 (Tarazi, Tomasini, & Baldessarini, 1998). While densities of these dopamine-related proteins tend to be relatively stable during adulthood there is a marked decrease in density of these receptors in the basal ganglia in old age (Giardino, 1996). Thus, adolescence is the final period of dopamine maturation before aging and alterations in this development will likely cause permanent neurobehavioral alterations that persist well through adulthood. This has been shown to be the case with permanent alterations in reinforcement processing and behavioral flexibility, behaviors associated with dopamine activity, being observed in rodent models of adolescent MeHg exposure (Boomhower & Newland, 2017, 2019).

Adolescent MeHg Exposure

Behavioral outcomes of exposure to MeHg during the sensitive adolescent period are broad, with notable impacts on behavioral flexibility and reinforcement sensitivity being noted in rodent models (Boomhower & Newland, 2017, 2019). Mice exposed to 3 ppm MeHg throughout adolescence displayed perseverative behavior on the second reversal of a spatial discrimination reversal task, comparable to impairments in the first reversal of this task in rats exposed to MeHg throughout gestation (Boomhower & Newland, 2017; Paletz et al., 2007). These mice exposed to MeHg during adolescence also displayed altered reinforcement processing, assessed using a

model called Mathematic Principles of Reinforcement, with lower response rates being emitted for reinforcement compared to non-exposure groups (Boomhower & Newland, 2019). Interestingly, mice exposed to MeHg during adolescence, similar to those exposed to MeHg during gestation, are sensitive to dopamine agonists with both long-term and acute exposure to *d*-amphetamine greatly effecting behavior in these procedures (Boomhower & Newland, 2017, 2019).

Adolescent exposure to MeHg has also been linked to behavior related to ADHD with notable improvements in impulsive choice being observed in both human and rodent models. Humans who consumed large amounts of fish in adolescence, likely concurrent with high intake of MeHg, and mice exposed to 0.3 ppm MeHg in drinking water throughout adolescence showed improvements in impulsivity (Boomhower & Newland, 2016; Butler et al., 2017). Alterations in motor activity, a proxy of hyperactivity, have also been observed in both human and animal models following high doses of MeHg. Exposure to a high dose of MeHg throughout postnatal and adolescent development, at 5 mg/kg, induces impaired performance on a rotarod—a rodent model of fine motor function (Sakamoto et al., 2004). As mentioned previously, impairment in gross motor function, such as ataxia, was observed in children exposed to high doses of MeHg in Iraq (Amin-zaki et al., 1978). This is not the case for sustained attention as childhood blood Hg levels in human children are not associated with attentional deficits (Boucher et al., 2012) and rats exposed to MeHg during adolescence, at either 0.5 or 5 ppm, do not exhibit altered attention in adulthood (Kendricks et al., 2020). However, the lack of attention deficits noted in animal models may be due to the lack of challenge associated with the attention task used in that study while the lack of observable alteration in attention in humans may be a result of the scales used to assess attention, which primarily rely on outsider rating. Unfortunately, observations of

memory alterations following adolescent MeHg exposure are scarce with one observation reporting improvements in memory in rats exposed to 0.5 ppm MeHg throughout adolescence (Kendricks et al., 2020). The paucity of research addressing long-term impacts of exposure to MeHg during adolescence means that the potential links between exposure to MeHg during this period and onset of ADHD symptoms is not well understood. Coupled with the conflicting observations linking gestational exposure to MeHg and ADHD diagnosis, it is unclear if MeHg exposure could be linked to ADHD in either human or animal models.

Review: Attention and Memory

Sustained attention and short-term remembering are closely associated processes (Fougnie, 2008). Sustained attention is facilitated by memory, with attention to a stimulus governed by both accurate detection of a stimulus as well as remembering the stimulus/response relation throughout the duration of time in which attention is to be maintained. Attention and short-term memory are also partially mediated by similar processes. Dopamine in the anterior cingulate cortex mediates attentional processes and dopamine in the medial temporal cortex and ventrolateral prefrontal cortex mediate short-term memory (Aalto et al., 2005). As mentioned previously, both inattention and impaired short-term memory are associated with ADHD symptomology, with inattention being more prevalent in adults diagnosed with ADHD (Ingram et al., 1999) and children diagnosed with ADHD tending to have higher prevalence of impairments in memory (Martinussen et al., 2005). However, associations between MeHg exposure and alterations in these behaviors is ill understood.

Assessment of inattention in animal models follows similar trends. As studied in 5-choice serial reaction time (5-CSRT) and two-choice visual signal detection (VSD) tasks, attention is defined as behavior under the control of a stimulus that is brief, infrequent, and unpredictable

(Bizot et al., 2015; Bushnell, 1998; Cherian et al., 2019; Openshaw, Thomson, Penninger, Pratt, & Morris, 2017; Paterson, Ricciardi, Wetzler, & Hanania, 2011). While the 5-CSRT task is a reliable measure of attention it is also often used to assess response inhibition, a component of motor impulsivity (Economidou et al., 2012), so these differing processes may be difficult to tease apart. VSD has been used in previous studies to assess the impacts of cholinergic and noradrenergic drugs on inattention in rodent models (Bushnell et al., 1997; Cherian et al., 2019) and may be sensitive enough to detect minor alterations in attention that could arise following adolescent MeHg exposure. However, the assessment of attention in VSD tasks is strengthened through the use of challenges to attentional processes—commonly altering the detectability of the stimulus used via either 1) altering the intensity of the stimulus (Bushnell et al., 1997; Levin et al., 2011) or 2) altering the duration of the stimulus (Cherian et al., 2019; Mohler et al., 2001) thus showing the importance of disruption of baseline behavior in the accurate detection of impaired attention.

The VSD procedure can be modified to assess short-term memory similar to other commonly used tasks in rodent models: namely delayed match-to-sample and delayed non-match-to-sample tasks (Sargisson & White, 2001, 2003). This modulation includes 1) an unpredictable pre-stimulus delay that allows for unpredictable presentation of the stimulus and 2) a variable post-stimulus delay that tests recall of whether a stimulus was presented. These modifications are used in the current study to assess both sustained attention and short-term memory concurrently.

Purpose

The purpose of this study is to assess interactions between adolescent MeHg exposure and adult onset of behaviors related to ADHD, namely impairments in sustained attention and

short-term memory, in a mouse model. Adolescence is a sensitive period in the development of dopamine systems with dopamine partially mediating both attentional processes and short-term memory. Acute and long-term exposure to MeHg produces irreversible alterations in dopamine neurotransmission and exposure to MeHg during adolescence has been found to alter behaviors mediated by dopamine, such as behavioral flexibility, reinforcement sensitivity, and impulsive choice (Boomhower & Newland, 2016, 2017, 2019). However, the unique contribution of MeHg on behavior related to ADHD following exposure throughout adolescence is poorly understood. This is important because of the possibility that attention deficits underlie some of the other deficits reported. In order to model these behaviors, mice were exposed to 0, 0.3, or 3 ppm MeHg in drinking water throughout the adolescent period and tested on a modified two-choice VSD task in adulthood. Behavior in the procedure was challenged by varying the duration of the visual signal, as in Cherian et al., (2019), to decrease stimulus discriminability. A novel distractor will also be introduced to further drive behavior away from the visual stimulus. Assessment of impacts of these alterations, as well as the contribution of MeHg in mediating these behaviors, will be done using Information Theoretic Approach.

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Chapter 2

Adolescent Methylmercury Exposure: Effects on Sustained Attention and Short-Term Remembering in Mice

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Abstract

Behavioral alterations due to adolescent exposure to the neurotoxicant, methylmercury (MeHg), have only recently been explored in animal models. In rodents, such alterations are related to permanent changes in dopamine neurotransmission and in behavior related to ADHD, such as impulsivity and motor activity. The current study uses a mouse model to observe sustained attention and short-term remembering by exposing mice to 0, 0.3, or 3 ppm methylmercury, with exposures distributed across littermates, from post-natal day 22-59, rodent adolescence. The mice were tested using a visual signal detection task in adulthood. Behavior was challenged by varying the duration of the visual signal and introducing a novel distractor. Attention in this task was altered by the duration of the visual signal, akin to previous studies, and by the novel distractor confirming the efficacy of this procedure in adequately capturing alterations in attention. However, mice exhibited no MeHg-related changes in sustained attention. Mice also exhibited no change in memory following adolescent MeHg exposure. The effects noted here differ from other studies using rats, suggesting that species differences may be important in assessing MeHg's development neurotoxicity.

Introduction

Neurobehavioral effects of exposure to environmental contamination are frequently observed in human populations following exposure during sensitive developmental periods, such as gestation, childhood, and adolescence (Boucher et al., 2012; Chevrier et al., 2009; Ha et al., 2009; Kim et al., 2013; Polańska et al., 2013; Van Wijngaarden et al., 2013) but unravelling the relative contributions of each developmental period is difficult to do in epidemiology studies. One such contaminant, MeHg, is consumed by mothers and their children when seafood is part of their regular diet (Burger & Gochfeld, 2004; Butler et al., 2017; Groth, 2010; Nielsen et al., 2014). Prenatal and early postnatal exposure to MeHg in human populations is correlated with changes in attention, impulsivity, and memory (Boucher et al., 2012; Grandjean et al., 2014; Stewart et al., 2006). In experimental models, changes in locomotor activity, reinforcement sensitivity, behavioral flexibility, and choice have been linked to prenatal exposure (Daré et al., 2003; Newland, Hoffman, Heath, & Donlin, 2013; Newland, Yezhou, Lögdberg, & Berlin, 1994; Paletz, Day, Craig-Schmidt, & Newland, 2007). These behaviors are closely linked to dopamine neurotransmission and activity of the prefrontal cortex. While developmental exposure to MeHg produces neurobehavioral deficits related to development of dopamine systems, behavioral outcomes following exposure during later stages of dopamine development, as is the case with adolescence, have only recently been observed in experimental models.

Adolescence is the last stage of cortical and basal ganglia development before aging. Receptor proliferation occurs in regions of the basal ganglia in rats, beginning with elevation of receptor density after PND 21, the beginning of early rodent adolescence (Adriani & Laviola, 2004; Spear, 2000). Subsequent pruning of receptors in the cortex occurs after PND 30 and continues into early adulthood (Tarazi & Baldessarini, 2000; Teicher et al., 1995). Similar

changes in dopamine neurotransmission in human and nonhuman species are correlated with risky decision making and impulsive choice (Andrzejewski et al., 2011; Dalley et al., 2008). These behaviors are often related to disorders associated with altered dopamine neurotransmission, such as ADHD which is marked by increased impulsivity, hyperactivity, and/or inattention deleterious to normal functioning (American Psychiatric Association, 2013). Patients diagnosed with ADHD have impaired dopamine signaling associated with a higher likelihood of increased DAT density in the basal ganglia (Cheon et al., 2003; Dougherty et al., 1999). Developmental exposure to MeHg is also related to symptoms of ADHD. Mice exposed to 0.3 ppm MeHg during adolescence exhibit decreased impulsive choice compared to animals exposed to 0 or 3 ppm MeHg (Boomhower & Newland, 2016) but higher ADHD diagnosis occurs in human children exposed to MeHg early in development (Cheuk & Wong, 2006). This interaction between MeHg and inattention or impaired memory, two components of ADHD, has only recently been explored in animal models (Kendricks et al., 2020).

Sustained attention, behavior under the control of a stimulus that occurs infrequently and unpredictably, is facilitated by processes of short-term memory, coincident with substantial overlap in neural processing (Fockert et al., 2001; Fougnie, 2008). Both inattention and impaired short-term memory are components of ADHD (Martinussen et al., 2005; Sagvolden et al., 2005) and related to changes in dopamine neurotransmission (Aalto et al., 2005). It is conceivable that inattention could be linked to MeHg-induced deficits like impulsivity or behavioral rigidity. However, interactions between MeHg and memory are highly variable. A reliable measure of sustained attention is the two-choice visual signal detection task which has been used previously to elucidate the impacts of cholinergic and noradrenergic systems on processes of sustained attention in animal models (Bushnell et al., 1997; Cherian et al., 2019; Rezvani et al., 2002,

2009). This procedure involves both accurate detection of a brief and unpredictable visual signal as well as accurate discriminability between the signal's presence and absence. In this procedure, a visual signal has a 50% chance of occurring within a variable window of time. This makes it unpredictable whether the signal will occur or not and, if the signal is to occur, it is unpredictable when it will occur. Attention to the signal would yield high reporting of the signal when it was presented, a high "hit" rate, as well as a high reporting of the signal's absence when it was not presented, a high "correct rejection" (CR) rate. Lapses in attention would result in inaccurately reporting that the signal was not presented when it was, a high "miss" rate, or inaccurately reporting that the signal was presented when it was not, a high "false alarm" (FA) rate. To ensure that the occurrence of the visual signal remains unpredictable randomized sets of delays occurring before (pre-signal) and after (post-signal) the visual signal are used. This task can be modified to assess short-term memory by introducing a large range of post-signal delays that would reduce memory of whether the signal occurred or not, similar to delay-match to sample tasks.

Reports describing the interaction between developmental MeHg exposure and both memory and attention often vary depending on 1) when exposure occurred, 2) the dose of exposure, 3) the species studied, and 4) the procedure used. Boucher et al. (2012) showed that while children exposed to MeHg during gestation exhibited impaired attention, exposure during childhood and adolescence had no such association. A similar lack of interaction between MeHg and inattention was observed in a rodent model of adolescent MeHg exposure (Kendricks et al., 2020). While improvements in memory have been observed in rats exposed to MeHg during adolescence (Kendricks et al., 2020) as well as non-human primates exposed to MeHg during

gestation (Gilbert, Burbacher, & Rice, 1993) such effects were not seen in rats who had impaired memory following gestational MeHg exposure (Albores-Garcia et al., 2016).

Due to the varied effects of MeHg and the overlap in processing between remembering and attention, it is important to observe these behaviors concurrently in different animal models following similar exposure regimens. To accomplish this, mice were trained on a modified visual signal detection task previously used to examine the impacts of attention and memory in a rat model (Kendricks et al., 2020). Typically, ranges of pre-signal delays in this task are less than 25s and post-signal delays span a very narrow range: up to 4s (Bushnell et al., 1997; Cherian et al., 2019; Mohler et al., 2001). In order to increase the unpredictability of the visual signal a range of pre-signal delays (from 0 to 74s) was used. To probe remembering of the signal's occurrence, post-signal delays of up to 29.3s were employed. In order to ascertain the validity of this task in assessing changes in attention and memory the detectability of the visual signal will be altered, as has been shown to reliably alter attention in previous reports (Cherian et al., 2019), and a novel distractor will be introduced, as distraction has been previously shown to impair memory (West, 1999).

Method

Subjects and Exposure

Thirty-five male C57BL/6 mice were purchased from ENVIGO (Indianapolis, IN). Mice arrived on PND 21 and were provided free access to standard mouse chow. For the first week, mice were housed in sets of four in ventilated Optimice® cages in an AAALAC-approved animal facility under a 12-12 light/dark cycle (lights on at 6am). After the first week, the mice were separated and pair-housed in partitioned cages for the remainder of the study. Mice were derived from twelve litters. Three male littermates were pseudorandomly divided into one of three

exposure groups: 0, 0.3, or 3 ppm MeHg dissolved in their drinking water. Because of a death unrelated to exposure, only eleven mice were in the 0.3 ppm group but the other groups had twelve. Exposure occurred from PND 22 through PND 59. On PND 60 water bottles for all animals were replaced with tap water (0 ppm). In order to calculate daily water consumption, water bottles were weighed 3-4 times each week. A sham bottle was weighed concurrently to estimate water spillage. Consumption was calculated as the mean of the pair's consumption except for the first week of exposure wherein consumption was calculated as the mean of the group of four's consumption, minus spillage. Body mass for all mice was maintained at approximately 25(±1)g during behavioral testing.

Apparatus

Behavioral sessions were conducted in Twelve Med Associates operant chambers modified for use with mice. Each chamber was equipped with two retractable front levers, one non-retractable rear lever, and two LED signal lights located above each of the two front levers. Between the two front levers a central house light was situated above a nose poke with a dipper that dispensed 0.01cc presentations of 3:1 water to sweetened condensed milk solution as reinforcement. Two Sonalert® generators with a high tone of 4500Hz and low tone of 2700Hz were located above the LED lights on the left and right sides of the front wall. Each chamber was encased in a sound attenuating box with a fan that provided white noise throughout experimental sessions. A computer in an adjacent room controlled all experimental sessions.

Lever-Press Training and Progressive Ratio

On PND 90, lever press training was established as described previously (Boomhower & Newland, 2017; Paletz et al., 2007; Reed et al., 2006). Training began on either the left or right front lever, counterbalanced across animals. The trained lever was extended into the chamber

concurrently with illumination of its corresponding LED. The lever remained extended for either 30s or until a response occurred, with each resulting in retraction of the lever, termination of the LED light, presentation of 0.01cc of milk, and onset of a 5 minute intertrial interval (ITI). After the mouse pressed the available lever 10 times, free reinforcement ended and a Fixed Ratio 1 (FR 1) schedule was introduced. Criterion was 40 responses under FR1 within a 60 min session. The mice were trained in overnight, 13 hr, training sessions. Failure to achieve 50 responses on the training lever after 5 consecutive sessions lead to handshaping, reinforcement of successive approximation, until response criterion were met. Three mice in the 0 ppm, three mice in the 0.3 ppm, and seven mice in the 3 ppm group required handshaping (a total of 13 mice).

After criteria were met for both front levers, mice were trained under intermittent reinforcement using a progressive ratio schedule. Mice were first required to respond under FR 1 until 12 reinforcers were obtained after which the FR requirement increased by 1 (i.e., FR 2). This continued until 12 reinforcers were obtained under a FR 5. Training occurred on both the right and left levers, with the lever that was trained first being counterbalanced across animals.

Visual Discrimination Training

After mice reached criterion in progressive ratio, they were trained to discriminate between the presence and absence of a visual signal. The mice were trained to respond on the left lever when the left LED was illuminated, a signal trial, or on the right lever when no LED was illuminated, a blank trial, via a set of fading procedures. The mice were first training to respond on the correct choice lever, with illumination of the left LED signaling when the left lever was correct, with the lever that was incorrect being faded in based on animal's individual accuracy. The mice were then trained to discriminate between the presence and absence of the left LED by first training responding on the left lever when the left LED was illuminated and responding on

the right lever when the right LED was illuminated. Illumination of the right LED was then faded out based on animals' individual accuracy. These stages are described in Appendix 1.

Visual Signal Detection (VSD) Training

Once animals achieved criterion in visual discrimination training, they were trained to respond on the left lever after a 1s illumination of the left LED and on the right lever when no LED was illuminated. In signal trials, a 1s low tone was followed by illumination of the left LED 0.3s prior to lever extension. During the first trial under VSD, the left LED in signal trials remained on for 30s after the extension of the levers or until a response occurred. The duration of blank trials was yoked to signal but no signal was presented. Correct responses, left lever press in signal trials or right press in blank trials, resulted in reinforcement. Incorrect responses and omissions, failure to respond within 30s of the levers extending, resulted in a time-out (TO) and correction trial. The duration the left LED was illuminated in signal trials decreased by 1% with each correct response until the duration was less than 0.7s after extension of the levers (i.e., 1s). After this, the duration the LED was illuminated prior to levers extending increased and the duration after the levers extended decreased by 10%/session until the LED was illuminated for 1s prior to the levers extending and terminated when the levers extended. Choice latency under VSD was maintained under percentile as described in Appendix 1.

After animals achieved over 75% accuracy for three consecutive sessions in VSD training the correction procedure was removed. Criterion was 3 consecutive sessions with over 75% accuracy with no correction. Twenty-eight mice reached this criterion (n = 10 for 0 and 0.3 ppm; n = 8 for 3 ppm) and one mouse (in 3 ppm group) advanced with two consecutive sessions. Analyses were performed with and without this animal to determine if this relaxed criterion altered behavior, see results.

Sustained Attention and Short-Term Remembering: Phases 1 and 2

Upon completion of VSD training, attention to the 1s LED was tested in a modified visual signal detection task, described previously (Kendricks et al., 2020). Mice were required to detect the presence or absence of a 1s LED following presentation of randomized pre- and post-signal delays. Short choice latencies continued to be maintained under percentile.

Trials in Phase 1 of sustained attention were cued by a 1s low tone occurring after a 10 ITI. A pre-signal delay, randomly selected without replacement from a pool of six delay per session, was presented after termination of the low tone. Two pools of six delays, ranging from 0.3-74s logarithmically spaced, were distributed across two sessions. The first set of delays included 0.3, 0.8, 1.4, 6.1, 16.5, and 74s while the second included 0.5, 2.2, 3.7, 10, 27.2, and 44.9s. After the pre-signal delay, either a 1s illumination of the left LED, signal trials, or a 1s yoked blank pause, blank trials, occurred. The 1s signal or blank pause was succeeded by the presentation of one of three post-signal delays (2, 3, or 4s) randomly selected without replacement. A response on the left lever when the left LED was illuminated or a response on the right lever when the signal was not illuminated resulted in reinforcement. Incorrect responses and omissions resulted in a TO. A trial in Phase 1 of sustained attention progressed as follows (Appendix 2): 10s inter-trial interval (ITI) → 1s low tone → Pre-signal delay (randomly selected without replacement) → 1s LED or 1s blank pause (pseudorandomly assigned with the same trial type not occurring 3 or more times in a row) → Post-signal delay (randomly selected without replacement) → levers extend →response→consequence.

After 12 sessions under Phase 1 of sustained attention, memory was probed by extending the range of post-signal delays to span 0.3 and 29.3s, spaced logarithmically. This is designated as Phase 2 (Fig. 2.1). The range of pre-signal delays remained the same as in Phase 1. Post-signal

delays were randomly selected from two sets of three delays each with each set alternating by session. The first set of delays were 0.3, 1.9, and 11.7s and the second set were 0.8, 4.7, and 29.3s. In order to ensure both sets of pre-signal delays and both sets of post-signal delays were presented evenly, pre- and post-signal delays were presented in a 2x2 fashion requiring 4 sessions to complete all delay pairings. Mice were required to complete 12 sessions under Phase 2 of sustained attention.

Signal Detection

After 12 sessions under Phase 2 of sustained attention, the mice's ability to detect the signal was challenged by varying the LED duration in signal trials. Behavior was probed with LED durations of 0.06, 0.13, 0.25, 0.5, and 1s, presented in pseudorandom order with the same order occurring for all animals. The duration of the pause in blank trials was yoked to these varying LED durations. The experimental criterion remained the same as in Phase 2 of sustained attention (Fig. 2.1), however, the number of presented pre-signal delays was reduced to six (0.3, 1.4, 3.7, 10, 27.2, and 74s) so that only one session was required to present all pre-signal delays and two sessions to present all post-signal delays. Thus all delay presentations required only two sessions. Each LED duration was tested twice with at least one day separating successive presentations. The 1s baseline LED duration was presented on intervening sessions.

Tactile Novel Distraction

To confirm that this task adequately captures sustained attention a novel distractor was introduced. The tactile distraction, a moderate sized toy (a plastic slide that was the same shape and size for all animals), was placed in the rear of the operant chamber in front of, but not obstructing, the non-retractable rear lever. The toy remained in the operant chamber throughout the experimental session allowing time for the animal to interact with the toy throughout the

behavioral session. Experimental parameters were the same as in "Signal Detection" (Fig. 2.1) so animals underwent two sessions with the toy in the rear of the chamber. At least one day separated successive presentations of the toy. Baseline data were collected on intervening behavioral sessions.

Data Analysis

All analyses and data management were performed using R (version 3.6.1). Accuracy was measured based on the proportion of correct responses in signal trials (Eq. 1) and the proportion of incorrect responses in blank trials (Eq. 2).

$$p(Hit) = \frac{Hit}{Hit+Miss} \quad \text{Eq. 1}$$

$$p(FA) = \frac{FA}{FA+CR} \quad \text{Eq. 2}$$

These are measures previously used to describe sustained attention in this signal detection paradigm (Bushnell et al., 1997). Log D, a measure of stimulus discriminability, and Log B, a measure of bias, were also observed. However, these measures provided no additional information that could not be interpreted from p(Hit) and p(FA) and, thus, are not presented here. Omissions and choice latency were also recorded during each phase of training. Data were Winsorized, a method of controlling for excessive influence of outliers without decreasing the number of data points (Wilcox, 1998).

Analyses were performed using an information theoretic (I-T) approach used previously to describe the behavioral impacts of environmental toxicants (Boomhower & Newland, 2016; Newland, 2019). In the I-T approach, the model that bests fits the observed data is identified by comparing its fit with that of other models in a candidate set using the Akaike Information Criterion (AICc), corrected for small sample sizes. The AICc provides information about the degree to which a given model in a set of models deviates from the observed data and the

outcome of the analysis is a probability that each model is the best of the candidate set. Because this determination is made in relation to all models in a candidate set, it is necessary to compare many models, with each model selected according to their theoretical relevance and plausibility.

Sets of models compared for all data included: 1) a null model in which slope and intercept predictions were based only on the grand mean; 2) linear models with main effects of MeHg, main effects of post-signal delay, and interactions between MeHg and post-signal delay; and 3) models including a quadratic term for post-signal delay to support a parabolic relation, again including main effects of MeHg, main effects of post-signal delay, and interactions between MeHg and post-signal delay. During challenges (both signal detection and distraction) the challenge condition was included as either a main effect or interaction. Models for signal detection had the duration of the LED as either linear or quadratic. Models for distraction had distraction as a factor. Models used for results (see below) are listed in Appendix 3. Final decisions were based on the model probability, w_i, which gives a determination of the probability that a specified model is the best model of the ones tested in the set. The model probability only accounts for tested models so any model that was not in the original set was not considered in the final determination.

Results

Body Mass and Consumption

Animals were exposed to either 0, 0.3, or 3 ppm MeHg in drinking water: confirmed by ICP-MS as 0, 0.28, and 2.67 ppm Hg, respectively. Body mass and water consumption were measured throughout the adolescent exposure period and were used to calculate the amount of MeHg consumed in the 0.3 and 3 ppm groups (Fig. 2.2). There were no MeHg-related differences in either body mass or water consumption throughout the adolescent period. MeHg

consumption leveled off at approximately 40 µg/kg/day for the 0.3 ppm group and 500 µg/kg/day for the 3 ppm. As previously reported, the MeHg dose was highest early in adolescence because of increased fluid consumption by these young mice accompanied by a lower body mass (Boomhower & Newland, 2016, 2017; Kendricks et al., 2020).

Discrimination and VSD Training

Advancement through each stage of discrimination training and through VSD training is shown in Fig. 2.3. The number of sessions required for each animal to advance to Phase 1 of sustained attention varied greatly with animals requiring between 37 and 154 sessions to reach final criterion. Six animals (2, 1, and 3 from the 0, 0.3, and 3 ppm groups, respectively) were removed from the study due to failure to acquire the VSD task. These animals are represented by the point at the end of VSD training in Fig. 2.3. It was determined that data for the animal that advanced under a relaxed criterion, described above, was an outlier and thus data from this subject (in the 3 ppm group) was also removed from analyses, described below. Thus, the final number of animals was 28 (10, 10, and 8 from the 0, 0.3, and 3 ppm groups, respectively).

Attention and Memory: Phase 1 and 2

Randomized pre-signal delays between 0.3 and 74s and random post-signal delays of 2, 3, and 4s were used during Phase 1 of sustained attention (Appendix 2). There were no MeHg-related effects on accuracy for any of the tested delays and these data are not shown.

Full forgetting functions in Phase 2 of sustained attention are shown in Fig. 2.4. A trend occurred where p(Hit) was slightly higher at the 1.9s delay (Fig. 2.4A), hereafter referred to as the training delay due to its correspondence with the shortest trained delay in Phase 1 of sustained attention (*i.e.*, 2s), as compared to the shortest (*i.e.*, 0.3s) delay. Values for p(Hit) dropped off at longer delays. The best three models describing the change in p(Hit) across post-

stimulus delay contained a quadratic term for delay (Table 2.1) and had a combined probability of greater than 0.99 (0.62 + 0.29 + 0.09) of being the best models for these data thus confirming the curvilinear appearance of the curve in Fig. 2.5A. Only the second and third best models included MeHg revealing no evidence of a MeHg effect on p(Hit) across delay.

While p(Hit) was affected by delay, p(FA) was not. Values for p(FA) (Fig. 2.4B) remained constant across all post-signal delays. As seen in Table 2.1, none of the models tested was distinguishable from one another or from the null model. Together, these results reveal no contribution of MeHg on attention or memory.

During Phase 2, sustained attention was defined by accuracy at the training delay or at the shortest post-signal delay of 0.3s (see also Sargisson & White, 2001). Values for p(Hit) were marginally elevated at the 1.9s delay as compared to the 0.3s delay (Fig. 2.5A) so both delays were considered for determination of attention deficits. Neither p(Hit) nor p(FA) (Fig. 2.5B) were affected by MeHg at either delay suggesting no MeHg-related effect on attention.

Attention and Memory: Signal Detection

The duration of the LED signal was varied to challenge behavior in this task and to confirm that performance reflected what would be expected of an attention task based off of previous reports (Cherian et al., 2019; Mohler et al., 2001). The hit rate, p(Hit), was greatly reduced as the LED duration shortened from 1.0s to 0.06s (Fig. 2.6A). The quadratic shape of the curve relating p(Hit) to post-signal duration remained in place, and even sharpened, with the effect of LED duration being exaggerated at post-signal delays shorter than the training delay. Values for p(FA) (Fig. 2.6B) increased at the 0.06s LED duration, showing diminished accuracy in reporting the absence of the signal when the signal duration was short. Further, p(FA) tended to improve linearly with increasing duration of the post-signal delay likely reflecting greater

tendency to report the absence of the signal when memory of whether the signal occurred is diminished. Model comparisons of these data are not shown.

Impairment in accuracy at short LED durations was most prominent at the 0.3s delay thus Fig. 2.7 shows the effect of LED duration at this delay. LED duration differentially affected p(Hit) at the 0.3s delay (Fig. 2.7A) with p(Hit) being greatest at the trained LED duration, 1s, and decreasing as the LED duration shortened, plateauing at slightly below chance when the LED was illuminated for less than 0.12s. This shift in p(Hit) was not affected by MeHg with the model including MeHg only having a 15% chance of being the best model of those observed (Table 2.2). All of the top models included the duration of the LED but none was better than another. Even the best model in the set, supporting a linear relation between LED and p(Hit), only had a 37% chance of being the best model, which is poor. Further, of the three best models both linear and parabolic relations between LED duration and p(Hit) were observed so even if LED was a contributing factor to decreasing accuracy the nature of the relation between LED duration and p(Hit) remains unclear given the models observed.

A more muted trend is seen with p(FA) (Fig. 2.7B). Values for p(FA) at the 0.3s delay were lowest for all animals at the 1s baseline LED duration and increased slightly at shorter LED durations. The best model for p(FA) included a linear relation between p(FA) and LED duration (w_i = 0.37) but this model did not include MeHg. This model was poor and did not substantially differ from the other models tested for p(FA). The top three models, with a cumulative probability of 0.65, had a term for LED duration and the null model is fourth best (Table 2.2). This offers little evidence of a role of LED duration on p(FA) at this delay. MeHg did not influence p(Hit) or p(FA) as the duration of the LED varied, further supporting a lack of MeHg

effect on attention. Further, the unique contribution of the LED duration on both p(Hit) and p(FA) was not determinable given the models tested.

Tactile Distraction

The tactile distraction reduced p(Hit) to at or below chance for all animals (Fig. 2.8A). The quadratic relation between post-signal delay and p(Hit) remained despite the downward shift in accuracy. This is supported by the best three models listed in Table 2.3 all showing a main effect of distraction and a main effect of post-signal delay including a quadratic term. The fourth best model includes an interaction between these terms. Two of the four best models include MeHg, but even taken together a model including MeHg would have little over 39% chance of being the best model to describe the shifts in p(Hit) during tactile distraction.

The tactile distraction decreased p(FA), thus reducing accuracy in reporting the absence of the signal, across all MeHg exposure groups and across all post-signal delays (Fig. 2.8B). While none of the models tested for p(FA) were substantially better than others, all of the best models tested, with a combined probability of over 99%, included the distraction and a quadratic term (the best five are shown as representative in Table 2.3). However, both post-signal delay and MeHg do not substantially contribute to the shifts seen in p(FA) when the distraction is present.

The peak in accuracy in the presence of the distraction also occurred at the training delay. In order to assess whether accuracy at the peak in the presence of the tactile distraction was influenced by MeHg, data at this delay were isolated. Neither p(Hit) (Fig. 2.9A) nor p(FA) (Fig. 2.9B) were affected by MeHg. Both, however, were affected by the tactile distraction. Together these results further support no contribution of MeHg on attention in this procedure.

Discussion

Sustained attention and short-term remembering were assessed in a mouse model of adolescent exposure to the contaminant, MeHg. MeHg did not alter the rate of acquisition of this task nor were there any MeHg-related alterations in either sustained attention or short-term remembering, in contrast to previous reports showing minor improvement in memory following developmental exposure in non-human primates (Gilbert et al., 1993) and in rats (Kendricks et al., 2020). This procedure was validated by several manipulations, including the introduction of varying signal durations to assess the impact of signal discriminability on attention and memory, the use of different pre-signal delays to ensure that the presence of the signal was unpredictable, the use of a range of post-signal delays to adequately assess forgetting, and the introduction of a novel distractor to disrupt processes of attention and memory. Short signal durations greatly diminished sustained attention in this study, akin to previous reports (Cherian et al., 2019; Mohler et al., 2001), but did not readily impact short-term remembering. The tactile distraction severely disrupted attention across all animals but did not interact with delay so memory may not have been altered.

There were no observable differences in body mass between exposure groups, as has been observed in previous reports of adolescent MeHg exposure in mice (Boomhower & Newland, 2016, 2017, 2019). The lack of difference in body mass between MeHg exposure groups minimizes differences in body fat among animals. This decreases the likelihood of MeHg being sequestered in body fat and, thus, decreases the risk of differences in behavior related to this accumulated MeHg rather than as a result of long-term impairments resultant from exposure to MeHg throughout adolescence. It also shows that the dose used was not so high as to cause growth retardation. The daily consumption of MeHg for the 0.3 ppm group in the current study

was akin to previous reports, at approximately 40 µg/kg/day, however, consumption in the 3 ppm group was slightly higher at 500 µg/kg/day versus the 400 µg/kg/day reported previously (Boomhower & Newland, 2016, 2017). This slight increase in MeHg consumption is unknown as the change in body mass and water consumption during this period is similar that reported previously by (Boomhower & Newland, 2016, 2017). The bitonic shape of the fluid consumption curve with age was also seen in earlier reports.

Sustained attention and short-term remembering were assessed using a modified visual signal detection task. The training procedure used to establish visual signal detection in this study yielded similar attrition rates as that observed in other studies using mice (Cherian et al., 2019; Mohler et al., 2001). This method did, however, result in great stimulus control as evidenced by the subjects' reliable discrimination between the presence and absence of the signal, the clear changes in behavior when the duration of the signal changed, and the short response latencies.

In order to adequately assess sustained attention and short-term remembering in this modified visual signal detection task, several methods were employed. First, a wide range of pre-signal delays was introduced in order to ensure that presentation of the visual signal was unpredictable. Second, the signal duration was slowly faded to short durations on an individualized basis according to an animals' accuracy. This ensured that the final duration of the signal, 1s, was brief without sacrificing behavior. Third, the duration of the signal was altered to provide information about the threshold of stimulus detection in this mouse model. Fourth, a novel distractor was introduced to determine if behavior under the visual stimulus was rigid. And finally, a wide range of post-signal delays was introduced to allow for generation of a forgetting

function. These methods yielded insight into both attention to the visual cue as well as memory of the cue after a delay in mice.

Sustained attention is behavior under the control of a brief and unpredictable signal and is marked by accuracy at either the shortest delay of 0.3s or at the training delay of 1.9s. Sustained attention reliably tracked signal duration at the 0.3s delay with accuracy falling off as the duration, and presumably the discriminability, of the signal decreased. This trend is in line with previous reports (Cherian et al., 2019). Further, accuracy at the training delay was greatly disrupted by the presence of a distraction. However, the lack of MeHg-related differences in behavior when the novel distractor was introduced, coupled with the lack of MeHg-related disruption in behavior when the duration of the visual signal was varied, suggests that both baseline attention as well as shifts in attention in mice exposed to MeHg are not disrupted. We conclude that MeHg did not alter sustained attention in this procedure, similar to a previous report in rats (Kendricks et al., 2020). This suggests that adolescent MeHg exposure may not interact with attentional processes in mice, similar to the lack of interaction noted in human populations between child blood Hg concentrations and attention deficits in childhood and adolescence (Boucher et al., 2012).

Short-term remembering in this procedure is defined as the change in accuracy as the delay to a chance to respond increases, as defined previously (Sargisson & White, 2003; White, 2001). Memory in the current procedure was not influenced by MeHg. Attempts to link MeHg exposure to forgetting have resulted in a variety of conclusions (Newland et al., 2008) perhaps reflecting weak effects or differences in species and methods used.

An explanation for the bitonic effect of delay on accuracy in this procedure is stimulus generalization (Sargisson & White, 2001). Temporal characteristics of the post-signal delay can

act as stimuli which govern behavior such that a generalization gradient may be generated with a peak occurring at the training delay and generalization defining the decrease in accuracy at delays falling away from the training delay; at both shorter and longer delays. Sargisson and White (2001) showed two contributions to this generalization/forgetting pattern: 1) generalization is defined by the peak of accuracy occurring at the training delay with corresponding decreases in accuracy at shorter and longer delays; 2) there is a steeper drop in accuracy at longer delays than at shorter delays with this drop being representative of forgetting. This same trend is observed here with a peak at the training delay of 1.9s and a steeper drop in p(Hit) at longer delays compared to shorter delays. Regardless, MeHg did not alter either aspect of accuracy across delay with both the peak of accuracy as well as the drop in accuracy at shorter and longer delays being the same across exposure groups.

The current procedure was designed to detect impacts of adolescent MeHg exposure on a hybrid sustained attention/short-term memory procedure in a mouse model. The parameters of this procedure were validated by several manipulations, including the use of varying signal durations, the introduction of novel distraction, and the use of a large array of post-signal delays. MeHg did not alter sustained attention, an effect that is in line with previous findings, nor did it alter short-term remembering, which further substantiates variable interactions between MeHg and memory across studies. The differences in effects on memory noted here compared to previous reports may be related to a difference in species used or the difference in training protocol employed. Further research is required to pull apart the nuances of these differences to better describe the impact of adolescent MeHg exposure on these behaviors across species.

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Tables

Table 2.1:
Model Comparisons: Phase 2 of Sustained Attention
Models compare impacts of MeHg and post-signal delay. Models with delay are either linear or contain a quadratic term to model shifts in p(Hit) or p(FA) across post-signal delay.

p(Hit)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
Delay^2	**162**	**5**	**-90.66**	**0**	**0.62**	**50.52**
MeHg + Delay^2	162	6	-89.11	1.55	0.29	50.83
MeHg * Delay^2	162	8	-86.70	3.96	0.09	51.82
p(FA)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
Null	**162**	**3**	**-353.79**	**0**	**0.25**	**179.97**
MeHg	162	4	-353.46	0.33	0.21	180.86
Delay	162	4	-353.21	0.58	0.19	180.73
MeHg + Delay	162	5	-352.86	0.93	0.16	181.62

Note: Models are listed in order of fit to the data: *N* = number of data points; *K* = number of parameters estimated; *AICc* = corrected Akaike Information Criterion; *ΔAICc* = difference between the best (smallest) and the given AICc; w_i = probability the model, *i*, is the best model; *LL* = log likelihood

Table 2.2:
Model comparisons: Signal Detection At 0.3s Post-Signal Delay
Models compare impacts of MeHg and signal duration ("LED"). Linear and parabolic models were tested to describe shifts in p(Hit) or p(FA) across signal duration.

p(Hit)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
LED	**139**	**4**	**-99.38**	**0**	**0.37**	**53.84**
LED^2	139	5	-98.61	0.77	0.25	54.53
MeHg + LED	139	5	-97.56	1.82	0.15	54.00
p(FA)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
LED	**139**	**4**	**-178.28**	**0**	**0.37**	**93.29**
LED^2	139	5	-176.49	1.79	0.15	93.47
MeHg + LED	139	5	-176.13	2.15	0.13	93.29
Null	139	3	-176.08	2.20	0.12	91.13

Note: Models are listed in order of fit to the data: *N* = number of data points; *K* = number of parameters estimated; *AICc* = corrected Akaike Information Criterion; *ΔAICc* = difference between the best (smallest) and the given AICc; w_i = probability the model, *i*, is the best model; *LL* = log likelihood

Table 2.3:
Model comparisons: Distraction
Models compare impacts of MeHg and tactile distraction ("Distract). Linear and parabolic models were tested to describe shifts in p(Hit) or p(FA) across post-signal delay.

p(Hit)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
Delay^2 + Distract	**336**	**6**	**-237.47**	**0**	**0.44**	**124.86**
MeHg(Delay^2 + Distract)	336	10	-236.09	1.38	0.22	128.39
Delay^2 + Distract + MeHg	336	7	-235.52	1.95	0.17	124.93
Delay^2 * Distract	336	8	-235.35	2.12	0.15	125.90
p(FA)						
Model	*N*	*K*	*AICc*	*ΔAICc*	w_i	*LL*
Distract	**336**	**4**	**-546.94**	**0**	**0.36**	**277.53**
Delay + Distract	336	5	-545.30	1.64	0.16	277.74
Distract + MeHg	336	5	-544.90	2.04	0.13	277.54
Delay * Distract	336	6	-544.34	2.60	0.10	278.30
Distract * MeHg	336	6	-544.07	2.87	0.09	278.16

Note: Models are listed in order of fit to the data: *N* = number of data points; *K* = number of parameters estimated; *AICc* = corrected Akaike Information Criterion; *ΔAICc* = difference between the best (smallest) and the given AICc; w_i = probability the model, *i*, is the best model; *LL* = log likelihood

Figures

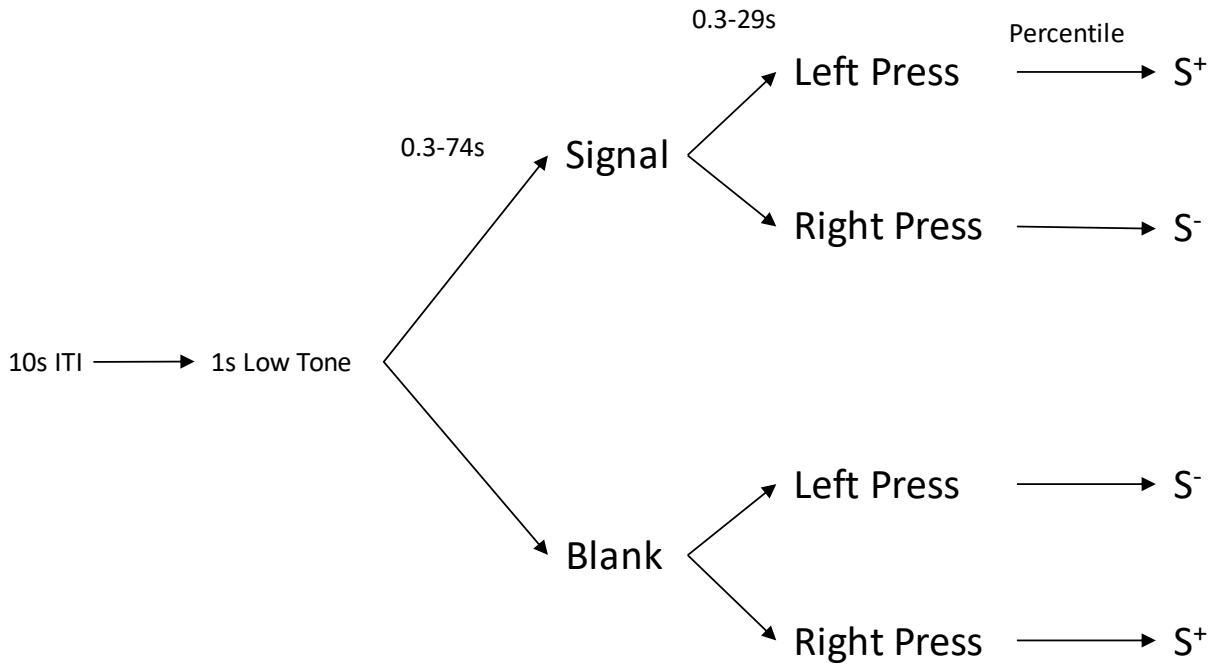


Figure 2.1: Schematic of the procedure used in Phase 2 of sustained attention, signal detection, and novel distraction. Sessions were cued by a 1s low tone followed by a pre-stimulus delay ranging between 0.3 and 74s. In "signal" the LED was illuminated after the pre-stimulus delay and in "blank" a pause occurred, yoked to the stimulus duration. A randomly selected post stimulus delay, between 0.3 and 29.3s, followed after either the LED stimulus or blank pause. Correct responses resulted in milk as S^+ while incorrect responses and omissions resulted in a 3s timeout as S^-. Pre- and post-stimulus delays were spaced logarithmically. Choice latency was under percentile. Figure modified from Rezvani et al., (2002).

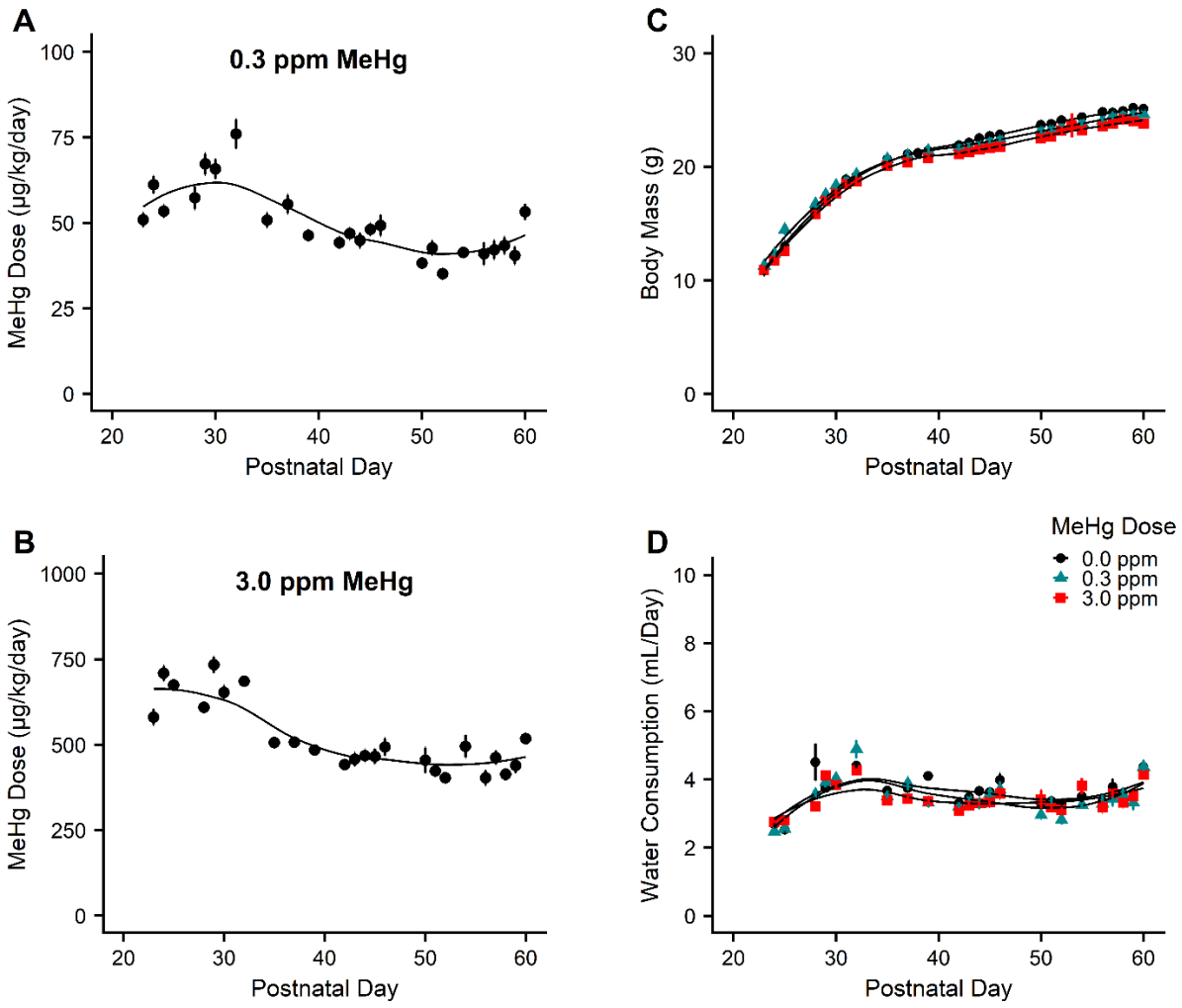


Figure 2.2: Body mass and water consumption throughout the adolescent exposure period (PND22-60). MeHg exposure for the 0.3 ppm (A) and 3 ppm (B) animals peaked early in adolescence before dropping off after PND 30. This change was tracked by the increase in body mass (C) and plateau in water consumption (D) seen for all animals after PND30. Colored markers between C and D are MeHg exposure groups for C and D. Values are means ± SEM.

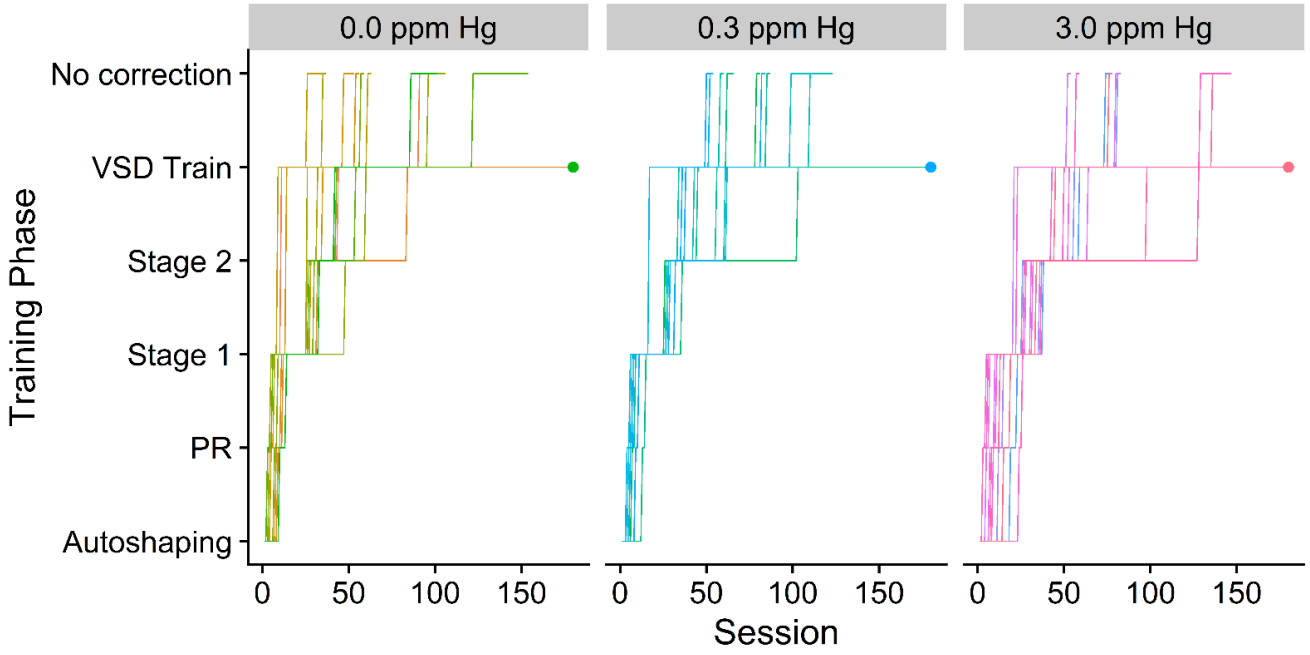


Figure 2.3: Progression through training from lever press training (autoshaping) to final entry into Phase 1 of sustained attention. Lines represent individual subjects. Each upward increment occurs on the session the subject progressed to the next training phase. The point at the end of VSD training denotes the time at which attrition occurred for those animals that failed to acquire the attention procedure. Data are grouped by MeHg exposure.

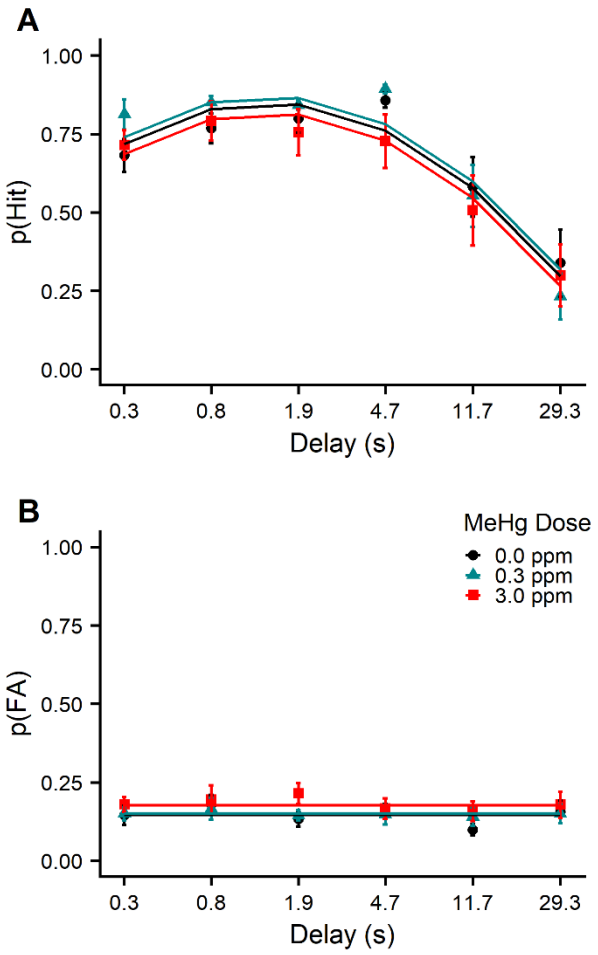


Figure 2.4: Accuracy across exposure groups for Phase 2 of sustained attention. (A) p(Hit) was affected by post-signal delay with a notable peak at the 1.9s delay, the training delay. (B) p(FA) was not affected by delay. Neither p(hit) nor p(FA) were affected by MeHg. Lines are based on model fits. Colored markers represent MeHg groups. Values are means ± SEM.

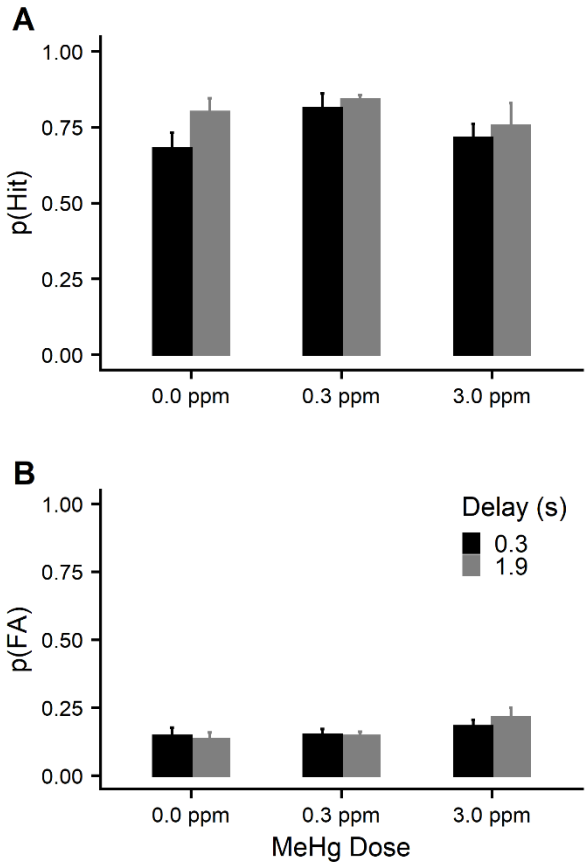


Figure 2.5: Accuracy at the 0.3s (black) and 1.9s (gray) delays during Phase 2 of sustained attention. (A) p(Hit) was higher at the 1.9s delay compared to the 0.3s delay while (B) p(FA) was relatively constant across both delays. Neither p(Hit) nor p(FA) were altered by MeHg at either delay. Values are means ± SEM.

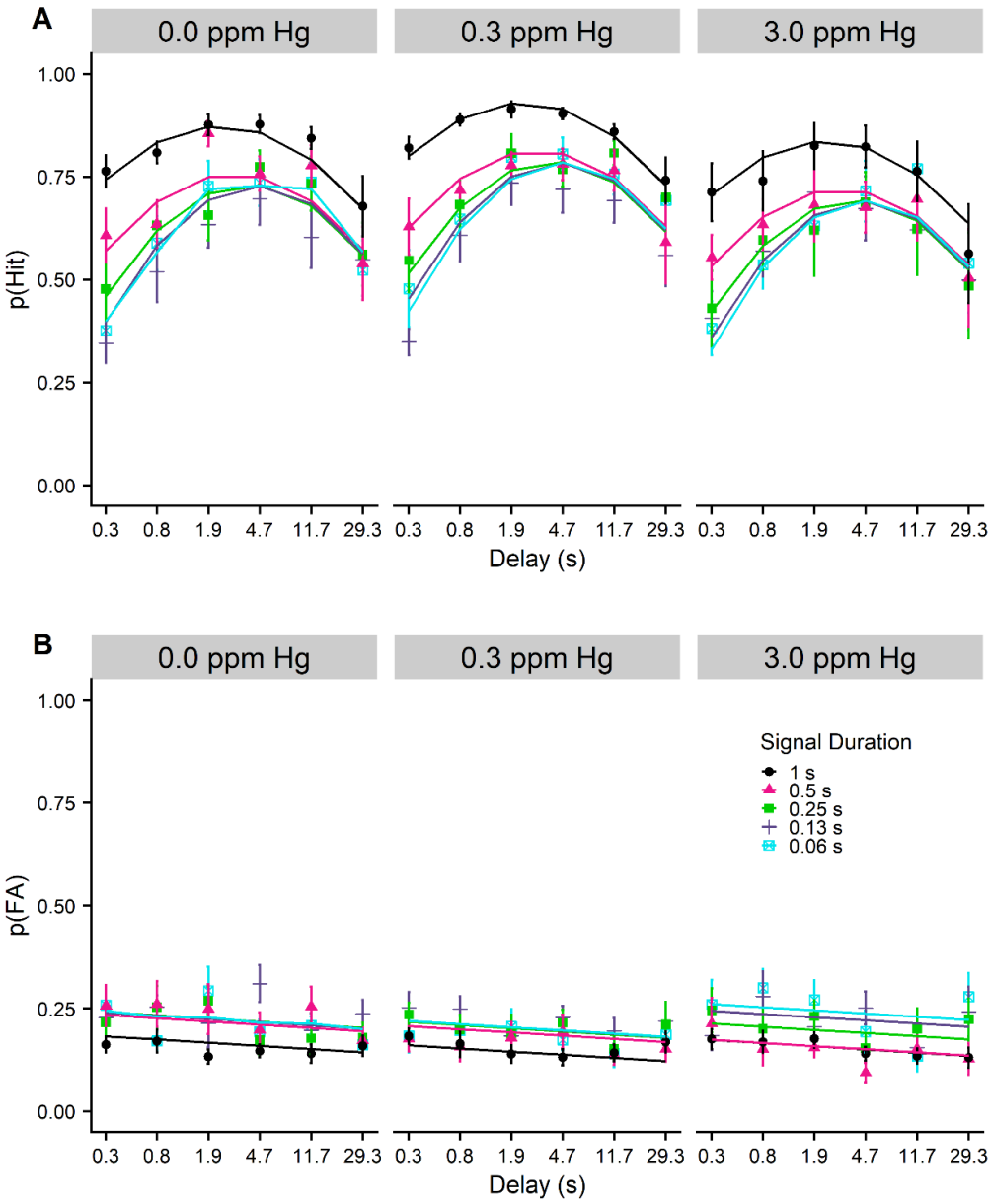


Figure 2.6: Detection of varying LED signal durations across post-signal delays. (A) Shorter LED durations reduced p(Hit) at the 0.3s post-signal delay, but not at longer post-signal delays. (B) Shorter LED durations yielded smaller p(FA). Lines are based on model fits. Values are means ± SEM.

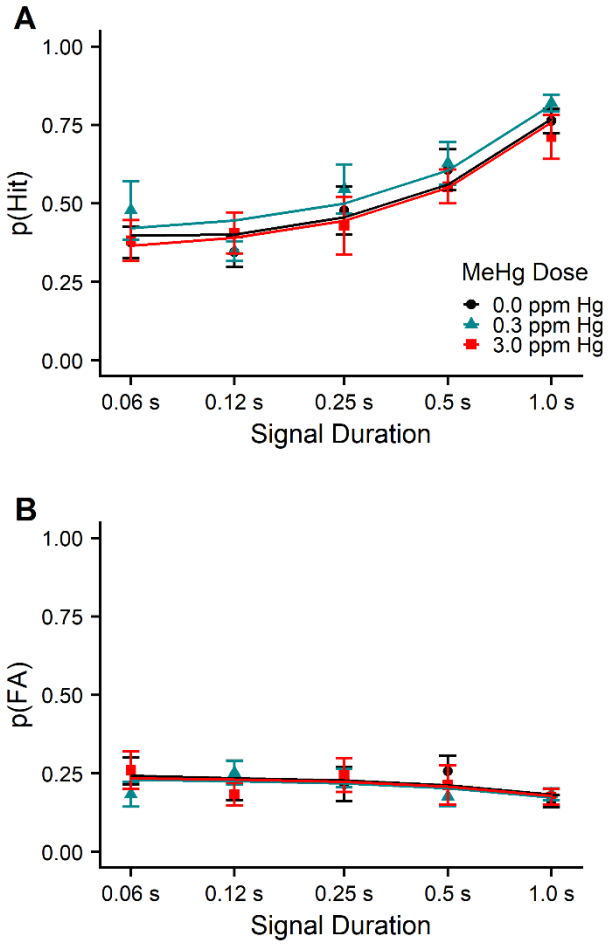


Figure 2.7: Accuracy across LED signal durations at the 0.3s delay. Accuracy for all groups decreased with decreasing signal duration until performance plateaued to chance at the 0.12s signal duration. (A) p(Hit) was highest at the training duration of 1s while (B) p(FA) was the smallest at this duration. Lines are based on model fits. Values are means ± SEM.

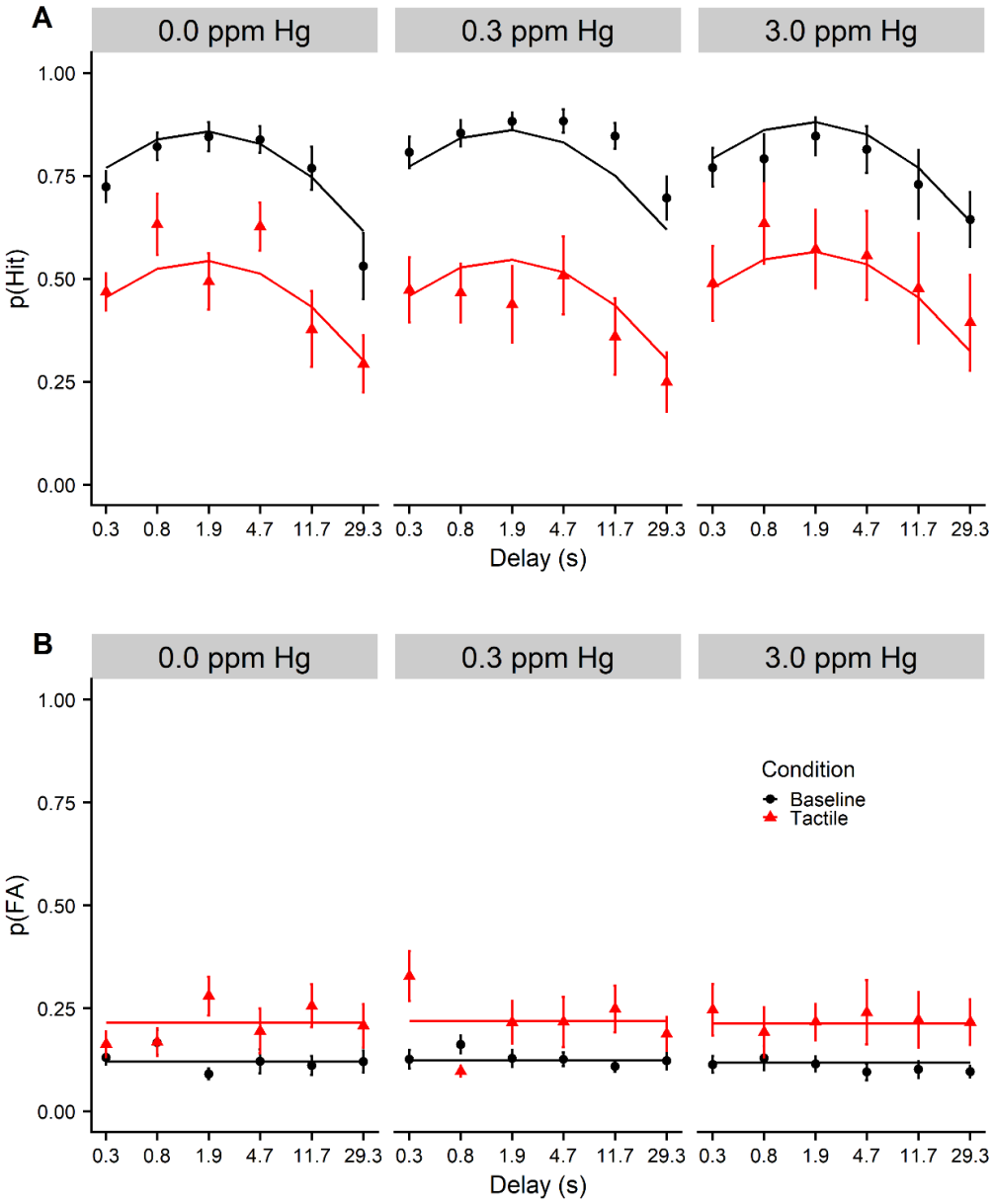


Figure 2.8: Accuracy during the distractor task. (A) The tactile distraction greatly impaired p(Hit) across all MeHg groups at all post-signal delays. (B) The distraction similarly impaired p(FA) for all animals at all delays. Lines are based on model fits. Color marker in (B) denotes conditions for (A) and (B). Values are means ± SEM.

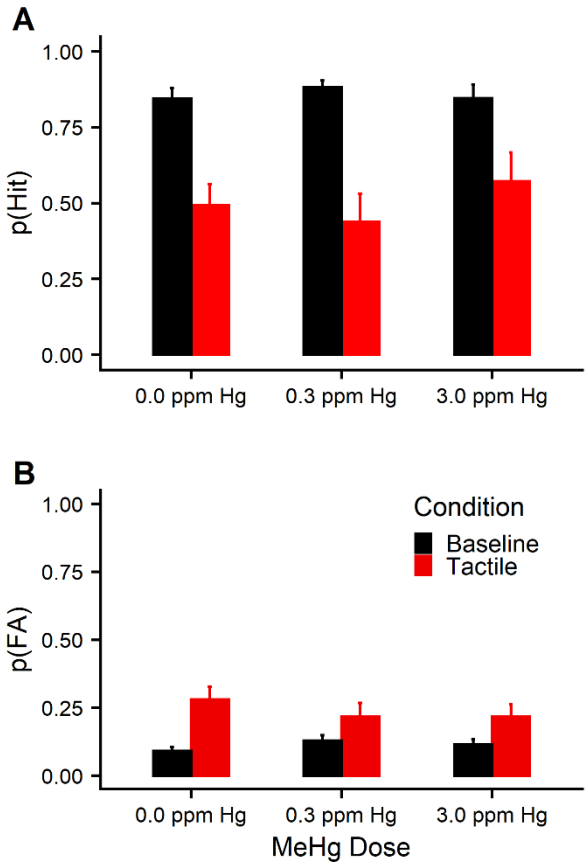


Figure 2.9: Accuracy at the 1.9s post-signal delay for baseline (black) and tactile distraction (red). Both p(Hit) (A) and p(FA) (B) were greatly impaired by the tactile distraction. This impairment was consistent across MeHg exposure groups. Values are means ± SEM.

Appendix 1: Supplementary Information

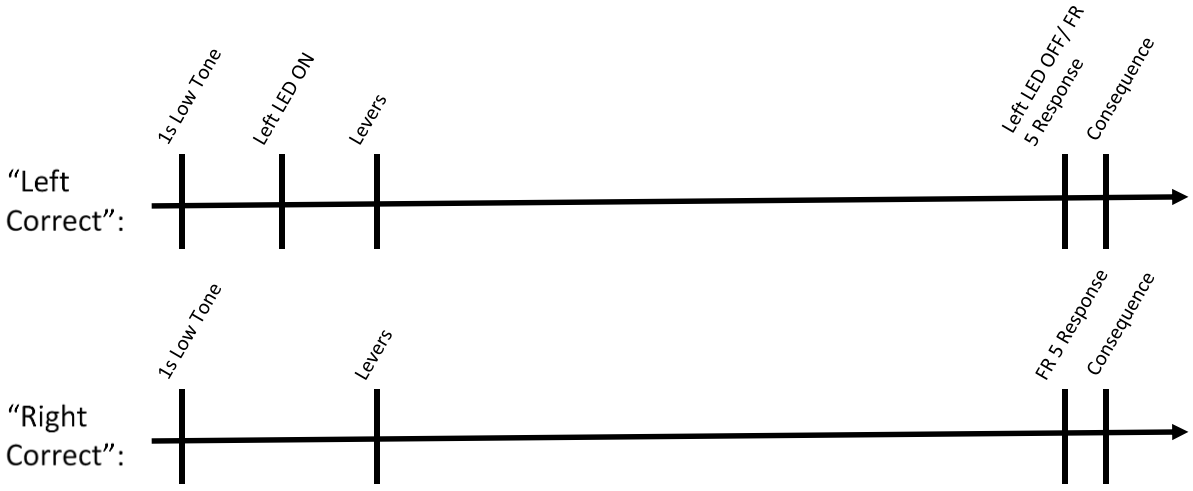
Visual Discrimination by Fading:

In Stage 1 of discrimination training the incorrect response alternative was faded in based on accuracy. Training began with either the left lever or right lever being designated as "correct", counterbalanced across animals. When left was correct the left LED was illuminated, when right was correct no LED was illuminated. During a trial, both levers were extended into the chamber with the "correct" lever remaining extended until five responses occurred on that lever and the "incorrect" lever retracting after 1s. Five consecutive responses (FR 5) on the "correct" lever resulted in reinforcement while a single response on the "incorrect" lever prior to retraction resulted in a time-out (TO). Each correct response increased the duration that the "incorrect" lever was extended into the chamber by 10% while each incorrect response decreased this duration by 10%. Failure to achieve the FR 5 within 30s of lever extension was counted as an omission and lead to a 3s TO. The "correct" lever switched when 80% accuracy was achieved. Once both levers were trained, the "correct" lever began to alternate within a session.

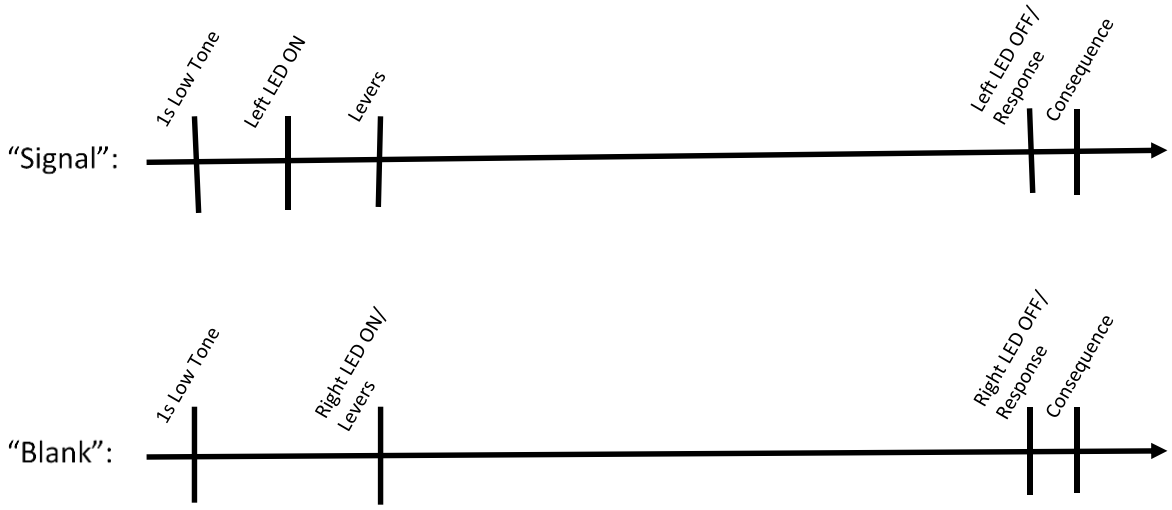
Inadequate accuracy and difficulties controlling choice latencies arose in Stage 1, prompting an alternative procedure wherein choice latency was controlled by a percentile schedule. This is designated as Stage 2. Seven mice achieved accurate signal discrimination following Stage 1 and advanced directly into signal detection training (see VSD, main text). Twenty-eight animals underwent Stage 2 in which illumination of the left LED signaled that a

left response would yield reinforcement and of the right LED signaled a right response would yield reinforcement. The left LED was always illuminated 0.3s prior to both levers extending while the right LED occurred simultaneously with the levers. Incorrect responses, right press when the left LED was illuminated or vice versa, and omissions, not responding within 30s, resulted in a TO followed by a correction procedure in which the same trial type was repeated. Trials progressed as follows: 10s inter-trial interval (ITI) → 1s low tone → 0.6s pause (left LED illuminates after 0.3s when left response is correct)→ levers extend (right LED is illuminated if right response is correct) →response→consequence. The duration that the right LED in "blank" trials was illuminated decreased by 1% with each correct response until the duration was less than 0.1s wherein it was terminated. Criterion was three consecutive sessions with over 75% accuracy after termination of the right LED in "blank" trials.

Training during Stage 2 of discrimination occurred under a percentile schedule for choice latency wherein a correct response was not reinforced unless the choice latency, the time between lever extending and a response, was shorter than the 75^{th} percentile of the previous 10 latencies. This allowed for control of short response latencies based on an animal's performance and maintained a consistent 75% reinforcement rate during behavioral sessions. Choice latencies that were too long in correct trials were not treated as incorrect responses but resulted in a return to the ITI without reinforcement.

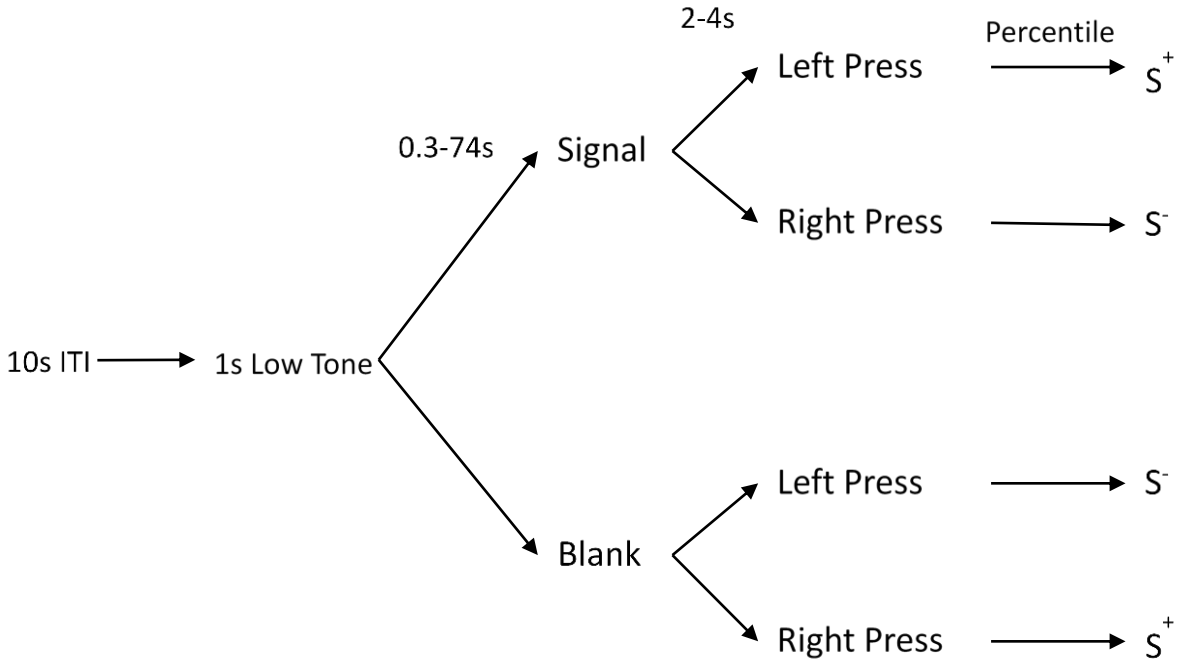


Trial Progression during Stage 1 of Discrimination Training. When the left lever was designated as "correct", the left LED was illuminated and five responses on the left lever resulted in reinforcement and an increase in the duration of time the right lever was inserted. A single response on the right lever when left is "correct" resulted in a 3s timeout and shortening of the duration of time the right lever was inserted. The reverse was true when the right lever was designated as "correct", but no LED was illuminated.



Trial progression during Stage 2 of Discrimination Training. During signal trials, the left LED was illuminated and a response on the left lever resulted in reinforcement under a percentile schedule for the choice latency. A response on the right lever resulted in a 3s timeout and correction procedure. During blank trials, the right LED was illuminated and a response on the right lever resulted in reinforcement under a percentile schedule. Correct responses in signal and blank trials resulted in a 1% decrease in the duration the right LED was illuminated.

Appendix 2: Phase 1 Sustained Attention



Schematic of the procedure used in Phase 1 of sustained attention. Sessions were cued by a 1s low tone followed by a randomly selected pre-stimulus delay (0.3-74s, log spaced). In "signal" trials the left LED was illuminated for 1s following termination of the pre-stimulus delay. In "blank" there was a 1s pause but no stimulus light was presented. A randomly selected post-stimulus delay (2-4s) was presented following either the 1s LED or blank pause. Correct responses resulted in milk as S^+. Incorrect responses and omissions resulted in a 3s timeout as S^-. Choice latency was under percentile. Figure modified from Rezvani et al., (2002).

Appendix 3: List of Models Comparisons

Models for Table 1: Phase 2 Sustained Attention

No.	Model Description
1	Null
2	Delay
3	MeHg
4	Delay + MeHg
5	Delay * MeHg
6	$Delay^2$
7	$Delay^2$ + MeHg
8	$Delay^2$ * MeHg

Models for Table 2: Signal Detection at 0.3s Delay

No.	Model Description
1	Null
2	LED
3	MeHg
4	LED + MeHg
5	LED * MeHg
6	LED^2
7	LED^2 + MeHg
8	LED^2 * MeHg

Models for Table 3: Distraction

No.	Model Description
1	Null
2	Delay
3	MeHg
4	Condition
5	Delay + MeHg
6	Delay * MeHg
7	Condition + MeHg
8	Condition * MeHg
9	Delay + Condition
10	Delay * Condition
11	MeHg (Delay + Condition)
12	MeHg + Delay + Condition
13	MeHg * Delay * Condition
14	$Delay^2$
15	$Delay^2$ + MeHg
16	$Delay^2$ * MeHg
17	$Delay^2$ + Condition
18	$Delay^2$ * Condition
19	$Delay^2$ + Condition + MeHg
20	$Delay^2$ * Condition * MeHg
21	MeHg ($Delay^2$ + Condition)