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Methylphenidate alleviates manganese-induced impulsivity but not distractibility

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ABSTRACT

Recent studies from our lab have demonstrated that postnatal manganese (Mn) exposure in a rodent model can cause lasting impairments in fine motor control and attention, and that oral methylphenidate (MPH) treatment can effectively treat the dysfunction in fine motor control. However, it is unknown whether MPH treatment can alleviate the impairments in attention produced by Mn exposure. Here we used a rodent model of postnatal Mn exposure to determine whether (1) oral MPH alleviates attention and impulse control deficits caused by postnatal Mn exposure, using attention tasks that are variants of the 5-choice serial reaction time task, and (2) whether these treatments affected neuronal dendritic spine density in the medial prefrontal cortex (mPFC) and dorsal striatum. Male Long-Evans rats were exposed orally to 0 or 50 Mn/kg/d throughout life starting on PND 1, and tested as young adults (PND 107–115) on an attention task that specifically tapped selective attention and impulse control. Animals were treated with oral MPH (2.5 mg/kg/d) throughout testing on the attention task. Our findings show that lifelong postnatal Mn exposure impaired impulse control and selective attention in young adulthood, and that a therapeutically relevant oral MPH regimen alleviated the Mn-induced dysfunction in impulse control, but not selective attention, and actually impaired focused attention in the Mn group. In addition, the effect of MPH was qualitatively different for the Mn-exposed versus control animals across a range of behavioral measures of inhibitory control and attention, as well as dendritic spine density in the mPFC, suggesting that postnatal Mn exposure alters catecholaminergic systems modulating these behaviors. Collectively these findings suggest that MPH may hold promise for treating the behavioral dysfunction caused by developmental Mn exposure, although further research is needed with multiple MPH doses to determine whether a dose can be identified that ameliorates the dysfunction in both impulse control and selective attention, without impairing focused attention.

1. Introduction

Studies of children and adolescents have linked late prenatal and early postnatal manganese (Mn) exposure with inattention, impulsivity, hyperactivity, oppositional behaviors, and impaired fine motor control (Bhang et al., 2013; Bouchard et al., 2007; Claus Henn et al., 2010; Crinella, 2003; Ericson et al., 2007; Farias et al., 2010; Lucchini et al., 2012; Oulhote et al., 2014; Sanders et al., 2015; Takser et al., 2004). Similarly, animal studies have reported that early postnatal Mn exposure causes abnormalities in behavior, learning/memory, and locomotor activity (Golub et al., 2005; Kern et al., 2010; McDougall et al., 2008), but until recently none had established impacts on attention and fine motor function to corroborate the associations reported in the human studies. However, our recent reports provided the first evidence that early postnatal Mn exposure can cause lasting disruption of attentional and fine motor function, with specific impairments in attentional preparedness, selective attention, and arousal regulation, and that the presence and severity of these deficits varied with the dose and duration of Mn exposure (Beaudin et al., 2017, 2013).

The effects of developmental Mn exposure on attentional function are particularly important, because attention is one of the three major co-active processes of the working brain (along with memory and activation), upon which most other cognitive functions depend (Bell

Abbreviations: MPH, methylphenidate

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and Deater-Deckard, 2007). Moreover, attentional dysfunction, including attention deficit hyperactivity disorder (ADHD), is the most prevalent neurodevelopmental disorder among children, affecting ~5–10% of all U.S. children between 6 and 17 years of age, with 2–3-times more males affected than females (Feldman and Reiff, 2014; Kaiser et al., 2015; Willcutt, 2012). ADHD encompasses three subtypes (American Psychiatric Association, 2013): (1) ADHD predominantly inattentive (ADHD-I); (2) ADHD predominantly hyperactive-impulsive (ADHD-H), and (3) ADHD combined type (ADHD-C). The ADHD-I subtype is the most prevalent subtype, affecting 5.1–5.7% of children up through 18 years. The etiology of attentional dysfunction (including ADHD) is unclear, although studies suggest that it is associated with hypo-functioning of catecholaminergic systems within the cortico-striatal loop (Arnsten, 2010; Brennan and Arnsten, 2008), and that its incidence is increased by exposure to environmental agents such as cigarette smoke, Mn, lead, alcohol, and PCBs (Abbott and Winzer-Serhan, 2012; Beaudin et al., 2007; Braun et al., 2006; Burt, 2009; Crinella, 2003; Eubig et al., 2010; Newman et al., 2007).

Methylphenidate (MPH), an inhibitor of the dopamine and norepinephrine transporters, is one of the drugs most commonly used to treat ADHD in children and adolescents (Robison et al., 1999; Wigal et al., 2010; Zito et al., 2000). Studies have shown that therapeutic doses of MPH not only improve symptoms of inattention and impulsivity in humans and animal models of ADHD (Blum et al., 2011; Cao et al., 2012; Kantak et al., 2008; Mohamed et al., 2011; Zhu et al., 2007), but that they also ameliorate manual skill impairment in ADHD children with co-existing developmental coordination disorder (ADHD/DCD) (Bart et al., 2013). Consistent with this latter finding, our recent study showed that oral MPH fully alleviated the fine motor dysfunction caused by Mn exposure in a rodent model (Beaudin et al., 2015).

The present study was designed to determine whether oral methylphenidate (MPH, Ritalin) also effectively alleviates the impairments in attention and impulse control caused by postnatal Mn exposure, using attention tasks that are variants of the 5-choice serial reaction time task (5-CSRTT). The attention tasks are well-accepted animal homologues of clinical tests used to assess MPH effects on attentional function and inhibitory control in children and adults with ADHD (e.g., Bari et al., 2008; Robbins, 2002). The phenotype of lasting behavioral dysfunction exhibited in our animal model of early postnatal Mn exposure, including deficits in selective and focused attention, arousal regulation, and fine motor function (Beaudin et al., 2017, 2013), is consistent with clinical evidence showing that children with attentional problems often perform poorly on motor skills tests (Brossard-Racine et al., 2012; Fliers et al., 2010; Lavasani and Stagnitti, 2011; Pitcher et al., 2003; Watemberg et al., 2007). In light of our prior study showing that oral MPH fully alleviated the fine motor deficits caused by elevated Mn exposure (Beaudin et al., 2015), we hypothesized that MPH would also effectively treat the attentional dysfunction of the Mn-exposed animals, thereby provide evidence that catecholaminergic dysfunction contributed to those Mn deficits.

2. Methods

2.1. Subjects

Forty Long-Evans male rats were used in the study. All subjects were born at the University of California, Santa Cruz over a 2 day period from 18 primiparous pregnant Long-Evans rats (acquired at gestational day 18; Charles River, USA). Twelve–24 hours after parturition (designated PND 1, birth = PND 0), litters were sexed, weighed, and culled to eight pups per litter such that each litter was comprised of 5–6 males per litter and the remainder females. Litters were balanced by treatment so that only one male/litter was assigned to a particular treatment condition. The study used a 2 × 2 factorial design, with the four treatment groups designated as Control + Vehicle, Mn + Vehicle, Control + MPH, and Mn + MPH (n = 10 males/treatment).

Animals (dams and weaned pups) were fed Harlan Teklad rodent chow #2018 (reported by the manufacturer to contain 118 mg Mn/kg), and housed in polycarbonate cages at a constant temperature (21 ± 2 °C). Animals were maintained on a reversed 10:14 h light/dark cycle with lights off at 6:00 AM and on at 8:00 PM. After weaning on PND 22 animals were pair-housed by treatment group assignment. Animals were weighed daily throughout the study. All aspects of testing and feeding were carried out during the active (dark) phase of the animals' diurnal cycle. The decision to test only males was based on the evidence that males are more sensitive than females to developmental Mn neurotoxicity (Kern et al., 2010; Lucchini et al., 2012; Takser et al., 2003), and attentional dysfunction is 2–3-times more prevalent in boys than girls (Feldman and Reiff, 2014; Willcutt, 2012). All animal care and treatments were approved by the institutional IACUC, and adhered to NIH guidelines set forth in the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Animals were food restricted starting on PND 45 in preparation for behavioral testing, as described previously (Beaudin et al., 2015, 2017, 2013). Briefly, animals were placed in individual feeding cages and provided a measured amount of food each day, ranging from 14 to 17 g as the animals grew, so that their body weights were maintained at ~90–95% of free-feeding weights. Animals were fed daily immediately after completing behavioral testing and allowed 2 h to consume their daily food allotment. Throughout the study, the amount of food provided was altered on an individual basis if there was evidence of aberrant weight loss or gain.

2.2. Mn exposure protocol

Neonatal rats were orally exposed to Mn doses of 0 or 50 mg Mn/kg/d starting on PND 1 throughout life. For dosing during PND 1–21, Mn was delivered once daily directly into the mouth of each pup (~20 μL/dose) via a micropipette fitted with a flexible polyethylene pipet tip (Fisher Scientific, Santa Clara, CA, USA). Control animals received the vehicle solution. For this, a 225 mg Mn/mL stock solution of $MnCl_2$ was prepared by dissolving $MnCl_2{\cdot}4H_2O$ with Milli-Q™ water; aliquots of the stock solution were diluted with a 2.5% (w/v) solution of the natural sweetener stevia to facilitate oral dosing of the pups. Oral Mn exposure post-weaning (PND 22 – end of study) occurred via the animals' drinking water. For this, a 42 mg Mn/mL stock Mn solution was prepared fresh weekly as above and diluted with tap water to a final concentration of 420 μg Mn/mL in a polycarbonate carboy. The stock solutions were made fresh weekly, and water bottles were refilled with fresh water two to three-times per week. Water bottle weights were recorded at refilling to determine water intake per cage, and daily Mn intake per kg body weight was estimated based on daily measured body weights of the two rats housed per cage. Drinking water Mn concentrations were adjusted weekly as needed to maintain target daily oral Mn intake levels of 50 mg/kg/d based on measured water intake rates. This Mn exposure regimen is relevant to children exposed to elevated Mn via drinking water, diet, or both; pre-weaning exposure to 50 mg Mn/kg/d produces a relative increase in Mn intake that approximates the increase reported in infants and young children exposed to Mn-contaminated water or soy-based formulas (or both) (Beaudin et al., 2017, 2013; Kern et al., 2010; Kern and Smith, 2011). Chronic oral exposure to the same daily Mn dose was maintained after weaning via drinking water, to model the situation where children may continue to suffer chronic elevated Mn exposures from a variety of environmental sources (e.g., contaminated well water, dust, etc.) (Bouchard et al., 2011; Lucas et al., 2015; Oulhote et al., 2014).

2.3. Methylphenidate treatment

Methylphenidate hydrochloride (MPH) (Sigma-Aldrich Inc., St-Louis, MO) was administered orally once per day over a 16 day drug treatment period. Doses of 0 or 2.5 mg MPH/kg/d were administered