Adolescent Methylmercury Exposure: Behavioral Mechanisms and Protective Effects of Sodium Butyrate and Environmental Enrichment

by

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Abstract

Whether exposure to methylmercury (MeHg), an environmental neurotoxicant, during adolescence alters the brain and behavior is only beginning to be explored. The continued maturation of mechanisms that control neuronal gene expression, such as DNA methylation and histone modifications, may predispose the adolescent brain and behavior to be particularly susceptible to MeHg exposure. In Experiment 1, male C57BL6/n mice were exposed to 0, 0.3, and 3.0 ppm MeHg (n = 12 each) via drinking water from postnatal days 21 to 60 (murine adolescence). As adults, mice were trained to lever press under an ascending series of fixed-ratio schedules of milk reinforcement. Adolescent MeHg exposure dose-dependently decreased estimates of response-reinforcer coupling and minimum response time relative to controls. Further, adolescent MeHg increased maximum response rates in exposed mice relative to controls. In Experiment 2, the protective effects of sodium butyrate (NaB; a histone deacetylase inhibitor) and environmental enrichment on MeHg-induced behavioral impairment were examined. Male C57BL6/n mice were assigned to control, NaB, or environmental enrichment and within each of these treatment conditions were given either 0 or 3.0 ppm MeHg. Adolescent MeHg exposure again decreased estimates of response-reinforcer coupling but did not significantly alter minimum response time. Chronic NaB also decreased response-reinforcer coupling. These data suggest that behavioral mechanisms of adolescent MeHg exposure may be related to motoric capacity and the impact of reinforcement on prior responses.

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Chapter 1

Literature Review

Adolescence: a vulnerable period of neurobehavioral development

Adolescence is a period of dramatic neurobiological and behavioral maturation and is implicated in the etiology of a variety of psychiatric disorders (Chambers, Taylor, & Potenza, 2003; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). In humans, adolescence is considered to range between 12 and 20 years of age based on brain development, though it is widely regarded that the beginning and end points of adolescence are graded (Spear, 2000, 2007b). During this period, the number of synapses peaks and decreases with age in a region-specific manner (Huttenlocher & Dabholkar, 1997; Lister et al., 2013). The neural fluctuations that occur across adolescence have been highly conserved phylogenetically in that they appear in many mammalian species, including humans, monkeys, and rodents (Spear, 2007b). In rodents, the adolescent period ranges between postnatal day (PND) 21 and 60 (Laviola, Macrì, Morley-Fletcher, & Adriani, 2003; Spear, 2000) and is marked by both an overproduction of synapses followed by region-specific pruning (De Felipe, Marco, Fairén, & Jones, 1997; Lister et al., 2013). Importantly, the brain circuitry that supports choice and motivation, primarily dopamine (DA) signaling, completes its development during adolescence.

The DA neurotransmitter system undergoes maturation throughout adolescence. The density of postsynaptic DA receptors in the rat prefrontal cortex (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Tarazi & Baldessarini, 2000) and striatum (Andersen, Rutstein,

Benzo, Hostetter, & Teicher, 1997; Teicher, Andersen, & Hostetter, 1995) peaks around PND 40 and decreases into adulthood. Expression of DA transporter, a presynaptic protein that removes DA from the synapse, in the nucleus accumbens and striatum also increases dramatically throughout adolescence, reaching stable levels at approximately PND 60 and steadily decreasing into adulthood (Purkerson-Parker, McDaniel, & Moser, 2001; Tarazi, Tomasini, & Baldessarini, 1998). These adolescent-onset neurobiological changes co-occur with a variety of maladaptive behavior.

The adolescent period is marked by a pattern of poor choices and impaired executive functioning. Human adolescence is associated with increased risky (Chambers & Potenza, 2003) and impulsive choices (Green, Fry, & Myerson, 1994) and a higher likelihood of using and abusing prescription and illicit drugs relative to other age groups (Johnston, O'Malley, Miech, Bachman, & Schlenberg, 2014). The rodent adolescent period is also a time of enhanced maladaptive behavior, including increased delay discounting (Pinkston & Lamb, 2011) and reduced cognitive flexibility in reversal-learning and extradimensional-shift procedures (Newman & McGaughy, 2011) that are ameliorated with age. Because the adolescent brain and behavior are in a state of flux, many regard this period as being highly vulnerable to neurotoxic substances (Chambers et al., 2003; Spear, 2007a) that may give rise to enduring psychological dysfunction later in life. Thus, understanding how neurotoxic perturbations during adolescence affect psychological functioning in adulthood is important for the creation of therapeutic interventions and treatments (Andersen, 2003). One toxicant that is ubiquitous in the environment, disrupts brain development, and results in long-lasting neurobehavioral alterations is methylmercury.

Methylmercury and the developing brain

Human exposure to methylmercury (MeHg), an environmental neurotoxicant found in fish, is a public-health concern worldwide. The developing brain is especially susceptible to MeHg (Rice & Barone, 2000), with gestational MeHg exposure producing profound cognitive and behavioral impairment in both humans (Axelrad, Bellinger, Ryan, & Woodruff, 2007; Harada, 1978; National Research Council, 2000) and nonhumans (Newland, Reed, & Rasmussen, 2015). In a rat model, gestational MeHg exposure slows the acquisition of choice (Newland, Reile, & Langston, 2004) and increases perseverative errors following spatial- and visual-discrimination reversals (Paletz, Day, Craig-Schmidt, & Newland, 2007; Reed, Paletz, & Newland, 2006). Gestational MeHg exposure also increases response rates under fixed- and progressive-ratio schedules of reinforcement (Paletz, Craig-Schmidt, & Newland, 2006; Reed, Banna, Donlin, & Newland, 2008), suggesting MeHg enhances the impact of reinforcement on operant responding. Indeed, the hypothesis that gestational MeHg exposure enhances reinforcer efficacy (Newland et al., 2015) is consistent with a large body of literature demonstrating MeHg-induced alterations in DAergic signaling.

The DA neurotransmitter system is particularly vulnerable to MeHg exposure both before and after birth. MeHg increases the synthesis of DA (Tiernan, Edwin, Goudreau, Atchison, & Lookingland, 2013) and shunts it along an alternative, cytotoxic metabolic pathway *in vitro* (Tiernan et al., 2015). MeHg both increases DA efflux from the presynaptic neuron (Dreiem, Shan, Okoniewski, Sanchez-Morrissey, & Seegal, 2009; Faro, Do Nascimento, Alfonso, & Durán, 1998; Faro, Do Nascimento, San José, Alfonso, & Durán, 2000; Kalisch & Racz, 1996; Tiernan et al., 2015) and inhibits the DA transporter *in vitro* and *in vivo* (Dreiem et al., 2009; Faro, do Nascimento, Alfonso, & Durán, 2002). These MeHg-induced disruptions in DA

signaling are consistent with the evidence of behavioral impairment following gestational MeHg exposure and are irreversible. Indeed, adult rats exposed to MeHg *in utero* are more sensitive to the DA transporter blockers *d*-amphetamine (Rasmussen & Newland, 2001) and cocaine relative to controls (Reed & Newland, 2009) long after exposure has ended, suggesting irreversible effects of MeHg on the regulation of synaptic DA. It is well-established that gestational MeHg exposure can have long-lasting neurobehavioral effects that extend well into aging and senescence (Newland, Reed, & Rasmussen, 2015). Exploring whether other developmental periods are vulnerable to MeHg exposure, such as adolescence, is critical for public health.

Adolescents may be particularly at risk of MeHg exposure for a number of reasons. First, relative to other age groups, human adolescents consume more fish, such as tuna (Nielsen, Aoki, Kit, & Ogden, 2015), which can have high mercury concentrations (Tran, Barraj, Smith, Javier, & Burke, 2004; Wang et al., 2013). Second, adolescents are encouraged to consume even more fish for health reasons (Gidding et al., 2005). Finally, the consumption of high-mercury seafood is related to blood-mercury levels in adolescents (Nielsen et al., 2015). These reports demonstrate that adolescents consume food that contains mercury and consume more of these foods relative to other age groups, suggesting adolescents may be particularly at risk of MeHg exposure. However, the long-term behavioral consequences of adolescent MeHg exposure have not been explored until recently.

In a series of experiments, our laboratory has demonstrated that the adolescent brain and behavior is susceptible to MeHg exposure. We have developed an exposure regimen in which 21-day-old mice are given water bottles containing 0.3 or 3.0 ppm MeHg until 60 days of age (Boomhower & Newland, 2016). This exposure regimen encompasses the murine adolescent period and produces brain-mercury levels associated with behavioral impairment in past work

with gestational exposures (Newland, Reed, LeBlanc, & Donlin, 2006; Newland & Reile, 1999). Behavioral testing begins on PND 90 at which time mice have now matured to adulthood and brain mercury has returned to trace levels (Boomhower & Newland, 2016). Adolescent MeHg exposure delivered in this manner increases the number of trials needed to transition through a spatial-discrimination reversal and spatial-to-visual discrimination (extradimensional shift) (Boomhower & Newland, 2017). The rate of the transition in the extradimensional shift is also significantly reduced following adolescent MeHg exposure (Boomhower & Newland, 2017). Further, adolescent MeHg exposure dose-dependently reduces choice for a larger reinforcer relative to a smaller reinforcer (Boomhower & Newland, 2016). These experiments suggest that MeHg exposure during adolescence can produce long-lasting behavioral effects related to choice in adulthood. The underlying behavioral mechanisms that permit MeHg-induced impairments in choice remain unclear though. For example, adolescent MeHg exposure may alter reinforcer efficacy as with gestational exposures (Newland et al., 2015), or it may change some other aspect of reinforcement or behavior. A detailed analysis of potential behavioral mechanisms altered by adolescent MeHg exposure is necessary for a better understanding of MeHg's behavioral effects and how to prevent or treat them.

Modeling operant responding with Mathematical Principles of Reinforcement

One method that could parse the behavioral effects of adolescent MeHg exposure is the use of a theoretically-driven model called Mathematical Principles of Reinforcement (MPR) (Killeen, 1994; Killeen & Sitomer, 2003). MPR posits that three fundamental processes govern operant (voluntary) behavior. First, specific activation of behavior is increased by reinforcer presentation, and this can be reflected in the number of responses that are supported by a reinforcer. Second, motor capabilities and the physics of response devices limit the maximum

rate of responding. Finally, a contingency linking reinforcement and the behavior that precedes it, called coupling, causes the reinforcer to increase both target and non-target responses. The strength of coupling decreases as a function of events or time between a response and the delivery of a reinforcer. For fixed-ratio schedules of reinforcement, MPR predicts response rate (*b*, responses/sec) as a function of fixed ratio (*n*) using Eq. 1 (Killeen & Sitomer, 2003):

$$b = \frac{1 - e^{-\lambda\delta n}}{\delta} - \frac{n}{\delta a} \quad (1)$$

where *a* represents specific activation and is a measure of reinforcer value, δ is the minimum time to complete a target response, and λ is the rate at which the coupling of reinforcement to a response (target or non-target) decreases as a function of events or time preceding the reinforcer.

Changes in the parameters of MPR as a result of adolescent MeHg exposure would reveal specific behavioral mechanisms of MeHg's effects. Based on our prior work with an impulsive-choice procedure (Boomhower & Newland, 2016), adolescent MeHg exposure may alter the coupling rate (λ), which is associated with impulsivity in mice (Pope, Boomhower, Hutsell, Teixeira, & Newland, 2016). Further, adolescent MeHg may alter reinforcer value (*a*), as our past work has shown more trials are required to transition through a spatial discrimination reversal and extradimensional shift following adolescent MeHg exposure (Boomhower & Newland, 2017). Parsing the motivational (a), motoric (δ), and memorial (λ) effects of early-life MeHg exposure would greatly contribute to our understanding of the psychological impact of MeHg. Nevertheless, the mechanism through which MeHg exposure early in life gives rise to delayed neurobehavioral damage remains largely unknown. One way adolescent MeHg exposure could affect behavior later in life is by altering gene expression in brain areas that underlie choice and motivation.

Neuroepigenetic modifications as a mechanism of adolescent methylmercury toxicity

Abnormal modifications to the epigenome as a result of neurotoxicant exposure can produce long-lasting neurobehavioral dysfunction. DNA winds around and interacts with proteins called histones in the cell nucleus. Regulation of neuronal genes is dynamically controlled in part by biochemical modifications to histone tails, which can promote or prevent gene expression (Day & Sweatt, 2011, 2012). For example, histone acetylation plays a crucial role in neuronal development, synaptic plasticity, learning, and memory (Day & Sweatt, 2012; Levenson et al., 2004; Miller, Campbell, & Sweatt, 2008). Acetylated sites of particular histone tails "relaxes" the bond between DNA and the histone complex, permitting other proteins access to DNA and allowing genes to be expressed. Conversely, removal of acetyl groups by histone deacetylases causes DNA to bind tightly to histones, preventing gene expression. The continued incorporation of histone proteins into chromatin of the frontal cortex follows a strict developmental time course with histone accumulation stabilizing during mid-adolescence in both humans and mice (Maze et al., 2015). Thus, the adolescent period may be especially sensitive to xenobiotic-induced histone modifications that suppress neuronal gene expression (e.g., histone deacetylation).

Regulation of neuronal genes is controlled also by direct alterations to DNA bases via methylation. DNA methylation describes the covalent attachment of a methyl group to a cytosine base (Day & Sweatt, 2010). Typically, DNA methylation occurs at cytosines adjacent to guanines (CpG dinucleotides), and when many CpG dinucleotides are methylated in the transcriptional start site of a neuronal gene, expression of this gene is suppressed (Day & Sweatt, 2011). DNA methylation in the frontal cortex increases across the lifespan (Hernandez et al., 2011; Jaffe et al., 2016; Lister et al., 2013; Numata et al., 2012), with the greatest increase in

DNA methylation occurring in adolescence for both humans and mice (Lister et al., 2013). Because the adolescent period marks a time of continued maturation of chromatin both in terms of histone modifications (Maze et al., 2015) and DNA methylation (Lister et al., 2013), enhancing supressive neuroepigenetic marks during this time can have long-lasting consequences on behavior and aging (e.g., Marioni et al., 2015). One way neuroepigenetic dysregulation could occur is by exposure to MeHg early in development.

MeHg exposure has been linked to distorted gene expression and epigenetic dysregulation in some model systems, including cultured cells, nonhuman animals, and humans (Robinson et al., 2011). MeHg reduces DNA methylation at genes underlying cell death in neural stem cells (Bose, Onishchenko, Edoff, Lang, & Ceccatelli, 2012). Altered DNA methylation has also been noted in cerebral tissue of juvenile mink (Basu et al., 2013) and in human cord blood (Bakulski et al., 2015; Cardenas et al., 2017) following MeHg exposure. Genes that underlie synaptic plasticity and learning, such as the brain-derived neurotrophic factor (*Bdnf*) gene, appear to be susceptible to early-life MeHg exposure as well. For example, gestational MeHg exposure is correlated with reduced *Bdnf* expression in the adult rat hippocampus, with a concurrent decrease in histone H3 acetylation and increase in DNA methylation at the *Bdnf* promoter (Onishchenko, Karpova, Sabri, Castrén, & Ceccatelli, 2008). Thus, developmental MeHg exposure may produce its neurobehavioral effects in part by increasing neuroepigenetic marks that suppress gene expression. One way to determine whether epigenetic dysregulation in the brain may be a mechanism of adolescent MeHg exposure is through treatments known to affect the epigenome. Sodium butyrate, a histone deactylase (HDAC) inhibitor, and environmental enrichment both impart beneficial neuroepigenetic profiles that could prevent or reverse MeHg-induced epigenetic dysregulation.

Protective effects of sodium butyrate and environmental enrichment

Though named for their inhibitory actions on histone deacetylases (HDACs), sodium butyrate (NaB) and other HDAC inhibitors prevent repressive neuroepigenetic markers in multiple ways. HDAC inhibitors not only indirectly increase histone acetylation, but also reverse DNA methylation at CpG islands via upstream effects on DNA methyltransferases (Sarkar et al., 2011; Xu, Parmigiani, & Marks, 2007). Both acute and chronic administration of NaB promotes the differentiation of neural stem cells (Balasubramaniyan et al., 2006) and production of neurotrophic factors *in vivo* (Varela et al., 2015). As NaB and other HDAC inhibitors enhance synaptic plasticity and memory formation (Crosio, Heitz, Allis, Borrelli, & Sassone-Corsi, 2003; Day & Sweatt, 2012; Levenson et al., 2004; Vecsey et al., 2007), HDAC inhibitors possess substantial promise as treatments for cognitive impairment (Day & Sweatt, 2012; Gräff & Tsai, 2013). Indeed, NaB rescues neurobehavioral deficits in animal models of both normal (Peleg et al., 2010; Reolon et al., 2011) and diseased aging (Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Kilgore et al., 2010; Rane et al., 2012) as well as following neurotoxicant exposure (Sharma & Sharma, 2013; Song, Kanthasamy, Anantharam, Sun, & Kanthasamy, 2010). Whether the behavioral effects of exposure to other neurotoxicants, such as MeHg, are reversible by chronic treatment with NaB are unknown. Another potentially protective treatment against MeHg's effects and one that confers both a beneficial neuroepigenetic and neuroanatomical profile is environmental enrichment.

Providing laboratory animals with an enriched environment enhances brain function, learning, and memory relative to standard or impoverished housing conditions (Dong & Greenough, 2004; Nithianantharajah & Hannan, 2006). Environmental enrichment typically consists of group housing animals and providing them access to running apparatuses (e.g., a

wheel), tunnels and burrows, elevated platforms, and toys (Nithianantharajah & Hannan, 2006). Under these conditions, animals display enhanced dendritic branching, production of neurotrophic factors, synaptogenesis, and synaptic plasticity relative to standard housing (Baroncelli et al., 2010; Branchi, Karpova, D'Andrea, Castrén, & Alleva, 2011). Environmental enrichment-induced alterations in neuroanatomy likely arise in part from changes in neuroepigenetic marks that promote gene expression, such as enhanced histone acetylation and reduced DNA methylation (Arai & Feig, 2011; Branchi et al., 2011; Mychasiuk et al., 2012). These beneficial neurobiological effects manifest in enhanced learning and memory in animal models of diseased aging (Fischer et al., 2007) and developmental disorders (Dong & Greenough, 2004) following environmental enrichment. In regard to drug and neurotoxicant exposure, environmental enrichment during adolescence rescues both behavioral and neuroepigenetic abnormalities. For example, exposure to enriched environments during adolescence rescues lead-induced decreases in *Bdnf* expression and Morris water maze performance (Guilarte, Toscano, McGlothan, & Weaver, 2003) as well as prevents phencyclidine-induced deficits in HDAC regulation, social behavior, and memory in rodents (Koseki et al., 2012). Whether environmental enrichment reverses the behavioral effects of exposure to other neurotoxicants, such as MeHg, is unknown.

The present study

The current experiments were designed to further describe the behavioral effects of adolescent MeHg exposure using Mathematical Principles of Reinforcement and determine the degree to which sodium butyrate and environmental enrichment prevented these effects. In Experiment 1, the effects of adolescent MeHg exposure on MPR's parameters—namely, reinforcer value (a), motoric capacity (δ), and the rate at which coupling decreases as a function

of time (λ)—will be assessed under a multiple fixed-ratio procedure. Based on prior work in our lab (Boomhower & Newland, 2016, 2017), adolescent MeHg exposure most likely will alter reinforcer value and saturation rate. In Experiment 2, the protective effects of sodium butyrate and environmental enrichment on the behavioral impairments caused by adolescent MeHg exposure will be assessed. As some evidence suggests MeHg has neuroepigenetic effects (Onishchenko et al., 2008), sodium butyrate and environmental enrichment should prevent any neuroepigenetic-mediated behavioral effects caused by adolescent MeHg exposure.

References

Andersen, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews*, *27*(1–2), 3–18. http://doi.org/10.1016/S0149-7634(03)00005-8

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., & Teicher, M. H. (1997). Sex differences in dopamine receptor overproduction and elimination. *Neuroreport*, *8*(6), 1495–1498. http://doi.org/10.1097/00001756-199704140-00034

Andersen, S. L., Thompson, A., Rutstein, M., Hostetter, J., & Teicher, M. (2000). Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse*, *37*(2), 167–169. http://doi.org/10.1002/1098-2396(200008)37:2<167::AID-SYN11>3.0.CO;2-B

Arai, J. A., & Feig, L. A. (2011). Long-lasting and transgenerational effects of an environmental enrichment on memory formation. *Brain Research Bulletin*, *85*(1–2), 30–35. http://doi.org/10.1016/j.brainresbull.2010.11.003

Axelrad, D. A., Bellinger, D. C., Ryan, L. M., & Woodruff, T. J. (2007). Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. *Environmental Health Perspectives*, *115*, 609–15.

Bakulski, K. M., Lee, H. J., Feinberg, J. I., Wells, E. M., Brown, S., Herbstman, J. B., … Daniele Fallin, M. (2015). Prenatal mercury concentration is associated with changes in DNA methylation at TCEANC2 in newborns. *International Journal of Epidemiology*, *44*(4), 1249–1262. http://doi.org/10.1093/ije/dyv032

Balasubramaniyan, V., Boddeke, E., Bakels, R., K??st, B., Kooistra, S., Veneman, A., & Copray, S. (2006). Effects of histone deacetylation inhibition on neuronal differentiation of

embryonic mouse neural stem cells. *Neuroscience*, *143*(4), 939–951. http://doi.org/10.1016/j.neuroscience.2006.08.082

Baroncelli, L., Braschi, C., Spolidoro, M., Begenisic, T., Sale, a, & Maffei, L. (2010). Nurturing brain plasticity: impact of environmental enrichment. *Cell Death and Differentiation*, *17*(7), 1092–1103. http://doi.org/10.1038/cdd.2009.193

Basu, N., Head, J., Nam, D. H., Pilsner, J. R., Carvan, M. J., Chan, H. M., … Scheuhammer, A. M. (2013). Effects of methylmercury on epigenetic markers in three model species: Mink, chicken and yellow perch. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *157*(3), 322–327. http://doi.org/10.1016/j.cbpc.2013.02.004

Boomhower, S. R., & Newland, M. C. (2016). Adolescent methylmercury exposure affects choice and delay discounting in mice. *Neurotoxicology*, *57*, 136–144.

Boomhower, S. R., & Newland, M. C. (2017). Effects of adolescent exposure to methylmercury and d-amphetamine on reversal learning and an extradimensional shift in male mice. *Experimental and Clinical Psychopharmacology*, *25*, 64–73.

Bose, R., Onishchenko, N., Edoff, K., Lang, A. M. J., & Ceccatelli, S. (2012). Inherited effects of low-dose exposure to methylmercury in neural stem cells. *Toxicological Sciences*, *130*(2), 383–390. http://doi.org/10.1093/toxsci/kfs257

Branchi, I., Karpova, N. N., D'Andrea, I., Castrén, E., & Alleva, E. (2011). Epigenetic modifications induced by early enrichment are associated with changes in timing of induction of BDNF expression. *Neuroscience Letters*, *495*(3), 168–172. http://doi.org/10.1016/j.neulet.2011.03.038

Cardenas, A., Rifas-Shiman, S., Godderis, L., Duca, R., Navas-Acien, A., Litonjua, A., … Baccarelli, A. (2017). Prenatal Exposure to Mercury: Associations with Global DNA

Methylation and Hydroxymethylation in Cord Blood and in Childhood. *Environmental Health Perspectives*, 1–10.

Chambers, R. A., & Potenza, M. N. (2003). Neurodevelopment, impulsivity, and adolescent gambling. *Journal of Gambling Studies*, *19*, 53–84.

Chambers, R. A., Taylor, J. R., & Potenza, M. N. (2003). Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *The American Journal of Psychiatriy*, *160*(6), 1041–1052.

Costello, E. J., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry*, *60*(8), 837–844. http://doi.org/10.1001/archpsyc.60.8.837

Crosio, C., Heitz, E., Allis, C. D., Borrelli, E., & Sassone-Corsi, P. (2003). Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. *Journal of Cell Science*, *116*(Pt 24), 4905–4914. http://doi.org/10.1242/jcs.00804

Day, J. J., & Sweatt, J. D. (2010). DNA methylation and memory formation. *Nature Neuroscience*, *13*(11), 1319–23.

Day, J. J., & Sweatt, J. D. (2011). Epigenetic mechanisms in cognition. *Neuron*, *70*(5), 813–29.

Day, J. J., & Sweatt, J. D. (2012). Epigenetic treatments for cognitive impairments. *Neuropsychopharmacology*, *37*(1), 247–260. http://doi.org/10.1038/npp.2011.85

De Felipe, J., Marco, P., Fairén, A., & Jones, E. G. (1997). Inhibitory synaptogenesis in mouse somatosensory cortex. *Cerebral Cortex*, *7*(7), 619–634. http://doi.org/10.1093/cercor/7.7.619

Dong, W. K., & Greenough, W. T. (2004). Plasticity of nonneuronal brain tissue: Roles in

developmental disorders. *Mental Retardation and Developmental Disabilities Research Reviews*, *10*(2), 85–90. http://doi.org/10.1002/mrdd.20016

Dreiem, A., Shan, M., Okoniewski, R. J., Sanchez-Morrissey, S., & Seegal, R. F. (2009). Methylmercury inhibits dopaminergic function in rat pup synaptosomes in an age-dependent manner. *Neurotoxicology and Teratology*, *31*(5), 312–317.

Faro, L., do Nascimento, J., Alfonso, M., & Durán, R. (2002). Mechanism of action of methylmercury on in vivo striatal dopamine release: Possible involvement of dopamine transporter. *Neurochemistry International*, *40*(5), 455–465.

Faro, L., Do Nascimento, J., Alfonso, M., & Durán, R. (1998). Acute administration of methylmercury changes in vivo dopamine release from rat striatum. *Bulletin of Environmental Contamination and Toxicology*, *60*(4), 632–638.

Faro, L., Do Nascimento, J. L. M., San José, J. M., Alfonso, M., & Durán, R. (2000). Intrastriatal administration of methylmercury increases in vivo dopamine release. *Neurochemical Research*, *25*(2), 225–229.

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L.-H. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature*, *447*(7141), 178–182. http://doi.org/10.1038/nature05772

Gräff, J., & Tsai, L.-H. (2013). Histone acetylation: molecular mnemonics on the chromatin. *Nature Reviews Neuroscience*, *14*(2), 97–111. http://doi.org/10.1038/nrn3427

Green, L., Fry, A. F., & Myerson, J. (1994). Discounting of delayed rewards: A life-span comparison. *Psychological Science*, *5*, 33–36.

Guilarte, T. R., Toscano, C. D., McGlothan, J. L., & Weaver, S. A. (2003). Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead

exposure. *Annals of Neurology*, *53*(1), 50–56. http://doi.org/10.1002/ana.10399

Harada, M. (1978). Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology*, *18*(2), 285–8.

Hernandez, D. G., Nalls, M. A., Gibbs, J. R., Arepalli, S., van der brug, M., Chong, S., … Singleton, A. B. (2011). Distinct DNA methylation changes highly correlated with chronological age in the human brain. *Human Molecular Genetics*, *20*(6), 1164–1172. http://doi.org/10.1093/hmg/ddq561

Huttenlocher, P., & Dabholkar, A. (1997). Regional differences in synaptogenesis in human cerebral cortex. *The Journal of Comparative Neurology*, *178*(July 1995), 167–178. http://doi.org/10.1002/(SICI)1096-9861(19971020)387:2<167::AID-CNE1>3.0.CO;2-Z

Jaffe, A. E., Gao, Y., Deep-Soboslay, A., Tao, R., Hyde, T. M., Weinberger, D. R., & Kleinman, J. E. (2016). Mapping DNA methylation across development, genotype and schizophrenia in the human frontal cortex. *Nature Neuroscience*, *19*(1546–1726 (Electronic)), 40–47. http://doi.org/10.1038/nn.4181

Johnston, L. D., O'Malley, P. M., Miech, R. A., Bachman, J. G., & Schlenberg, J. G. (2014). *Monitoring the Future national results on adolescent drug use: 1975-2013: Overview key findings on adolescent drug use*. Ann Arbor.

Kalisch, B. E., & Racz, W. J. (1996). The effects of methylmercury on endogenous dopamine efflux from mouse striatal slices. *Toxicology Letters*, *89*(1), 43–49. http://doi.org/10.1016/S0378-4274(96)03787-3

Kilgore, M., Miller, C. A., Fass, D. M., Hennig, K. M., Haggarty, S. J., Sweatt, J. D., & Rumbaugh, G. (2010). Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology*, *35*(4), 870–

80. http://doi.org/10.1038/npp.2009.197

Killeen, P. R. (1994). Mathematical principles of reinforcement. *Behavioral and Brain Sciences*, *17*(1), 105–172.

Killeen, P. R., & Sitomer, M. T. (2003). MPR. *Behavioural Processes*, *62*(1–3), 49–64.

Koseki, T., Mouri, A., Mamiya, T., Aoyama, Y., Toriumi, K., Suzuki, S., … Nabeshima, T. (2012). Exposure to enriched environments during adolescence prevents abnormal behaviours associated with histone deacetylation in phencyclidine-treated mice. *International Journal of Neuropsychopharmacology*, *15*(10), 1489–501. http://doi.org/10.1017/S1461145711001672

Laviola, G., Macrì, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews*, *27*(1–2), 19–31.

Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. a., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *Journal of Biological Chemistry*, *279*(39), 40545–40559. http://doi.org/10.1074/jbc.M402229200

Lister, R., Mukamel, E. A., Nery, J. R., Urich, M., Puddifoot, C. A., Johnson, N. D., … Ecker, J. R. (2013). Global epigenomic reconfiguration during mammalian brain development. *Science*, *341*(6146), 1237905. http://doi.org/10.1126/science.1237905

Marioni, R. E., Shah, S., McRae, A. F., Chen, B. H., Colicino, E., Harris, S. E., … Deary, I. J. (2015). DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biology*, *16*(1), 25. http://doi.org/10.1186/s13059-015-0584-6

Maze, I., Wenderski, W., Noh, K.-M., Bagot, R. C., Tzavaras, N., Purushothaman, I., … Allis, C.

D. (2015). Critical role of histone turnover in neuronal transcription and plasticity. *Neuron*, *87*(1), 77–94. http://doi.org/10.1016/j.neuron.2015.06.014

Miller, C. A., Campbell, S. L., & Sweatt, J. D. (2008). DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. *Neurobiology of Learning and Memory*, *89*(4), 599–603. http://doi.org/10.1016/j.nlm.2007.07.016

Mychasiuk, R., Zahir, S., Schmold, N., Ilnytskyy, S., Kovalchuk, O., & Gibb, R. (2012). Parental enrichment and offspring development: Modifications to brain, behavior and the epigenome. *Behavioural Brain Research*, *228*(2), 294–298. http://doi.org/10.1016/j.bbr.2011.11.036

National Research Council. (2000). *Toxicological effects of methylmercury*. Washington, D.C.: National Academy Press. http://doi.org/10.17226/9899

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), 710–20.

Newland, M. C., Reed, M. N., & Rasmussen, E. (2015). A hypothesis about how early developmental methylmercury exposure disrupts behavior in adulthood. *Behavioural Processes*, *114*, 41–51.

Newland, M. C., & Reile, P. a. (1999). Blood and brain mercury levels after chronic gestational exposure to methylmercury in rats. *Toxicological Sciences*, *50*(1), 106–16.

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

Newman, L., & McGaughy, J. (2011). Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Developmental Psychobiology*, *53*(4), 391–401.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews. Neuroscience*, *7*(September), 697–709. http://doi.org/10.1038/nrn1970

Numata, S., Ye, T., Hyde, T. M., Guitart-Navarro, X., Tao, R., Wininger, M., … Lipska, B. K. (2012). DNA methylation signatures in development and aging of the human prefrontal cortex. *American Journal of Human Genetics*, *90*(2), 260–272. http://doi.org/10.1016/j.ajhg.2011.12.020

Onishchenko, N., Karpova, N., Sabri, F., Castrén, E., & Ceccatelli, S. (2008). Long-lasting depression-like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. *Journal of Neurochemistry*, *106*(3), 1378–1387. http://doi.org/10.1111/j.1471-4159.2008.05484.x

Paletz, E. M., Craig-Schmidt, M. C., & Newland, M. C. (2006). Gestational exposure to methylmercury and n-3 fatty acids: Effects on high- and low-rate operant behavior in adulthood. *Neurotoxicology and Teratology*, *28*(1), 59–73.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R. C., … Fischer, A. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science*, *328*(5979), 753–756. http://doi.org/328/5979/753 [pii]\r10.1126/science.1186088

Pinkston, J. W., & Lamb, R. J. (2011). Delay discounting in C57BL/6J and DBA/2J mice: Adolescent-limited and life-persistent patterns of impulsivity. *Behavioral Neuroscience*, *125*(2), 194–201.

Pope, D. A., Boomhower, S. R., Hutsell, B. A., Teixeira, K., & Newland, M. C. (2016). Chronic cocaine exposure in adolescence: Effects on spatial discrimination reversal, delay

discounting, and performance on fixed-ratio schedules in mice. *Neurobiology of Learning and Memory*, *130*, 93–104. http://doi.org/10.1016/j.nlm.2016.01.017

Purkerson-Parker, S., McDaniel, K. L., & Moser, V. C. (2001). Dopamine transporter binding in the rat striatum is increased by gestational, perinatal, and adolescent exposure to heptachlor. *Toxicological Sciences*, *64*(2), 216–23.

Rane, P., Shields, J., Heffernan, M., Guo, Y., Akbarian, S., & King, J. A. (2012). The histone deacetylase inhibitor, sodium butyrate, alleviates cognitive deficits in pre-motor stage PD. *Neuropharmacology*, *62*(7), 2408–2411. http://doi.org/10.1016/j.neuropharm.2012.01.026

Reed, M. N., Banna, K. M., Donlin, W. D., & Newland, M. C. (2008). Effects of gestational exposure to methylmercury and dietary selenium on reinforcement efficacy in adulthood. *Neurotoxicology and Teratology*, *30*(1), 29–37.

Reolon, G. K., Maurmann, N., Werenicz, A., Garcia, V. A., Schröder, N., Wood, M. A., & Roesler Rafael, R. (2011). Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats. *Behavioural Brain Research*, *221*(1), 329–332. http://doi.org/10.1016/j.bbr.2011.03.033

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

Robinson, J. F., Theunissen, P. T., van Dartel, D. A. M., Pennings, J. L., Faustman, E. M., & Piersma, A. H. (2011). Comparison of MeHg-induced toxicogenomic responses across in vivo and in vitro models used in developmental toxicology. *Reproductive Toxicology*, *32*(2), 180–188. http://doi.org/10.1016/j.reprotox.2011.05.011

Sarkar, S., Abujamra, A. L., Loew, J. E., Forman, L. W., Perrine, S. P., & Faller, D. V. (2011).

Histone deacetylase inhibitors reverse CpG methylation by regulating DNMT1 through ERK signaling. *Anticancer Research*, *31*(9), 2723–2732.

Sharma, B., & Sharma, P. M. (2013). Arsenic toxicity induced endothelial dysfunction and dementia: Pharmacological interdiction by histone deacetylase and inducible nitric oxide synthase inhibitors. *Toxicology and Applied Pharmacology*, *273*(1), 180–188. http://doi.org/10.1016/j.taap.2013.07.017

Song, C., Kanthasamy, A., Anantharam, V., Sun, F., & Kanthasamy, a G. (2010). Environmental neurotoxic pesticide increases histone acetylation to promote apoptosis in dopaminergic neuronal cells: relevance to epigenetic mechanisms of neurodegeneration. *Molecular Pharmacology*, *77*(4), 621–632. http://doi.org/10.1124/mol.109.062174

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*(4), 417–463.

Spear, L. P. (2007a). Assessment of adolescent neurotoxicity: Rationale and methodological considerations. *Neurotoxicology and Teratology*, *29*(1), 1–9.

Spear, L. P. (2007b). The developing brain and adolescent-typical behavior patterns: An evolutionary approach. In E. Walker, J. Bossert, & D. Romer (Eds.), *Adolescent Psychopathology and the Developing Brain: Integrating Brain and Prevention Science* (pp. 9–30). New York: Oxford University Press.

Tarazi, F., & Baldessarini, R. (2000). Comparative postnatal development of dopamine D1, D2, and D4 receptors in rat forebrain. *International Journal of Developmental Neuroscience*, *18*(1), 29–37.

Tarazi, F., Tomasini, E. C., & Baldessarini, R. J. (1998). Postnatal development of dopamine and serotonin transporters in rat caudate-putamen and nucleus accumbens septi. *Neuroscience*

Letters, *254*(1), 21–24. http://doi.org/10.1016/S0304-3940(98)00644-2

Teicher, M. H., Andersen, S. L., & Hostetter, J. C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Developmental Brain Research*, *89*, 167–172.

Tiernan, C. T., Edwin, E. A., Goudreau, J. L., Atchison, W. D., & Lookingland, K. J. (2013). The role of de novo catecholamine synthesis in mediating methylmercury-induced vesicular dopamine release from rat pheochromocytoma (PC12) cells. *Toxicological Sciences*, *133*(1), 125–132. http://doi.org/10.1093/toxsci/kft025

Tiernan, C. T., Edwin, E. A., Hawong, H. Y., Rios-Cabanillas, M., Goudreau, J. L., Atchison, W. D., & Lookingland, K. J. (2015). Methylmercury impairs canonical dopamine metabolism in rat undifferentiated pheochromocytoma (pc12) cells by indirect inhibition of aldehyde dehydrogenase. *Toxicological Sciences*, *144*(2), 347–356. http://doi.org/10.1093/toxsci/kfv001

Varela, R. B., Valvassori, S. S., Lopes-Borges, J., Mariot, E., Dal-Pont, G. C., Amboni, R. T., … Quevedo, J. (2015). Sodium butyrate and mood stabilizers block ouabain-induced hyperlocomotion and increase BDNF, NGF and GDNF levels in brain of Wistar rats. *Journal of Psychiatric Research*, *61*, 114–121. http://doi.org/10.1016/j.jpsychires.2014.11.003

Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., … Wood, M. A. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. *The Journal of Neuroscience*, *27*(23), 6128–6140. http://doi.org/10.1523/JNEUROSCI.0296-07.2007

Xu, W. S., Parmigiani, R. B., & Marks, P. (2007). Histone deacetylase inhibitors: molecular

mechanisms of action. *Oncogene, 26*, 5541–5552. http://doi.org/10.1038/sj.onc.1210620

Chapter 2

Methylmercury (MeHg), an environmental neurotoxicant primarily found in fish, produces neurobehavioral impairment when exposure occurs in gestation. Whether other developmental periods, such as adolescence, display enhanced vulnerability to the behavioral effects of MeHg exposure is only beginning to be explored. In Experiment 1, male C57BL6/n mice were exposed to 0, 0.3, and 3.0 ppm MeHg (n = 12 each) via drinking water from postnatal days 21 to 60 (murine adolescence). As adults, mice were trained to lever press under an ascending series of fixed-ratio schedules of milk reinforcement. Adolescent MeHg exposure dose-dependently decreased estimates of response-reinforcer coupling and minimum response time relative to controls. In Experiment 2, the protective effects of sodium butyrate (NaB; a histone deacetylase inhibitor) and environmental enrichment on MeHg-induced behavioral impairment were examined. Male C57BL6/n mice were assigned to control, NaB, or environmental enrichment and within each of these treatment conditions were given either 0 or 3.0 ppm MeHg during adolescence (n = 12 in each cell). Adolescent MeHg exposure again decreased estimates of response-reinforcer coupling but did not significantly alter minimum response time. Chronic NaB also decreased response-reinforcer coupling. These data suggest that the behavioral mechanisms of adolescent MeHg exposure and chronic sodium butyrate may be related to the impact of reinforcement on prior responses, and that MeHg's effects are not reversed by environmental enrichment or sodium butyrate.

Adolescent Methylmercury Exposure and Chronic Sodium Butyrate Decrease the Impact of Reinforcement on Responding in Mice

The adolescent period is a time of dynamic psychological and neurobiological maturation in mammals (Spear, 2000). The density of neural connections and amount of gray matter decreases in the frontal cortex (Giedd et al., 1999) and dopamine-receptor pruning occurs in the midbrain (Teicher, Andersen, & Hostetter, 1995). These changes co-occur with increased risky and impulsive choices (Chambers & Potenza, 2003), decreased executive functioning (Newman & McGaughy, 2011), and a higher likelihood of substance abuse (Johnston, O'Malley, Miech, Bachman, & Schlenberg, 2014). This state of flux escalates the vulnerability of adolescents to neurotoxic substances (Chambers, Taylor, & Potenza, 2003) and can result in enduring psychological dysfunction later in life. Thus, understanding how neurotoxic perturbations during adolescence affect behavior in adulthood is important.

Methylmercury (MeHg) is an environmental neurotoxicant found in fish and is a significant public health concern (National Research Council, 2000). In animal models, gestational exposure to doses of MeHg that encompass human exposures impairs reversal learning and decision making in adulthood (Newland, Paletz, & Reed, 2008; Newland, Reile, & Langston, 2004; Reed, Paletz, & Newland, 2006), suggesting deficiencies in executive functioning (Newland, Reed, & Rasmussen, 2015). However, the long-term behavioral effects of adolescent MeHg exposure remain virtually unexplored. This is especially troubling since human adolescents both consume more fish and have higher blood mercury concentrations compared to other age groups (Nielsen, Aoki, Kit, & Ogden, 2015; Sichert-Hellert, Wicher, & Kersting, 2009). For these reasons, our laboratory has begun examining the behavioral toxicity of adolescent MeHg exposure. We have reported that mice exposed to environmentally-relevant

doses of MeHg during adolescence require more trials to transition through a spatial-discrimination reversal and spatial-to-visual discrimination (extradimensional shift) (Boomhower & Newland, 2017). Adolescent MeHg exposure also dose-dependently reduces choice for a larger-immediate reinforcer relative to a smaller-immediate reinforcer (Boomhower & Newland, 2016). These experiments suggest that MeHg exposure during adolescence can produce long-lasting behavioral effects related to choice in adulthood but the behavioral mechanisms underlying these effects remain unclear. For example, adolescent MeHg exposure may alter reinforcer efficacy as with gestational exposures (Newland et al., 2015), or it may change some other aspect of reinforcement or behavior.

Mathematical Principles of Reinforcement (MPR) offers one way to characterize fundamental behavioral consequences of chemical exposure (Killeen, 1994; Killeen & Sitomer, 2003). MPR posits that three fundamental processes govern operant (voluntary) behavior: (a) the presentation of a reinforcer increases activation of behavior, (b) the maximal rate at which a response can be produced is limited by the minimum time it takes to complete that behavior, and (c) a contingency between behavior and reinforcement causes both target and non-target responses to become coupled to the reinforcer. The coupling strength decreases as a function of events or time between a response and reinforcer delivery, to produce a delay-of-reinforcement gradient. For fixed-ratio schedules of reinforcement, MPR predicts response rate (*b*, responses/sec) as a function of fixed-ratio size (*n*) using Eq. 1 (Killeen & Sitomer, 2003):

$$b = \frac{1 - e^{-\lambda\delta n}}{\delta} - \frac{n}{\delta a} \quad (1)$$

where *a* represents specific activation and is a measure of reinforcer value, δ is the minimum response time, and λ is the rate at which the coupling of reinforcement to a response (target or

non-target) decreases with time. MeHg-induced changes in these parameters would reveal specific behavioral mechanisms of MeHg's effects.

A growing literature implicates neuroepigenetic alterations in mediating the toxic effects of developmental MeHg exposure (Bakulski et al., 2015; Basu et al., 2013; Bose, Onishchenko, Edoff, Lang, & Ceccatelli, 2012; Cardenas et al., 2017; Onishchenko, Karpova, Sabri, Castrén, & Ceccatelli, 2008; Robinson et al., 2011). If true, then therapeutic treatments that counteract suppressive epigenetic markers should prevent MeHg-induced behavioral impairment. Drugs that directly inhibit histone deacetylase (HDAC) and indirectly inhibit DNA methylation (Sarkar et al., 2011; Xu, Parmigiani, & Marks, 2007), such as sodium butyrate (NaB), possess substantial promise as treatments for cognitive impairment (Day & Sweatt, 2012). Indeed, NaB prevents behavioral deficits caused by neurotoxicant exposure by promoting histone acetylation and neuronal gene expression (Sharma & Sharma, 2013). Another potential treatment is environmental enrichment. As opposed to standard-housing conditions (i.e., individually-housed animals with access to food, water, and bedding), environmental enrichment includes group-housing animals and providing them access to running wheels, tunnels, burrows, elevated platforms, and toys (Nithianantharajah & Hannan, 2006). Exposure to enriched environments during adolescence prevents lead-induced decreases in neuronal gene expression and maze performance (Guilarte, Toscano, McGlothan, & Weaver, 2003) and phencyclidine-induced deficits in HDAC regulation, social behavior, and memory in rodents (Koseki et al., 2012). A reversal of MeHg-induced behavioral deficits by NaB or environmental enrichment would implicate alterations of the neuroepigenome in adolescent MeHg's neurotoxicity.

Experiment 1

Experiment 1 was designed to describe the behavioral effects of adolescent MeHg exposure using Mathematical Principles of Reinforcement (Eq. 1; Killeen & Sitomer, 2003). The effects of adolescent MeHg exposure on MPR's parameters—namely, reinforcer value (a), motoric capacity (δ), and the rate at which coupling between a response and reinforcer decreases as a function of time (λ)—were assessed under a multiple fixed-ratio procedure. Based on our prior work with an impulsive-choice procedure (Boomhower & Newland, 2016), we hypothesized that adolescent MeHg exposure will alter the coupling rate (λ), a change that is associated with impulsivity in mice (Pope, Boomhower, Hutsell, Teixeira, & Newland, 2016). Further, adolescent MeHg may alter reinforcer value (a), as our past work has shown more trials are required to transition through a spatial discrimination reversal and extradimensional shift following adolescent MeHg exposure (Boomhower & Newland, 2017).

Method

Subjects and exposure

Thirty-six male C57BL/6n mice were purchased from a commercial vendor (Envigo, Indianapolis, IN). The mice were derived from 12 litters, with each of 3 littermates assigned to one of the three dose groups (see below). These mice have previously been reported on (Boomhower & Newland, 2016). Briefly, all mice were pair-housed and maintained on a 12-hr light/dark cycle (lights on at 6:00 AM) in a temperature- and humidity-controlled, AAALAC-accredited animal facility. Upon arrival, 21-day-old littermates were divided among three MeHg-exposure groups (via drinking water): 0 ppm (control), 0.3 ppm, and 3.0 ppm MeHg. Thus the litter was the statistical unit. MeHg was delivered as methylmercuric chloride ($MeHgCl_2$) dissolved in drinking water, and exposure occurred from postnatal day (PND) 21 through 59. All

water was replaced with tap water on PND 60. Dosing was calculated by weighing water bottles, and sham water bottles were weighed to account for spillage. The low (0.3 ppm) and high (3.0 ppm) doses of MeHg corresponded to approximately 40 and 400 μg/kg/day of MeHg in mice, respectively (Boomhower & Newland, 2016, 2017). The dose range of MeHg used here has been associated with neurobehavioral impairment in past work (Boomhower & Newland, 2016, 2017). Mice were maintained at 25 (± 1) g body mass by restricting daily food intake to 2.4 (± .02) g chow. The Auburn University Institutional Animal Care and Use Committee approved all procedures.

Apparatus

Twelve standard operant chambers (Med Associates, St. Albans, VT) modified for mice were used for data collection. Each chamber was equipped with two retractable levers on a front wall panel. Situated between the two levers was an alcove where a dipper system delivered .01-cc presentations of a 3:1 water and sweetened-condensed milk solution (hereafter, milk). Two Sonalert® tone generators (high tone: 4500 Hz, low tone: 2700 Hz) were located at the top of the front wall. A sound-attenuating cubicle enclosed each chamber. All experimental contingencies were controlled within 0.01-sec resolution by a computer in an adjacent room. Each mouse was assigned a particular chamber for the duration of the study. Mice were divided into four squads that ran at approximately the same time every day (±15 min) Monday through Friday. The number of mice in each exposure group was counterbalanced across chambers and squads.

Procedure

All mice had prior experience lever pressing as described in Boomhower and Newland (2016). At approximately PND 300, mice were trained under a multiple fixed-ratio (FR) schedule of reinforcement based on Reilly (2003) with some modifications. A session was

divided into six components, each associated with a different FR schedule and signaled by a unique, high/low-alternating tone (Table 1). The FR schedule was increased across components in the following order: FR 1, 5, 15, 30, 60, and 120. At the beginning of a session, one lever was inserted into the chamber and the tones sounded. Upon completion of the FR requirement, the lever retracted, the tones were extinguished, and 3 sec of access to milk was made available. The lever was then re-inserted into the chamber. A component ended after the delivery of 12 reinforcers or 10 min, whichever came first. Components were separated by 30 sec during which the lever was retracted and tones were extinguished. The multiple FR schedule was in effect for 40 sessions to allow responding to stabilize.

Data analysis

Responding under the multiple FR schedule among exposure groups was assessed by averaging response rates, calculated as the number of responses divided by the duration of the component (in seconds), for each subject from the last 10 sessions. Eq. 1 was fit to individual-subject response-rate functions using nonlinear least-squares regression. Response rates were compared among groups using a repeated-measures ANOVA with exposure and FR as the within-subjects variables. Estimates of a, δ, and λ were compared across exposure groups using a repeated-measures ANOVA. Planned post-hoc comparisons were conducted between MeHg-exposed animals and controls.

Results and Discussion

Figure 1 shows mean response rates as a function of FR for the MeHg exposure groups along with best fits of Eq. 1 (solid lines). Individual-subject data for two mice from each exposure group also are shown to demonstrate the range of goodness of fit of Eq. 1: one was the best fit and one was the worst fit for each exposure group. One 3.0-ppm MeHg exposed mouse, was a statistical outlier due to his low rate of responding (Fig. 1, lower right panel) so his data

are not included in the mean for the 3.0-ppm group. There were main effects of both FR [$F(5, 197) = 37.95$, $p < .001$] and exposure [$F(2, 197) = 7.53$, $p < .001$] in that response rates were a bitonic function of FR and MeHg-exposed mice responded more quickly on average. There was no significant FR X exposure interaction [$F(10, 197) = .36$, $p = .96$]. Eq. 1 provided a good fit of all individual-subject data with the mean (SEM) pseudo R^2 value being 0.94 (0.03) for all groups.

Figure 2 shows mean parameter estimates of λ, δ, and a for the MeHg exposure groups. The outlier is denoted as an open circle. Estimates of λ were significantly different among the exposure groups [$F(2, 33) = 4.16$, $p = .02$], and post-hoc comparisons revealed that mice in the 3.0-ppm group had higher estimates of λ, or saturation rates, compared to controls ($p < .05$). Thus, the coupling between responses and reinforcers decreased more rapidly for the MeHg-exposed mice. Stated differently, the probability that a prior response was strengthened by a reinforcer decreased more quickly across time following MeHg exposure. Estimates of δ were significantly different among exposure groups [$F(2, 33) = 9.15$, $p < .001$]. Post-hoc comparisons revealed that the 0.3- and 3.0-ppm groups had lower estimates of δ, or faster minimum response times, relative to controls ($p < .02$). This was evident in higher maximum response rates ($1/\delta$) in exposed mice. It can be noted that the inclusion of the statistical outlier (see above) in the 3-ppm group renders δ estimates for this group statistically similar to Controls' δ estimates. Estimates of a were not significantly different among exposure groups [$F(2, 33) = 0.89$, $p = .42$], indicating that the value of a reinforcer was similar following adolescent MeHg exposure.

As revealed by MPR (Killeen & Sitomer, 2003), adolescent exposure to MeHg manifested as alterations in response-reinforcer coupling (λ) and minimum response time (δ) in adulthood. The finding that adolescent MeHg exposure diminishes the impact of reinforcement on prior responding is consistent with prior work showing that adolescent MeHg exposure

impairs the second spatial-discrimination reversal and an extradimensional shift in mice (Boomhower & Newland, 2017). Further, an enhancement in psychomotor responding following MeHg exposure is consistent with past work showing gestationally-exposed rats respond more quickly on fixed- (Paletz, Craig-Schmidt, & Newland, 2006) and progressive-ratio schedules of reinforcement (Reed, Banna, Donlin, & Newland, 2008). These findings suggest that adolescent MeHg exposure may produce long-lasting distortions in both the ability of a reinforcer to strengthen past responses and psychomotor responding.

Because the behavioral effects of adolescent MeHg exposure observed in Experiment 1 are subtle, they require replication. Further, one way adolescent MeHg exposure could come to alter adult behavior is through changes in the neuroepigenome. If the effects of MeHg on saturation rate and minimum response time can be reversed using treatments with known neuroepigenetic effects, such as sodium butyrate and environmental enrichment, then this would suggest a neuroepigenetic mechanism of MeHg behavioral toxicity. Replicating the effects of Experiment 1 and testing whether neuroepigenome-altering treatments confer therapeutic effects would provide information on the functional (behavioral) significance and potential mechanisms of adolescent MeHg toxicity.

Experiment 2

Developmental MeHg exposure may produce its neurobehavioral effects in part by increasing neuroepigenetic marks that suppress gene expression (Onishchenko et al., 2008). One way to determine whether epigenetic dysregulation in the brain may be a mechanism of adolescent MeHg exposure is through treatments known to affect the epigenome. Sodium butyrate (NaB), a histone deacetylase (HDAC) inhibitor, and environmental enrichment both impart beneficial neuroepigenetic profiles that could prevent or reverse MeHg-induced

behavioral dysregulation. In past work, NaB has reversed behavioral effects of neurotoxicant exposure by promoting histone acetylation and neuronal gene expression (Sharma & Sharma, 2013). Environmental enrichment during adolescence also prevents lead-induced decreases in neuronal gene expression and maze performance (Guilarte et al., 2003). Whether NaB and environmental enrichment alters reinforcer value, motoric capacity, or response-reinforcer coupling as described by Mathematical Principles of Reinforcement is unknown. Determining the effects of these treatments on specific mechanisms of operant behavior is important for the design of future therapeutic interventions.

A second goal of Experiment 2 is to replicate the effects of adolescent MeHg exposure on saturation rate, minimum response time, and specific activation observed in Experiment 1. The procedure used is novel to neurotoxicology, so we wished to determine the robustness of the effects reported. As with any novel approach, it is always possible that effects could have arisen by chance, even with the *p* values reported here (Nuzzo, 2014). Therefore, we sought to conduct a second study under slightly different conditions.

Method

Subjects and exposure

Seventy-two male C57Bl/6n mice derived from 24 litters were purchased from a commercial vendor (Envigo, Indianapolis, IN). Past work has demonstrated that rodents reduce food and water consumption during shipment from commercial vendors (see Obernier & Baldwin, 2006), so there was a concern that water consumption may be abnormally elevated in mice immediately upon arrival. Thus, mice arrived at our facility on PND 23, and half of the litters (i.e., 36 mice) were exposed to 3 ppm MeHg beginning on PND 24. MeHg was delivered as methylmercuric chloride dissolved in drinking water. The other litters received control (tap)

water. Dosing was calculated by weighing water bottles, and sham water bottles were used to control for spillage. On PND 63, MeHg bottles were removed and replaced with tap water, thus MeHg exposure spanned PND 24-62 (see Figure 3). All mice were given *ad libitum* access to food until behavioral testing, which began on PND 100. Two weeks prior to behavioral testing, mice were maintained at a body mass of 25 (± 1) g body mass by food restricting daily chow intake to 2.4 (± 0.2) g. All mice were maintained under a 12-hr light/dark cycle (lights on at 6:00 AM) in a temperature- and humidity-controlled, AAALAC-approved animal facility. The Auburn University Institutional Animal Care and Use Committee approved all procedures.

Therapeutic treatments

Upon arrival (PND 23), littermates in both exposure groups were divided among three treatment conditions: control, sodium butyrate (NaB), and environmental enrichment. Control mice were individually-housed and received an i.p. saline (10 mL/kg) injection once daily for 14 days prior to behavioral testing. This began on PND 86. NaB mice were individually-housed and received an acute i.p. injection of NaB (0.6 g/kg) once daily for 14 days prior to behavioral testing, a regimen similar to past work (Kim, Leeds, & Chuang, 2009; Rane et al., 2012). Sodium butyrate (Sigma) was dissolved in saline. Control and NaB mice were housed in Optimice® cages with 37.5-in^2 floor space (Animal Care Systems, Centennial, CO). Enrichment mice were group-housed (5-6 mice/cage) from PND 23 to PND 85. Cages were 18 (l) x 14 (w) x 8 (h) in. with 252-in^2 floor space. Each cage was equipped with a running wheel, chewable bone, elevated platform, two toys, and a configurable hut and tunnel. The configuration of the hut and tunnel were changed every week, and the two toys were exchanged every week for two novel toys. The environmental-enrichment conditions described above were based on past work (Guilarte et al., 2003; Nithianantharajah & Hannan, 2006). On PND 86, Enrichment mice were individually-

housed in Optimice® cages for the remainder of the study and given an i.p. injection of saline (10 mL/kg) once daily for 14 days prior to behavioral testing.

Apparatus

The same apparatuses were used as in Experiment 1.

Procedure

All mice had previous experience lever pressing under a spatial-discrimination-reversal and visual-discrimination procedure, identical to Boomhower and Newland (2017). At approximately PND 250, mice were trained under the multiple fixed-ratio procedure described in Experiment 1. The number of mice from each exposure and treatment group were counterbalanced across chambers and session time. One 0-ppm MeHg mouse (Control) and four 3-ppm MeHg mice (Control, $n = 1$; NaB, $n = 2$; EE, $n = 1$) were euthanized before behavioral testing for reasons unrelated to the present experiments.

Data Analysis

Similar to Experiment 1, Eq. 1 was fit to individual-subject response-rate functions using nonlinear least-squares regression. Response rates were analyzed using a linear-mixed effects (LME) model with group and FR as fixed effects and litter as a random effect. Estimates of a, δ, and λ were compared across groups using a LME model with group as a fixed effect and litter as a random effect. LME was chosen because it is able to model incomplete repeated-measures data more effectively than does traditional repeated-measures analysis of variance.

Results and Discussion

Figure 4 shows MeHg consumption across adolescence for each MeHg exposure group. The dose of MeHg was highest at the beginning of exposure and gradually decreased to about 400 μg/kg/day. This pattern of dosing was similar to that seen in Experiment 1 (see Boomhower

& Newland, 2016) and Boomhower and Newland (2017). Neither treatment with NaB nor environmental enrichment significantly altered MeHg dosing across adolescence.

Figure 5 shows mean response rates and best fits of Eq. 1 for each treatment group. There was a main effect of FR [$F(5, 365) = 73.40$, $p < .001$], which was reflected in a bitonic relation between response rates and FR size. There was also a main effect of exposure group [$F(5, 365) = 2.69$, $p = .02$] in that MeHg-exposed mice as well as mice who experienced environmental enrichment had higher response rates overall, though this effect was more muted than in Experiment 1. There was no significant FR X exposure interaction [$F(25, 365) = .46$, $p = .99$]. Eq. 1 fit individual response-rate functions well with a mean pseudo $R^2 = 0.94$ (*SD*: .06) across all groups.

Figure 6 shows mean parameter estimates from Eq. 1 for each exposure and treatment group. Estimates of saturation rate (λ) were significantly different among groups [$F(5, 60) = 2.18$, $p = .05$] with post-hoc comparisons revealing a significant increase in λ following MeHg exposure alone and NaB administration alone relative to the 0-ppm Control group (p's $< .05$). Further, the MeHg-induced increase in saturation rate remained significant relative to 0-ppm Control following treatment with NaB and environmental enrichment. Neither minimum response time (δ) [$F(5, 60) = 1.66$, $p = .16$] nor specific activation (a) [$F(5, 60) = .74$, $p = .60$] estimates were significantly altered relative to 0-ppm Control.

The finding that adolescent MeHg exposure increases saturation rate, or the degree to which prior responses are coupled to reinforcers, was replicated in Experiment 2. Further, MeHg-exposed mice tended to have higher response rates than unexposed mice similar to Experiment 2, but this was more muted than in Experiment 1 and was not reflected in a statistically significant decrease in minimum response time. Mice in Experiment 2 were

approximately 50 days younger than mice in Experiment 1 at the time of testing. Mice in Experiment 1 also experienced a delay-discounting procedure beforehand (Boomhower & Newland, 2016), whereas mice in Experiment 2 experienced a spatial-discrimination-reversal procedure. These age- and experience-related differences might explain why the effects on minimum response time were not replicated in Experiment 2. Of all three parameter estimates from MPR, the ability of reinforcers to strengthen past responding appears to be most sensitive to adolescent MeHg exposure. The MeHg-induced increase in λ estimates persisted following chronic treatment with NaB and exposure to an enriched environment, suggesting no therapeutic benefits of these treatments under these levels and durations. Chronic NaB treatment alone, however, produced a significant increase in saturation rate relative to 0-ppm Controls, an effect that was similar in magnitude to MeHg's. This finding suggests a role for the neuroepigenome in mediating response-reinforcer coupling.

General Discussion and Conclusions

The effects of adolescent methylmercury (MeHg) exposure on reinforcer value, motoric capacity, and the coupling of responses to reinforcement in mice were evaluated using Mathematical Principles of Reinforcement (Killeen & Sitomer, 2003) in two separate experiments. Further, the possible therapeutic effects of sodium butyrate (NaB) and environmental enrichment (EE) were examined to test the hypothesis that MeHg behavioral toxicity was due in part to altered neuroepigenetics. Overall, MPR described response-rate functions collected from individual mice quite well and captured alterations in responding following the various exposures and treatments.

In both Experiment 1 and 2, adolescent MeHg exposure increased saturation rate (λ), the rate at which response-reinforcer coupling decreased across time. Stated differently, adolescent

MeHg exposure decreased the number of responses that were strengthened by reinforcers. Reduced response-reinforcer coupling following adolescent MeHg exposure could explain past work reporting MeHg-induced impairments in choice. Adolescent MeHg dose-dependently reduced sensitivity to a larger reinforcer compared to a smaller reinforcer in these mice (Boomhower & Newland, 2016), and a single dose of MeHg (3 ppm) increased the number of trials required to transition through repeated spatial-discrimination reversals and an extradimensional shift (Boomhower & Newland, 2017). These findings suggest a degradation in the ability of a reinforcer to strengthen prior responses and is consistent with past work showing that saturation rate (λ) is correlated with impaired performance on spatial discrimination reversals in mice (Pope et al., 2016).

The finding that adolescent MeHg exposure diminishes the impact of reinforcement on previous responses implicates neural substrates linked to motivation in adolescent MeHg toxicity. The nucleus accumbens, its projections and interaction with the prefrontal cortex, and dopamine signaling are important for reward processing (Everitt & Robbins, 2005; Fiorillo, Tobler, & Schultz, 2003). Lesions of the rat nucleus accumbens core increase a parameter similar to saturation rate (λ) (Bezzina et al., 2008), whereas rat orbital prefrontal-cortex lesions are associated with reductions in specific activation (a) (Kheramin et al., 2005). Changes in the parameters of Mathematical Principles of Reinforcement are also intimately linked with dopaminergic function, as both neuroleptics and amphetamines can alter saturation rate, minimum response time, and specific activation (Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000; Reilly, 2003). Our data suggest adolescent MeHg exposure interferes with the coupling of behavior to reinforcement, which is linked to nucleus-accumbens and prefrontal-cortex function. The finding that the effects of reinforcement on behavior are impaired following adolescent

MeHg exposure is consistent with Newland et al.'s (2015) hypothesis that developmental MeHg exposure impacts reinforcer processing, primarily through MeHg's effects on dopamine. Though Newland et al.'s (2015) review relied on models of gestational MeHg exposure, the present study suggests that adolescent MeHg exposure also contributes to alterations in brain-behavior function.

Minimum response times (δ) were decreased by adolescent MeHg exposure in Experiment 1, particularly under the 0.3-ppm dose, but were not significantly altered in Experiment 2. Reduced minimum response time manifests as increased maximum response rate, which is correlated with $1/\delta$ both theoretically (Killeen & Sitomer, 2003) and experimentally (Hutsell & Newland, 2013). Past work in gestationally-exposed rats showed that MeHg increases response rates under fixed-ratio (Paletz et al., 2006) and progressive-ratio schedules of reinforcement (Reed et al., 2008). In Experiment 1, both doses of MeHg reduced minimum response time. The greatest decrease in minimum response time occurred after 0.3 ppm MeHg, and the 3.0-ppm dose of MeHg also produced a significant decrease in δ estimates following the removal of a statistical outlier. Thus, it is unclear why the same dose of MeHg in Experiment 2 did not produce a significant reduction in minimum response time. We have noted previously dose-specific behavioral effects of adolescent MeHg exposure in the cohort of animals used in Experiment 1. Specifically, delay sensitivity was reduced in the 0.3-ppm group whereas mice in the 3-ppm group displayed similar estimates of delay sensitivity as controls (Boomhower & Newland, 2016).

Some evidence suggests that MeHg may exert its behavioral toxicity via abnormal modifications to the epigenome. Gestational MeHg exposure is correlated with reduced expression of the brain-derived neurotrophic factor gene (*Bdnf*) in the adult rat hippocampus,

with a concurrent decrease in histone H3 acetylation and increase in DNA methylation at the *Bdnf* promoter (Onishchenko et al., 2008). To test the hypothesis the MeHg neurobehavioral toxicity was due in part to altered neuroepigenetics, we examined whether sodium butyrate (NaB), a histone deacetylase inhibitor, and environmental enrichment (EE) prevented MeHg-induced changes in behavior. We found that neither NaB nor EE reversed MeHg-induced increases in saturation rate; rather, chronic NaB alone induced an increase in saturation rate similar to MeHg alone. The finding that NaB produced a behavioral effect similar to MeHg rather than reversing MeHg's effects is unclear. In nonhuman models, studies using NaB as a treatment for both normal (Peleg et al., 2010; Reolon et al., 2011) and diseased aging (Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Kilgore et al., 2010; Rane et al., 2012) as well as following neurotoxicant exposure (Sharma & Sharma, 2013; Song, Kanthasamy, Anantharam, Sun, & Kanthasamy, 2010) have noted a range of therapeutic effects. NaB induces differentiation in neurons (Balasubramaniyan et al., 2006; Hsieh, Nakashima, Kuwabara, Mejia, & Gage, 2004) and apoptosis in neuroblastoma cells (Nuydens et al., 1995; Rozental et al., 2004). Histone deacetylase inhibitors similar to NaB, such as nicotinamide and trichostatin A, arrest neural stem cell growth and induce apoptosis (Wang, Cheng, Wang, & Wen, 2012). Further characterization of the neuroprotective and neurotoxic effects of NaB, and other histone deacetylase inhibitors, will be necessary for future work.

Some evidence suggests that EE can protect against neurotoxicant-induced changes in neuronal gene expression and behavior in rodents. Specifically, exposure to enriched environments during adolescence prevents lead-induced decreases in *Bdnf* expression and maze performance (Guilarte et al., 2003) and phencyclidine-induced deficits in HDAC regulation, social behavior, and memory in rodents (Koseki et al., 2012). In the present study, EE did not

protect against adolescent MeHg-induced increases in saturation rate. Studies examining the effects of EE on reinforcement processing have reported mixed findings, mostly confined to differences in baseline rates of behavior between EE and control rodents (Stairs & Bardo, 2009). This could explain why saturation-rate estimates were elevated (though not significantly) for EE mice relative to independently-housed mice in Experiment 2.

Across two studies, exposure to environmentally-relevant levels of MeHg in adolescent mice reduced the degree to which responses were coupled to reinforcement in adulthood. We also found some evidence that adolescent MeHg exposure reduces minimum response time, particularly following exposure to 0.3 ppm MeHg, which is consistent with past work on developmental MeHg exposure's psychomotor effects. Treatment with sodium butyrate and exposure to an enriched environment did not reverse MeHg-induced changes in saturation rate (λ). The present study suggests adolescence is a time of both behavioral and neurobiological vulnerability to MeHg exposure. The finding that response-reinforcer coupling is reduced by MeHg exposure, and there is a lack of a therapeutic effect of sodium butyrate and environmental enrichment on MeHg-induced impairment, carry implications for the neurobehavioral mechanisms that permit adolescent MeHg toxicity.

Tables

Table 1. Tone durations for the FR procedure.

FR	Low/high tone durations (sec)
1	0.15/1.19
5	0.74/0.60
15	0.92/0.42
30	1.04/0.30
60	1.13/0.21
120	1.19/0.15

FR = fixed-ratio schedule of reinforcement

Figures

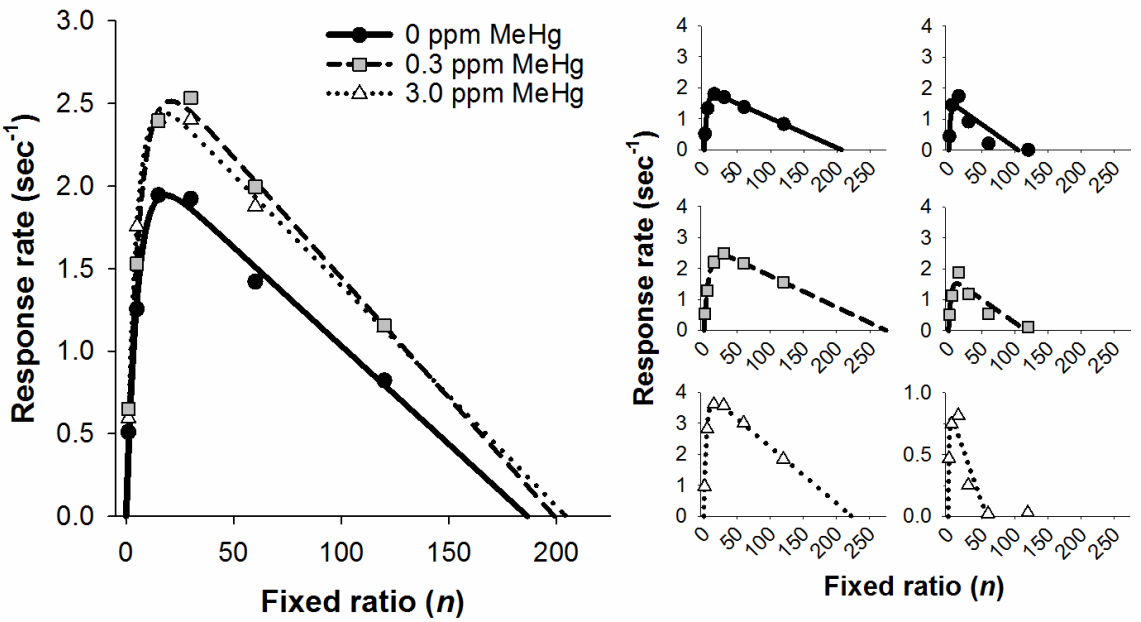

Figure 1. *Left panel*: Mean response rate as a function of fixed ratio for mice exposed to MeHg in adolescence. Lines represent mean predictions of Eq. 1. *Right panel*: Response rate (symbols) and predictions of Eq. 1 (lines) as a function of fixed ratio for individual mice. The left column shows data from mice with the best-fitting curves, and the right column shows data from mice with the worst-fitting curves. Note the Y-axis scaling in the lower-right panel.

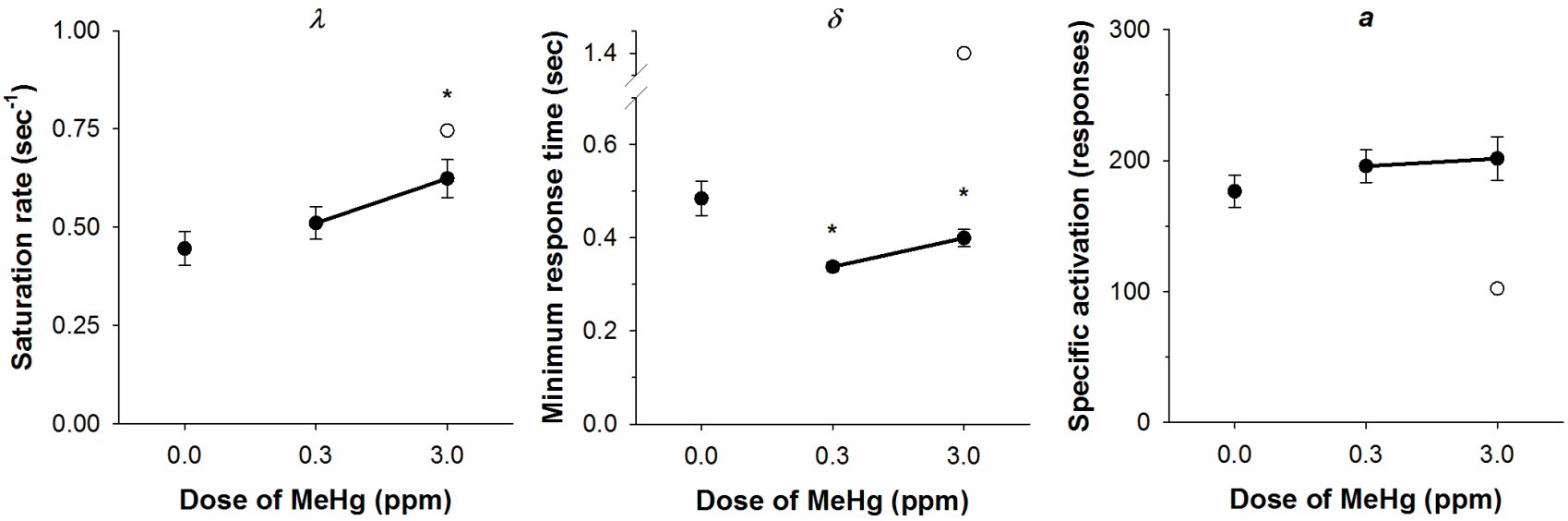

Figure 2. Mean (±SEM) parameter estimates from Eq. 1 as a function of dose of MeHg in adolescence. An outlier in the 3.0 ppm group is denoted as an open circle. *$p < .05$ relative to control

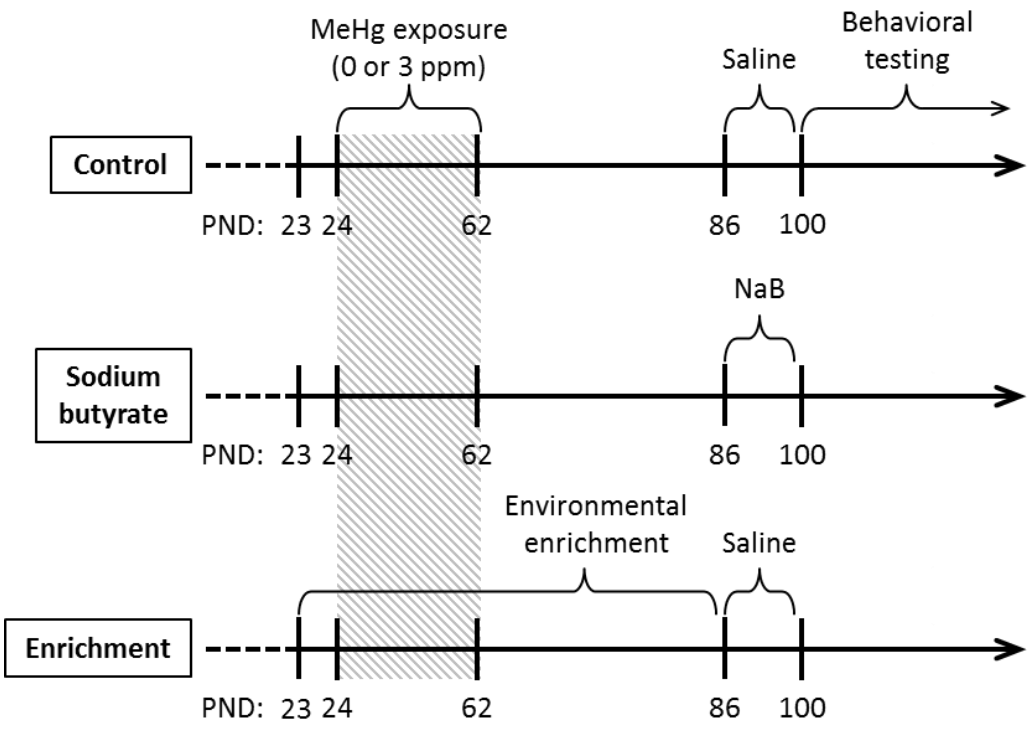

Figure 3. Timeline of exposures, treatments, and testing in Experiment 2. PND = postnatal day, NaB = sodium butyrate

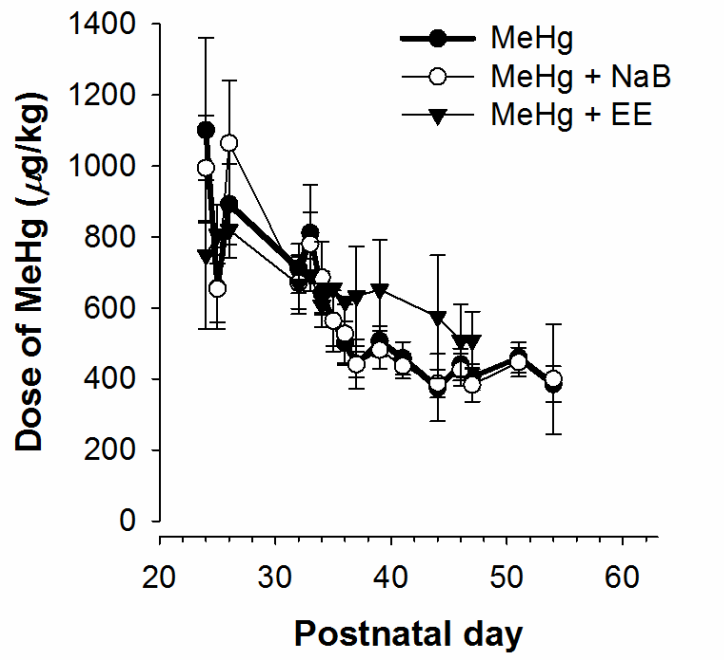

Figure 4. Mean (±SD) dose of MeHg as a function of postnatal day for each MeHg-exposure group in Experiment 2. Doses for the MeHg + EE group were calculated by averaging the intake of the entire cage. Note: all errors bars represent one SD rather than one SEM. EE = environmental enrichment, NaB = sodium butyrate

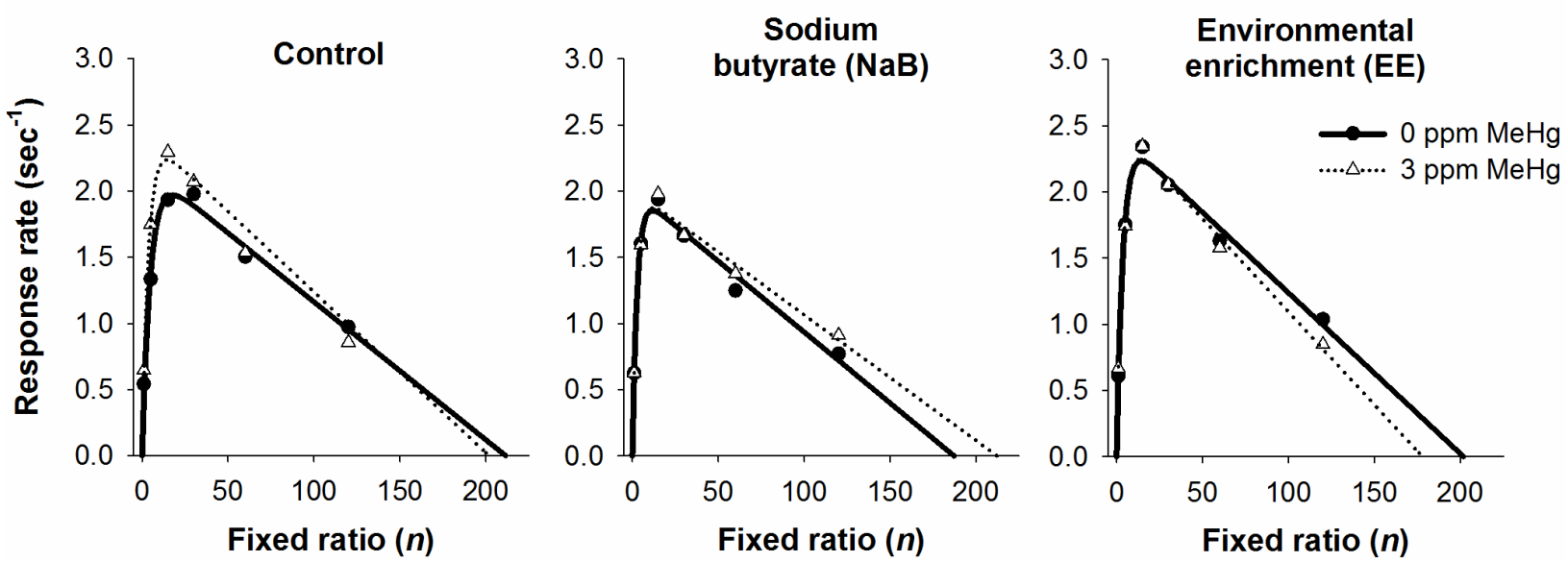

Figure 5. Mean response rate as a function of fixed ratio for mice exposed to 0 or 3 ppm MeHg in adolescence and treated with control (left panel), sodium butyrate (middle panel), or environmental enrichment (right panel) before behavioral testing. Lines represent mean predictions of Eq. 1.

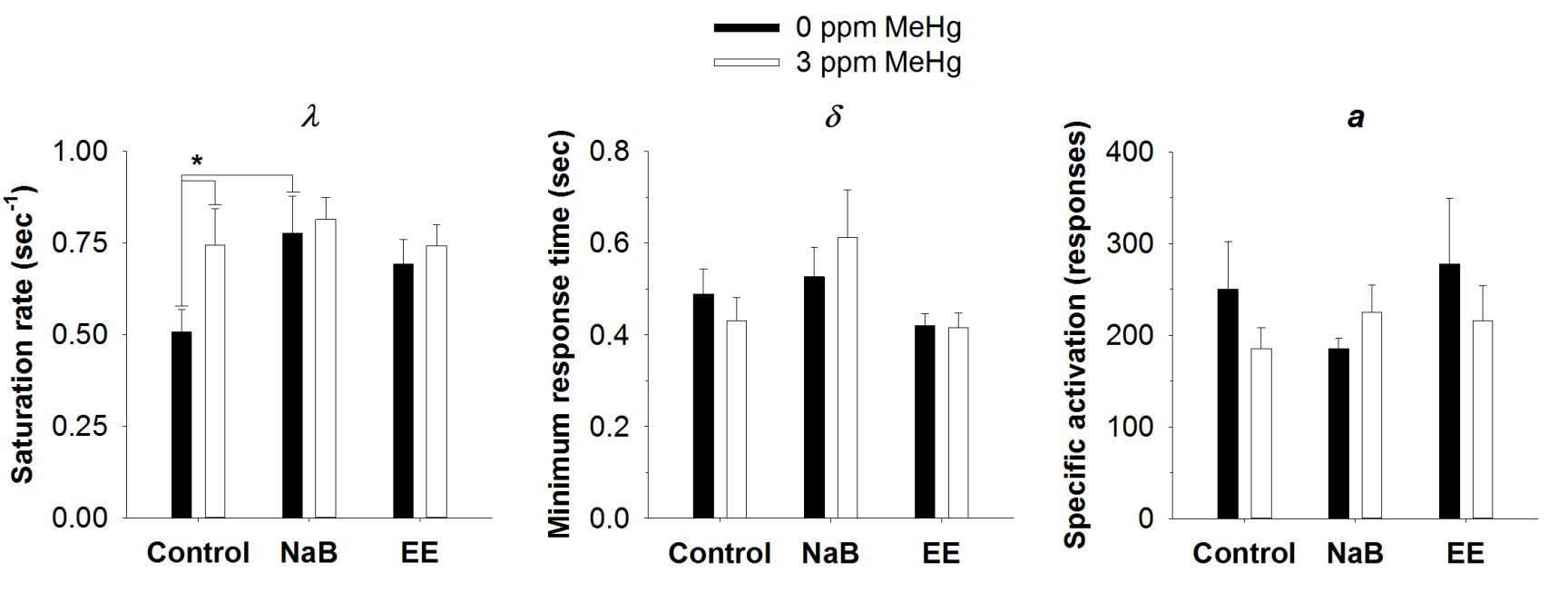

Figure 6. Mean (+SEM) parameter estimates from Eq. 1 for each treatment group. EE = environmental enrichment, NaB = sodium butyrate. *$p < .05$

References

Bakulski, K. M., Lee, H. J., Feinberg, J. I., Wells, E. M., Brown, S., Herbstman, J. B., … Daniele Fallin, M. (2015). Prenatal mercury concentration is associated with changes in DNA methylation at TCEANC2 in newborns. *International Journal of Epidemiology*, *44*(4), 1249–1262. http://doi.org/10.1093/ije/dyv032

Balasubramaniyan, V., Boddeke, E., Bakels, R., Kust, B., Kooistra, S., Veneman, A., & Copray, S. (2006). Effects of histone deacetylation inhibition on neuronal differentiation of embryonic mouse neural stem cells. *Neuroscience*, *143*(4), 939–951. http://doi.org/10.1016/j.neuroscience.2006.08.082

Basu, N., Head, J., Nam, D. H., Pilsner, J. R., Carvan, M. J., Chan, H. M., … Scheuhammer, A. M. (2013). Effects of methylmercury on epigenetic markers in three model species: Mink, chicken and yellow perch. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *157*(3), 322–327. http://doi.org/10.1016/j.cbpc.2013.02.004

Bezzina, G., Body, S., Cheung, T. H. C., Hampson, C. L., Deakin, J. F. W., Anderson, I. M., … Bradshaw, C. M. (2008). Effect of quinolinic acid-induced lesions of the nucleus accumbens core on performance on a progressive ratio schedule of reinforcement: Implications for inter-temporal choice. *Psychopharmacology*, *197*(2), 339–350. http://doi.org/10.1007/s00213-007-1036-0

Boomhower, S. R., & Newland, M. C. (2016). Adolescent methylmercury exposure affects choice and delay discounting in mice. *Neurotoxicology*, *57*, 136–144.

Boomhower, S. R., & Newland, M. C. (2017). Effects of adolescent exposure to methylmercury and d-amphetamine on reversal learning and an extradimensional shift in male mice. *Experimental and Clinical Psychopharmacology*, *25*, 64–73.

Bose, R., Onishchenko, N., Edoff, K., Lang, A. M. J., & Ceccatelli, S. (2012). Inherited effects of low-dose exposure to methylmercury in neural stem cells. *Toxicological Sciences, 130*(2), 383–390. http://doi.org/10.1093/toxsci/kfs257

Cardenas, A., Rifas-Shiman, S., Godderis, L., Duca, R., Navas-Acien, A., Litonjua, A., … Baccarelli, A. (2017). Prenatal Exposure to Mercury: Associations with Global DNA Methylation and Hydroxymethylation in Cord Blood and in Childhood. *Environmental Health Perspectives*, 1–10.

Chambers, R. A., & Potenza, M. N. (2003). Neurodevelopment, impulsivity, and adolescent gambling. *Journal of Gambling Studies*, *19*, 53–84.

Chambers, R. A., Taylor, J. R., & Potenza, M. N. (2003). Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *The American Journal of Psychiatriy*, *160*(6), 1041–1052.

Day, J. J., & Sweatt, J. D. (2012). Epigenetic treatments for cognitive impairments. *Neuropsychopharmacology*, *37*(1), 247–260. http://doi.org/10.1038/npp.2011.85

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience*, *8*(11), 1481–1489. http://doi.org/10.1038/nn1579

Fiorillo, C. D., Tobler, P. N., & Schultz, W. (2003). Discrete Coding of Reward Dopamine Neurons. *Science*, *299*(March), 1898–1902. http://doi.org/10.1126/science.1077349

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L.-H. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature*, *447*(7141), 178–182. http://doi.org/10.1038/nature05772

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., …

Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–3.

Guilarte, T. R., Toscano, C. D., McGlothan, J. L., & Weaver, S. A. (2003). Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. *Annals of Neurology*, *53*(1), 50–56. http://doi.org/10.1002/ana.10399

Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E., & Gage, F. H. (2004). Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *PNAS*, *101*(47), 16659–16664. http://doi.org/10.1073/pnas.0407643101

Hutsell, B. A., & Newland, M. C. (2013). A quantitative analysis of the effects of qualitatively different reinforcers on fixed ratio responding in inbred strains of mice. *Neurobiology of Learning and Memory*, *101*, 85–93.

Johnston, L. D., O'Malley, P. M., Miech, R. A., Bachman, J. G., & Schlenberg, J. G. (2014). *Monitoring the Future national results on adolescent drug use: 1975-2013: Overview key findings on adolescent drug use*. Ann Arbor.

Kheramin, S., Body, S., Herrera, F. M., Bradshaw, C. M., Szabadi, E., Deakin, J. F. W., & Anderson, I. M. (2005). The effect of orbital prefrontal cortex lesions on performance on a progressive ratio schedule: implications for models of inter-temporal choice. *Behavioural Brain Research*, *156*(1), 145–52. http://doi.org/10.1016/j.bbr.2004.05.017

Kilgore, M., Miller, C. A., Fass, D. M., Hennig, K. M., Haggarty, S. J., Sweatt, J. D., & Rumbaugh, G. (2010). Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology*, *35*(4), 870–80. http://doi.org/10.1038/npp.2009.197

Killeen, P. R. (1994). Mathematical principles of reinforcement. *Behavioral and Brain Sciences*,

17(1), 105–172.

Killeen, P. R., & Sitomer, M. T. (2003). MPR. *Behavioural Processes*, *62*(1–3), 49–64.

Kim, H. J., Leeds, P., & Chuang, D. M. (2009). The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. *Journal of Neurochemistry*, *110*(4), 1226–1240. http://doi.org/10.1111/j.1471-4159.2009.06212.x

Koseki, T., Mouri, A., Mamiya, T., Aoyama, Y., Toriumi, K., Suzuki, S., … Nabeshima, T. (2012). Exposure to enriched environments during adolescence prevents abnormal behaviours associated with histone deacetylation in phencyclidine-treated mice. *International Journal of Neuropsychopharmacology*, *15*(10), 1489–501. http://doi.org/10.1017/S1461145711001672

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. http://doi.org/10.1007/s002130000486

National Research Council. (2000). *Toxicological effects of methylmercury*. Washington, D.C.: National Academy Press. http://doi.org/10.17226/9899

Newland, M. C., Paletz, E. M., & Reed, M. N. (2008). Methylmercury and nutrition: Adult effects of fetal exposure in experimental models. *Neurotoxicology*, *29*(5), 783–801.

Newland, M. C., Reed, M. N., & Rasmussen, E. (2015). A hypothesis about how early developmental methylmercury exposure disrupts behavior in adulthood. *Behavioural Processes*, *114*, 41–51.

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

Newman, L., & McGaughy, J. (2011). Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Developmental Psychobiology*, *53*(4), 391–401.

Nielsen, S. J., Aoki, Y., Kit, B. K., & Ogden, C. L. (2015). More than half of US youth consume seafood and most have blood mercury concentrations below the EPA reference level, 2009-2012. *The Journal of Nutrition*, *145*(2), 322–327. http://doi.org/10.3945/jn.114.203786

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews. Neuroscience*, *7*(September), 697–709. http://doi.org/10.1038/nrn1970

Nuydens, R., Heers, C., Chadarevian, A., De Jong, M., Nuyens, R., Cornelissen, F., & Geerts, H. (1995). Sodium butyrate induces aberrant tau phosphorylation and programmed cell death in human neuroblastoma cells. *Brain Research*, *688*(1–2), 86–94. http://doi.org/10.1016/0006-8993(95)00514-Q

Nuzzo, R. (2014). Statistical errors: P values, the "gold standard" of statistical validity, are not as reliable as many scientists assume. *Nature*, *506*(7487), 150–152. http://doi.org/10.1136/bmj.1.6053.66

Obernier, J. A., & Baldwin, R. L. (2006). Establishing an appropriate period of acclimatization following transportation of laboratory animals. *ILAR Journal*, *47*(4), 364–369. http://doi.org/10.1093/ilar.47.4.364

Onishchenko, N., Karpova, N., Sabri, F., Castrén, E., & Ceccatelli, S. (2008). Long-lasting depression-like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. *Journal of Neurochemistry*, *106*(3), 1378–1387. http://doi.org/10.1111/j.1471-4159.2008.05484.x

Paletz, E. M., Craig-Schmidt, M. C., & Newland, M. C. (2006). Gestational exposure to

methylmercury and n-3 fatty acids: Effects on high- and low-rate operant behavior in adulthood. *Neurotoxicology and Teratology*, *28*(1), 59–73.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R. C., … Fischer, A. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science*, *328*(5979), 753–756. http://doi.org/328/5979/753 [pii]\r10.1126/science.1186088

Pope, D. A., Boomhower, S. R., Hutsell, B. A., Teixeira, K., & Newland, M. C. (2016). Chronic cocaine exposure in adolescence: Effects on spatial discrimination reversal, delay discounting, and performance on fixed-ratio schedules in mice. *Neurobiology of Learning and Memory*, *130*, 93–104. http://doi.org/10.1016/j.nlm.2016.01.017

Rane, P., Shields, J., Heffernan, M., Guo, Y., Akbarian, S., & King, J. A. (2012). The histone deacetylase inhibitor, sodium butyrate, alleviates cognitive deficits in pre-motor stage PD. *Neuropharmacology*, *62*(7), 2408–2411. http://doi.org/10.1016/j.neuropharm.2012.01.026

Reed, M. N., Banna, K. M., Donlin, W. D., & Newland, M. C. (2008). Effects of gestational exposure to methylmercury and dietary selenium on reinforcement efficacy in adulthood. *Neurotoxicology and Teratology*, *30*(1), 29–37.

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

Reilly, M. P. (2003). Extending mathematical principles of reinforcement into the domain of behavioral pharmacology. *Behavioural Processes*, *62*, 75–88.

Reolon, G. K., Maurmann, N., Werenicz, A., Garcia, V. A., Schröder, N., Wood, M. A., & Roesler Rafael, R. (2011). Posttraining systemic administration of the histone deacetylase

inhibitor sodium butyrate ameliorates aging-related memory decline in rats. *Behavioural Brain Research*, *221*(1), 329–332. http://doi.org/10.1016/j.bbr.2011.03.033

Robinson, J. F., Theunissen, P. T., van Dartel, D. A. M., Pennings, J. L., Faustman, E. M., & Piersma, A. H. (2011). Comparison of MeHg-induced toxicogenomic responses across in vivo and in vitro models used in developmental toxicology. *Reproductive Toxicology*, *32*(2), 180–188. http://doi.org/10.1016/j.reprotox.2011.05.011

Rozental, R., Faharani, R., Yu, Y., Johnson, J. M., Chan, S. O., & Chiu, F. C. (2004). Sodium butyrate induces apoptosis in MSN neuroblastoma cells in a calcium independent pathway. *Neurochemical Research*, *29*(11 SPEC. ISS.), 2125–2134. http://doi.org/10.1007/s11064-004-6886-9

Sarkar, S., Abujamra, A. L., Loew, J. E., Forman, L. W., Perrine, S. P., & Faller, D. V. (2011). Histone deacetylase inhibitors reverse CpG methylation by regulating DNMT1 through ERK signaling. *Anticancer Research*, *31*(9), 2723–2732.

Sharma, B., & Sharma, P. M. (2013). Arsenic toxicity induced endothelial dysfunction and dementia: Pharmacological interdiction by histone deacetylase and inducible nitric oxide synthase inhibitors. *Toxicology and Applied Pharmacology*, *273*(1), 180–188. http://doi.org/10.1016/j.taap.2013.07.017

Sichert-Hellert, W., Wicher, M., & Kersting, M. (2009). Age and time trends in fish consumption pattern of children and adolescents, and consequences for the intake of long-chain n-3 polyunsaturated fatty acids. *European Journal of Clinical Nutrition*, *63*(9), 1071–1075.

Song, C., Kanthasamy, A., Anantharam, V., Sun, F., & Kanthasamy, a G. (2010). Environmental neurotoxic pesticide increases histone acetylation to promote apoptosis in

dopaminergic neuronal cells: relevance to epigenetic mechanisms of neurodegeneration. *Molecular Pharmacology*, *77*(4), 621–632. http://doi.org/10.1124/mol.109.062174

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*(4), 417–463.

Stairs, D., & Bardo, M. (2009). Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. *Pharmacology Biochemistry and Behavior*, *92*(3), 377–382. http://doi.org/10.1016/j.pbb.2009.01.016.Neurobehavioral

Teicher, M. H., Andersen, S. L., & Hostetter, J. C. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Developmental Brain Research*, *89*, 167–172.

Wang, H., Cheng, H., Wang, K., & Wen, T. (2012). Different effects of histone deacetylase inhibitors nicotinamide and trichostatin A (TSA) in C17.2 neural stem cells. *Journal of Neural Transmission*, *119*(11), 1307–1315. http://doi.org/10.1007/s00702-012-0786-y

Xu, W. S., Parmigiani, R. B., & Marks, P. (2007). Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene*, *26*, 5541–5552. http://doi.org/10.1038/sj.onc.1210620