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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 mg 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

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dose can be identified that ameliorates the dysfunction in both impulse control and selective attention, without impairing focused attention.

Keywords

Manganese; methylphenidate; Ritalin; attention; impulsivity

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 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 yrs 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; Neuman 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.

Methods

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 hrs 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 14–17 grams 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 hrs 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.

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).

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 each day ~1 hr before behavioral testing using a food wafer delivery method, as described previously (Beaudin et al. 2015; Ferguson and Boctor 2009). Briefly, cookie wafers (Mini-Vanilla, Nabisco Inc.) were quartered and adulterated with

vehicle or MPH solution (~20 – 30 μL) to achieve the targeted daily drug dose per animal. The MPH solution was prepared fresh each day by dissolving the drug in normal saline solution. The vehicle and MPH-adulterated pieces of wafer were then placed in 24-well plates pre-labelled with the animal's identification number and specific time of delivery. At delivery, the pieces of wafer were transferred into individual food cups pre-labelled with the subject's ID number before being placed onto the floor of the animal's individual holding cage. Experimenters delivering the food cups confirmed that the rat ingested the entire wafer piece, which typically occurred within 10 s of delivery. This, or very similar, dosing regimens of MPH have been shown to be safe to adolescent rats and juvenile monkeys, to produce a blood MPH half-life of ~2 h in adult rats, and to improve learning and symptoms of inattention and impulsivity in animal models (Cao et al. 2012; Kuczenski and Segal 2002; Mohamed et al. 2011; Rodriguez et al. 2010; Thanos et al. 2015; Zhu et al. 2007), thereby mimicking the human pharmacokinetic profile and clinical use of MPH.

Testing apparatus

Eight identical automated MED Associates 5-CSRTT testing chambers (#MED-NP5L-OLF, Med Associates, Inc., St Albans, VT) were used to assess specific cognitive processes, including focused and selective attention, and inhibitory control, as described previously (Beaudin et al., 2016). Briefly, each testing chamber contained a curved aluminum wall equipped with five 2.5 × 2.5 cm response ports positioned 2 cm above the grid floor. Each port was fitted with a light-emitting diode that served as the visual cue, an infrared beam to register nose pokes, and pneumatic inlet and vacuum outlet ports to introduce and remove air-based odor distractors. Opposite the response wall was the food magazine wall that contained a 45 mg food pellet dispensing port fitted with an infrared beam to register nose pokes. The two side walls and ceiling were polycarbonate, and the floor was a grid of stainless steel rods. Each unit also contained a small house light and was enclosed in a sound attenuating cubicle.

Behavioral Testing

Behavioral testing began on ~PND 67, with food magazine and nose poke training for 1 week followed by two five-choice visual discrimination tasks with a fixed cue duration of 15 s and 1.0 s and no pre-cue delay. This was immediately followed by a series of attention tasks, including two focused attention tasks, and a selective attention task with olfactory distractors, as described below. The two focused attention tasks were administered during PND 85 – 106, following completion of the visual discrimination tasks. The first focused attention task, administered for 12 sessions (one test session/day), used variable pre-cue delays of 0, 3, 6, or 9 s and a fixed visual cue duration of 1 s. The second focused attention task, administered for 10 sessions, used variable pre-cue delays of 0, 3, or 6 s and variable visual cue durations of 0.5 or 1.0 s. The focused attention tasks are similar to tasks used in human and animal studies of attentional function and dysfunction (Robbins 2002; Winstanley et al. 2006); results from those tasks will be presented elsewhere.

All rats were weighed and tested 6 days/week throughout training and testing. Behavioral assessment occurred during the active (dark) period of the diurnal cycle at the same time each day and in the same chamber for each individual rat. A daily test session consisted of

150 trials or 60 minutes, whichever came first. Each trial sequence was initiated by the animal with a nose-poke in the food magazine port, followed by a 3 s turnaround time to allow the animal to reorient from the food magazine wall to the response wall; trial onset began after the 3 s turnaround time. All behavioral training and testing was conducted by individuals blind to the treatment condition of the subjects. All animals were maintained on a food restriction schedule with water available *ad lib* throughout behavioral assessment, as described previously (Beaudin et al. 2015, 2017, 2013).

Selective attention task with olfactory distracters

Selective attention can be defined as the ability to maintain a behavioral or cognitive set in the face of distracting or competing environmental stimuli (Petersen and Posner 2012). The final two tasks administered, following the focused attention tasks, were (1) the baseline attention task and (2) the selective attention task with olfactory distractors. These two tasks were administered for three test sessions (PND 107–109) and six test sessions (PND 110–115), respectively. The baseline attention task included variable pre-cue delays (3 or 4 s), and variable visual cue durations (0.5 or 1.0 s), both balanced across trials in each session. This task was followed by the selective attention task; the selective attention task was identical to the baseline attention task except that on one third of the trials in each session an olfactory distractor was presented 1 or 2 s after trial onset (i.e., 1 – 3 s before the visual cue).

Nine different scents were used as olfactory distractors; odorant distractors were prepared from pure liquid extracts of anise, maple, almond, peppermint, rum, orange, butter, cinnamon, and coconut (McCormick & Company, Inc., MD USA). Different concentrations of the pure liquid extracts ranging from 2.5% to 10% (v/v) were diluted with propylene glycol in a final volume of 200 mL liquid odorant. Twenty-five mL of the final solution was transferred into individual odor delivery jars mounted on the olfactory module on the outside wall of the sound attenuating cubicle housing the 5-CSRTT chamber. Scented air was delivered for ~1 s into a response port within the chamber, with the condition that the visual cue response port and olfactory distractor response port never coincided within a trial. Delivery of scented air used air pumps and computer-controlled solenoid valves for each chamber. Each response port contained an air inlet port and air evacuation port. During odor delivery, a vacuum pump fitted to the air evacuation port within the response port evacuated the scented air so that it remained within the area of the response port and did not permeate the greater testing chamber space.

Behavioral dependent measures

Recorded response types for attention tasks included premature responses as a measure of impulse control (responses made after trial onset but before presentation of the visual cue); correct responses as a measure of attentional accuracy (responses made to the correct port following presentation of the visual cue); incorrect responses (responses made to an incorrect port following presentation of the visual cue); and omission errors (failure to respond to any port within the 10 s response interval following presentation of the visual cue). Premature and incorrect responses and omission errors were not rewarded and were immediately followed by a 5 s ‘time-out’, in which the house light was turned off for 5 s. Perseverative responses, defined as responses made into the correct port after a correct

choice, were also tallied on each trial in a session. In addition, the latency for correct responses was recorded, as was the latency to retrieve the food pellet reward following correct responses. The calculated response outcomes were *percent accuracy*, calculated as # correct responses / (correct + incorrect) × 100; *percent premature*, calculated as # premature responses / (correct + incorrect + premature + omissions) × 100; *percent omissions*, calculated as # omissions / (correct + incorrect + premature + omissions) × 100. *Perseverative responses* were calculated as the total number of responses made into the correct port after a correct choice, across all trials in a test session.

Spine density analysis

Twenty-four hours after the final MPH dose, rats were deeply anesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were extracted, rinsed with Milli-Q water, and then prepared for Golgi–Cox staining using the rapid Golgistain kit (FD Neuroethologies, Inc., Ellicott City, MD). For this, the brains were placed in a Golgi–Cox solution and stored at room temperature in the dark for 14 d followed by 3 d in a 30% sucrose solution. Brains were cut into 250 μm coronal sections using a vibratome, mounted on gelatin-coated slides, and allowed to dry naturally at room temperature before staining within the next 12–24 hrs.

To be included in the analysis of spine density, the dendritic branch of a give neuron had to be well-impregnated and free of stain precipitations, blood vessels, and astrocytes. The medial PFC and dorsal striatum brain regions of interest were identified at 10× magnification using a Leica DM5500B widefield microscope fitted with a motorized stage and multi-point image acquisition. These two brain regions were selected for analysis because of the important role of the cortico-striatal loop in the control of attentional, impulse control, and motor response functions (Arnsten 2010; Brennan and Arnsten 2008). In the mPFC, five layer III pyramidal cells were selected from each hemisphere in the Cg 1 and 2 cortical areas (Paxinos and Watson 1998). Dendritic spines were counted live at 100× magnification from an ~50 μm segment of a single terminal tip (third-order) apical dendrite from each pyramidal cell, with the condition that the entire dendritic segment was within the focal plane range of the microscope. The exact length of counted dendrite was determined using the length measurement tool function of the microscope. Spine density was calculated as the number of spines per 10 μm of dendrite length. Spine density on terminal tip apical dendrites of medium-sized spiny neurons in the medial dorsal striatum was determined in the same manner, with the exception that ~20 – 30 μm dendritic segments were counted. All neuronal cell selection and spine counting were done by individuals blind to the treatment conditions of the rats.

Determination of blood and brain Mn levels

Blood and brain Mn concentrations were determined in the study animals at the completion of all behavioral testing (~PND 145). A prior study in a separate cohort of animals determined blood and brain Mn concentrations under the same Mn exposure conditions at PND 24 and PND 66 (7 – 8/treatment group and time point). In both cases, animals were euthanized via sodium pentobarbital overdose (75 mg/kg i.p.) and exsanguination, and whole blood (2 – 3 mL) was collected from the left ventricle of the surgically-exposed heart

and stored in EDTA Vacutainers at −20 °C for analyses. Whole brain was immediately removed, and the hind-brain region collected and stored at −80 °C for Mn concentration determinations. Tissues were processed for Mn concentrations using trace metal clean techniques, as previously described (Kern et al. 2010). Briefly, aliquots of whole blood were digested overnight at room temperature with 16N HNO_3 (Optima grade, Fisher Scientific), followed by addition of H_2O_2 and Milli-Q™ water. Digestates were centrifuged (15,000 × g for 15 min.) and the supernatant used for Mn analysis. For brain, aliquots of homogenized hind brain tissue (~200 mg wet weight) were dried then digested with hot 16N HNO_3, evaporated and redissolved in 1N HNO_3 for analyses. Manganese levels were determined using a Thermo Element XR inductively coupled plasma – mass spectrometer, measuring masses ^{55}Mn and ^{103}Rh, with rhodium added to samples as an internal standard. External standardization for Mn used certified SPEX standards (Spex Industries, Inc., Edison, NJ). National Institutes of Standards and Technology SRM 1577b (bovine liver) was used to evaluate procedural accuracy. The analytical detection limit for Mn in blood and brain was 0.04 and 0.015 ng/mL, respectively.

Statistical methods

The behavioral data were modeled by way of structured covariance mixed models (SAS Proc Glimmix with normal errors). The fixed effects of Mn exposure (0 and 50 mg Mn/kg/d) and MPH treatment (0 and 2.5 mg MPH/kg/d) were included in all models. In addition and depending on the outcome analyzed, the fixed effects of pre-cue delay, cue duration, session block, and distractor condition were also included. Session block represents a defined block of test session days, in which the total number of test sessions (e.g., 12) are divided into equal test session blocks (e.g., two session blocks of six test sessions per block). In all models, rat was used as the random effect to account for correlations of observations from the same animal. Statistical tests used a Sattherwaite correction. Plots of residuals by experimental conditions were used to examine the assumption of homogeneity. Additional random effects (variance components) for experimental conditions with high variance in the residuals across the levels of the factor (e.g. distractor condition) were added to achieve homogeneity. The distribution of each random effect was inspected for approximate normality and presence of influential outliers. The spine density data were analyzed similarly with the same Mn and MPH main effects, with the addition of brain region as a within-animal factor. Blood and brain Mn data were analyzed using a two-way ANOVA (SAS Proc GLM) and Tukey's post hoc test for pairwise comparisons. Data were log transformed before analysis if necessary to achieve normal distribution and variance homogeneity.

The significance level was set at $p \leq 0.05$, and p-values between 0.05 and 0.10 were considered to be trends and are presented if the pattern of findings aided in clarifying the nature of the Mn and MPH effects. Significant main effects or interaction effects were followed by single-degree of freedom contrasts. All analyses were conducted using SAS 9.4 for Windows on a mainframe computer or JMP 11.0 (SAS Institute, Cary, NC, USA).

Results

The results provided evidence for significant adverse effects of postnatal Mn exposure on impulse control (premature responses) and attention (response accuracy), as well as evidence that oral MPH treatment alleviated the Mn-induced dysfunction in impulse control but not attention. Furthermore, the results provided widespread evidence that the Mn-exposed animals responded differently than controls to MPH treatment, as seen in the measures of impulse control, perseverative responses, and attention (response accuracy and omission errors). We report first the evidence for adverse effects of Mn exposure, by comparing the Mn+Veh and Control+Veh groups, followed by comparison of the Mn+MPH and Control +Veh groups to determine whether MPH treatment alleviated the Mn dysfunction. Finally, the hypothesis that the Mn-exposed animals responded differently to MPH than the vehicle-treated animals was assessed by determining whether the statistical interaction of MPH and Mn exposure was significant; if significant, the nature of the MPH effect in the control and Mn-exposed groups was compared. Note that the statistical models for each dependent variable (e.g., premature responses, response accuracy) contained the same independent variables (Mn, MPH, distractor condition, pre-cue delay, cue duration, and session block), but only those independent variables that showed a statistical interaction with Mn and/or MPH are presented in the figures.

Mn exposure increased premature responses and impaired attentional accuracy, but did not affect perseverative responses or omission errors

Premature responses—In the baseline attention task, which did not involve olfactory distractors, there was no effect of Mn exposure on the percentage of premature responses; the Mn+Veh and Control+Veh groups did not differ ($p=0.43$). The interaction of Mn × MPH also was not significant ($F(1, 34)=0.31$, $p=0.58$) (Figure 1A).

The percentage of premature responses in the selective attention task was significantly increased with the presentation of olfactory distractors in all groups, as evidenced by a significant main effect of distraction condition ($F(2, 105)=136.96$, $p < 0.0001$), with the 1 s distraction condition (distractor presented 1 s into the trial; i.e., 2–3 s before the visual cue) causing the greatest increase in premature responses relative to the non-distraction condition ($p<0.0001$; Figure 1B). Further, there was a significant distraction condition × session block interaction ($F(2, 320)=37.47$, $p<0.0001$), reflecting that premature responses declined more over the two session blocks for the distraction than for the non-distraction condition (Figure 1B).

Although the distractors increased premature responding for both the control and Mn-exposed groups, the increase was greater for the Mn-exposed animals, reflecting their impaired impulse control in response to the distractors, relative to controls. This inference is supported by the significant 4-way interaction of Mn exposure × MPH treatment × distraction condition × session block ($F(2, 320)=8.99$, $p=0.0002$), followed by specific contrasts. Specifically, the percentage of premature responses was higher in the Mn+Veh group than Control+Veh in session blocks 1 and 2 for the 1 s distraction condition (p's = 0.008 and 0.03, respectively), and in session block 1 for the 2 s distraction condition ($p=0.01$) (Figure 1B). Note that there was no effect of Mn exposure on premature responses

for the non-distraction trials of the selective attention task (p's = 0.87 and 0.95 for session blocks 1 and 2, respectively; Figure 1B), indicating that the increased impulsivity of the Mn-exposed rats was specific to the distraction trials, and was most evident on the 1 s distraction condition trials, the condition in which the distractor most disrupted performance.

Perseverative response—Perseverative responses (defined as a nosepoke into the correct port after a correct response), were made on ~10% of the correct response trials. The analysis of perseverative responses revealed a borderline interaction of Mn exposure × MPH in the baseline attention task ($F(1, 32)=3.58$, $p=0.06$), and a significant interaction of Mn exposure × MPH treatment in the selective attention task ($F(1, 46)=5.85$, $p=0.01$) (Figure 2A, B). These interactions reflect the fact that, although Mn exposure alone did not affect perseverative responses in either task (i.e., Control+Veh vs Mn+Veh, p's>0.5 for both tasks), the control and Mn-exposed groups responded differently to MPH treatment (discussed below).

Response accuracy—In the baseline attention task (no olfactory distractors), the analysis of response accuracy revealed a significant main effect of visual cue duration ($F(1, 32)=70.56$, $p<0.0001$), reflecting that the briefer visual cues increased the attentional demands of the task, as intended (Figure 3A). In addition, there was a significant 3-way interaction of Mn exposure × MPH treatment × cue duration ($F(1, 32)=4.06$, $p=0.05$). As seen in Figure 3A, Mn exposure did not affect response accuracy in the absence of the drug, as indicated by contrasts comparing the Mn+Veh and Control+Veh groups for trials with a 0.5 s or a 1.0 s visual cue duration (p's = 0.36 and 0.81, respectively) (Figure 3A). The interaction was driven by a differential response of the Mn and control groups to the MPH treatment (discussed below).

In the selective attention task (Figure 3B), there was a significant main effect of distraction condition on response accuracy ($F(2, 105)=179.54$, $p<0.0001$), reflecting the marked reduction in attentional accuracy in all groups caused by the presentation of olfactory distractors, with the 2 s distractor condition causing the greatest impairment in response accuracy versus the non-distraction trials ($p<0.0001$). In addition, there was a significant interaction of Mn exposure × MPH treatment × distraction condition ($F(2, 105)=9.90$, $p=0.0001$). This interaction was driven in part by a trend for Mn exposure to *lower* response accuracy *only* in the 2 s distraction condition (Control+Veh vs Mn+Veh, $p=0.07$) (Figure 3B); there were no effects of Mn exposure on response accuracy in the 1 s distraction or the non-distraction condition trials (Control+Veh vs Mn+Veh, p's = 0.49 and 0.30, respectively). This interaction was also driven by the fact that the Mn and control groups responded differently to MPH treatment in the non-distraction condition trials (discussed below).

Omission errors—In the baseline attention task, which did not involve olfactory distractors, there was no effect of Mn exposure on the percentage of omission errors; the Mn +Veh and Control+Veh groups did not differ ($p=0.15$). The interaction of Mn × MPH also was not significant ($F(1, 33)=0.33$, $p=0.57$).

The analysis of percentage omission errors in the selective attention task revealed a main effect of distraction condition ($F(2, 43)=11.37$, $p=0.0001$); in all groups the incidence of

omission errors was significantly *higher* for the non-distraction condition than for the distraction conditions (Figure 4). This pattern likely reflects the substantial increase in premature responses caused by the presentation of the olfactory distractors, reflecting disinhibition of responding. Because premature responses occur, by definition, very early in the trial (before the visual cue is presented), the high rate of such responses necessarily means that errors committed later in the trial, such as omissions errors, cannot be made. Thus, trial conditions that result in a premature response (i.e., increased impulsivity) preclude the assessment of sustained attention errors, as was the case here.

There was also a trending 3-way interaction between Mn exposure × MPH treatment × distraction condition ($F(2, 43)=2.54$, $p=0.09$) (Figure 4). Contrasts revealed that Mn exposure (in the absence of MPH) did not affect omission errors, with the Control+Veh and Mn+Veh groups exhibiting similar omission error rates for all distraction conditions (p's = 0.67, 0.63, and 0.97 for no distractor, 1 s, and 2 s distraction conditions, respectively) (Figure 4). Instead, as described below, the trending Mn interaction arose from the differential effect of MPH treatment on Mn versus control groups for non-distraction trials.

Oral MPH treatment alleviated the impulse control, but not the attention deficits caused by Mn exposure

There were two instances in which the Mn-exposed animals were significantly impaired relative to controls: (1) The Mn-exposed animals exhibited *increased* premature responses on the 1 s and 2 s distraction condition trials (Figure 1B), and (2) they tended to have *lower* response accuracy on the 2 s distraction condition trials, as reported above (Figure 3B).

Oral MPH treatment alleviated the Mn deficit in impulse control in session block 1 of the selective attention task, in both the 1 and 2 s distraction conditions. The Mn+MPH group committed a *similar* rate of premature responses as the Control+Veh group for the 1 s and 2 s distraction condition trials during session block 1 (p's = 0.74 and 0.79, respectively), and significantly *fewer* premature responses than the Mn+Veh group for those two distraction conditions during session block 1 (p's = 0.01 and 0.02 respectively) (Figure 1B). The treatment efficacy of MPH did not persist into session block 2 of the 1 s distraction condition trials. Although the mean percentage of premature responses for the Mn+MPH group was lower than for the Mn+Veh group, this difference was not significant ($p = 0.57$), and the Mn+MPH group committed significantly *more* premature responses than the Control +Veh group ($p = 0.05$) (Figure 1B).

In contrast, oral MPH treatment *did not* alleviate the trending Mn deficit in attentional accuracy seen in the 2 s distraction condition in the selective attention task. In this condition, the response accuracy of the Mn+MPH group was significantly *lower* than that of the Control+Veh group ($p=0.01$), and similar to that of the Mn+Veh group ($p=0.83$) (Figure 3B).

Mn-exposed rats responded differently than controls to MPH in several behavioral measures

There were numerous instances across many of the behavioral outcomes in which the Mn-exposed animals responded differently than controls to MPH treatment. A differential drug response was seen for premature responses, perseverative responses, accurate responses, and

omission errors, and was seen in both the baseline attention and selective attention tasks. This inference was based on findings of a significant interaction of MPH and Mn exposure, followed by specific contrasts comparing the MPH effect in the control and Mn-exposed groups.

Premature responses—As noted above, the analysis of percentage premature responses for the selective attention task revealed a significant 4-way interaction of Mn exposure × MPH treatment × distraction condition × session block ($F(2, 320)=8.99$, $p=0.0002$). Contrasts revealed that the nature of the drug effect was very different for the Mn and control rats; in the 1 s distraction condition during session block 1 (the testing condition in which animals generally exhibited the highest rates of premature responses), MPH treatment significantly *increased* premature responses in the control animals ($p=0.02$; Figure 1B). In contrast, the drug significantly *reduced* premature responses in the Mn-exposed animals for the 1 s and the 2 s distraction conditions during session block 1 (p's = 0.01 and 0.03, respectively) (Figure 1B). These differential effects of MPH treatment on control and Mn-exposed animals occurred only during session block 1 of testing. As a result of this differential effect of MPH treatment on premature responses in the control versus Mn-exposed groups, the Mn+MPH group committed significantly *fewer* premature responses than the Control+MPH group for the 1 s distractor condition during session block 1 ($p=0.04$; Figure 1B).

Perseverative responses—As noted above, the analysis of perseverative responses revealed a borderline interaction of Mn exposure × MPH in the baseline attention task ($F(1, 32)=3.58$, $p=0.06$), and a significant interaction of Mn exposure × MPH treatment in the selective attention task ($F(1, 46)=5.85$, $p=0.01$) (Figure 2A, B). These interactions reflect the fact that the control and Mn-exposed groups responded differently to MPH treatment. In both tasks, perseverative responses in the Mn and control animals did not differ under the non-drug (vehicle) condition (p's = 0.53 and 0.89 for the baseline and selective attention tasks, respectively). However, in both tasks MPH reduced perseverative responding in the Mn-exposed animals, and increased it in the controls; specifically, in the baseline attention task MPH treatment tended to *reduce* perseverative responses in the Mn-exposed animals (Mn+Veh vs Mn+MPH, $p=0.07$), while in the selective attention task MPH treatment significantly *increased* perseverative responses by the control animals (Control+Veh vs Control+MPH, $p=0.01$) (Figure 2A, B). As a result, the Mn+MPH animals committed significantly fewer perseverative responses than their Control+MPH counterparts (p's = 0.04 and 0.0007 for the baseline and selective attention tasks, respectively) (Figure 2A, B).

Response accuracy—As noted above, both tasks revealed significant interactions involving Mn and MPH treatment, providing further evidence that attentional function was affected differently by MPH treatment in the Mn versus control groups. In the baseline attention task trials with the more demanding cue condition (0.5 s visual cue duration), MPH treatment significantly lowered response accuracy in the Mn-exposed animals ($p = 0.05$), but tended to increase it in the controls, with the result that response accuracy was significantly *lower* in the Mn+MPH group compared to the Control+MPH group (p's= 0.01) (Figure 3A).

The selective attention task also revealed evidence of a differential effect of MPH treatment on Mn versus control groups, specifically for trials with no distractor. For these trials, MPH significantly reduced response accuracy in the Mn-exposed animals (compared to the Mn +Veh group; p = 0.007) (Figure 3B), but did not affect accuracy in control animals (Control +MPH vs Control+Veh, p=0.36). As a result, the Mn and control groups did not differ in the vehicle condition, but did differ in response accuracy with MPH treatment (Mn+MPH vs. Control+MPH, p= 0.01) (Figure 3B).

Omission errors—As noted above, a trending 3-way interaction of Mn exposure × MPH treatment × distraction condition was seen for omission errors in the selective attention task (p=0.09, reported above). This trending interaction was driven in part by the fact that for non-distraction trials, MPH treatment significantly *increased* omission errors in Mn-exposed animals (p's = 0.01), but did not affect omission errors in the controls. As a result, although the Mn-exposed and control animals did not differ in the vehicle condition, the two groups tended to differ with MPH treatment (p= 0.07) (Figure 4).

Spine density of PFC and striatal neurons following Mn and MPH treatment

Following the completion of chronic MPH treatment, we assessed whether spine density of medial PFC pyramidal and dorsal striatal spiny neurons was affected by Mn exposure and MPH treatment, as evidence of neuroplastic changes in response to these treatments. The analysis of spine density (spines/10 μm dendrite length) revealed a significant 3-way interaction of Mn exposure × MPH treatment × brain region ($F(1, 20)=4.85$, $p=0.03$). Because this interaction, coupled with visual inspection of the data (Figure 5), indicated that the drug effect on the two groups differed by brain region, separate analyses were conducted on each brain region. Whereas the interaction of Mn × MPH was not significant in the striatum ($F(1, 15)=1.92$, $p=0.19$), this interaction was significant in the mPFC ($F(1, 15)=4.71$, $p=0.04$). In the mPFC, the drug tended to *increase* spine density in the Mn animals and *decrease* spine density in the controls. As a result, whereas the two groups did not differ in the vehicle condition, there was a significant difference between the Mn and control groups following MPH treatment (p=0.01).

Mn exposure produced environmentally relevant body Mn levels, with no effects on adult body weight

Blood Mn levels were measured in the animals immediately following the conclusion of behavioral testing (PND ~145), as an indication of body Mn levels after lifelong oral Mn exposure and chronic MPH treatment. Blood Mn levels in the Mn treated groups (Mn+Veh and Mn+MPH) were significantly elevated to ~160% of controls levels (Control+Veh and Control+MPH) ($F(1,35)=50.91$, $p<0.0001$). There was no measurable difference in blood Mn levels between MPH and vehicle (no MPH) groups ($F(1, 35)=1.49$, $p=0.23$), and no significant interaction between Mn × MPH treatments ($F(1, 35)=1.15$, $p=0.29$ (Table 1).

Finally, all groups gained weight over the course of MPH treatment ($F(4, 59) = 278.5$, $p<0.0001$). There was no effect of Mn exposure, MPH treatment, or of the interaction of these factors on adult body weights ($p>0.05$ for all).

Discussion

Our findings show that lifelong postnatal Mn exposure impairs impulse control and selective attention in young adulthood, and that a therapeutically relevant oral MPH regimen alleviated the Mn-induced dysfunction in impulse control. However, MPH treatment was not effective in ameliorating the deficit in selective attention in the Mn-exposed rats, and actually impaired the ability of these animals to focus attention on brief visual cues presented randomly in time and location. In addition, the effect of MPH across a range of behavioral and neural measures was qualitatively different for the Mn-exposed versus control animals, suggesting that postnatal Mn exposure alters catecholaminergic systems modulating inhibitory control and attention. These findings extend our previous studies, which showed that early postnatal Mn exposure altered focused and selective attention, arousal regulation, and fine motor function, and that the fine motor dysfunction was completely normalized by MPH treatment (Beaudin et al. 2015, 2017, 2013). Collectively these findings illuminate the basis of the behavioral deficits produced by elevated Mn exposure, and the potential for pharmacotherapeutic agents to treat these impairments in Mn-exposed children and adolescents.

Impulse control and selective attention are impaired by postnatal Mn exposure

The present study demonstrated that lifelong postnatal Mn exposure can increase distractor-induced impulsivity and impair selective attention in adulthood. In these tasks, increased impulsivity, or impaired impulse control, is inferred by the percentage of premature responses (i.e., nose-poke responses made following the start of the trial but before presentation of the visual cue). Evidence for this type of dysfunction in the Mn+Veh group is seen in the selective attention task, for the 1 s and 2 s distraction conditions during the first block of trials (Figure 1B). Notably, the impulse control deficit of the Mn-exposed animals emerged most prominently under the distraction conditions that elicited the largest overall increase in premature responses, and thus placed the greatest demand on impulse control ability. Consistent with this, Mn exposure had no adverse effect on premature responses on trials that were less demanding on impulse control ability, such as those in the baseline attention task and the non-distraction trials of the selective attention task, neither of which involved the presentation of olfactory distractors (Figure 1A, B).

Manganese exposure also impaired selective attention, based on the trend towards reduced response accuracy for the Mn+Veh group (vs Control+Veh group) for the 2 s distraction condition ($p=0.07$; Figure 3B). Impaired selective attention, or increased distractibility, can be inferred if the reduction in response accuracy produced by the presentation of the olfactory distractor, relative to the no distraction condition, is greater for the Mn+Veh group than controls, which is the case for the 2 s distraction condition. In the selective attention task, presentation of the distractor 2 s into the trial (i.e., 1 s or 2 s before the visual cue) produced the greatest reduction in response accuracy, and hence placed the greatest demand on selective attention. Our prior studies have shown that early and lifelong postnatal Mn exposure causes deficits in selective and focused attention, arousal regulation, and fine motor function (Beaudin et al. 2015, 2017, 2013), although we did not previously find evidence of impaired impulse control in Mn-exposed animals (Beaudin et al. 2017). The

reasons for these disparate results concerning Mn effects on impulse control is not clear, but one possible explanation is that the animals in our prior study had a much longer behavioral testing history, and hence were more highly trained to inhibit premature responding, perhaps therefore precluding the detection of group differences in this domain.

This study is among the first to demonstrate that postnatal Mn exposure can impair impulse control and selective attention, and as such sheds further light on the highly specific effects of postnatal Mn exposure on behavioral function in young adulthood (Beaudin et al. 2017, 2013; Golub et al. 2005; Kern et al. 2010; Kern and Smith 2011). Collectively, these findings provide crucial causal evidence of Mn-induced deficits in these functional domains, and they support the reported associations between Mn exposure and inattention, impulsivity, hyperactivity, oppositional behaviors, and fine motor deficits in children (Bhang et al. 2013; Bouchard et al. 2007, 2011; Crinella 2003; Ericson et al. 2007; Farias et al. 2010; Golub et al. 2005; Lucchini et al. 2012; Oulhote et al. 2014; Takser et al. 2004).

Oral MPH treatment alleviated the impulse control, but not the selective attention deficits caused by Mn exposure

Our findings indicate that daily oral MPH treatment alleviated the impairment in impulse control caused by postnatal Mn exposure, but not the attentional dysfunction. Specifically, the Mn+MPH group committed significantly *fewer* premature responses than the Mn+Veh group for the 1 s and 2 s distraction condition trials during session block 1 of the selective attention task, performing at a level that was not different from the Control+Veh group (Figure 1B). There is, however, some suggestion of diminished efficacy of MPH on impulse control with prolonged MPH treatment. This is evident in the selective attention task, where MPH treatment significantly reduced premature responses in the Mn+MPH group to levels comparable to the Control+Veh group in the first block of trials for the 1 s and 2 s distraction conditions, but not in the second block of trials for the 1 s distraction condition (Figure 1B).

In contrast, the drug did not alleviate the dysfunction in selective attention produced by Mn exposure. Manganese exposure in this study tended to reduce response accuracy in the 2 s distraction condition (the condition placing the greatest demand on selective attention; see Figure 3B), but accuracy was not improved by oral MPH treatment. Moreover, there was evidence that under testing trial conditions that did not involve the presentation of olfactory distractors MPH treatment produced detrimental effects on focused attention in the Mn animals. Specifically, MPH treatment significantly reduced response accuracy and increased omission errors in the Mn group for the non-distraction trials of the selective attention task (Figures 3B, 4), and reduced response accuracy in these same animals for trials of the attention baseline task with the briefest visual cue (Figure 3A).

Thus, in summary, oral MPH treatment alleviated the impairments in impulse control (present study) and fine motor function (Beaudin et al. 2015) produced by postnatal Mn exposure, whereas it did not ameliorate the selective attention dysfunction, and actually induced an impairment in focused attention in the Mn-exposed animals. This pattern of results suggests either that these different areas of dysfunction have different underlying neural mechanisms, and/or that the effective dose of MPH varies across these functional deficits in Mn-exposed rats. This latter suggestion is consistent with evidence of the

differential efficacy of MPH on the different sub-types of ADHD (i.e., ADHD-H, ADHD-I, and ADHD-C), with noticeable improvement reported for tasks requiring impulse control, and either variable or no improvement noted for tasks requiring sustained/selective attention (Blum et al. 2011; Gardner et al. 2008; Grizenko et al. 2010; Lajoie et al. 2005). Moreover, studies have also shown that drugs used to treat attentional dysfunction, such as MPH and atomoxetine (a selective NET inhibitor), have more potent actions within the PFC than in subcortical areas (Berridge et al. 2006), and that the behavioral response to these pharmacological therapies follows an inverted-U dose-response function, whereby moderate doses significantly improve performance, whereas higher doses impair performance (Arnsten 2011; Arnsten and Dudley 2005; Levy 2009; Newman et al. 2008). In light of this, it may be that the oral MPH dose used here exceeded the efficacious dose for alleviating Mn-caused attention deficits.

Mn-exposed rats responded differently than controls to MPH on several behavioral measures

The Mn-exposed rats responded differently than controls to MPH treatment for many of the behavioral outcomes, including premature responses, perseverative responses, accurate responses, and omission errors, in both the baseline attention and selective attention tasks. For example, premature and perseverative responses were both reduced by MPH in the Mn-exposed animals, whereas both measures were *increased* by MPH in controls (Figure 1B, 2A, B; summarized in Table 2). These latter findings in the control animals are consistent with previous studies reporting that manipulations which increased extracellular catecholamines (presumably to supraoptimal levels) led to inhibitory control deficits in rodents in the 5-CSRTT (Baarendse and Vanderschuren 2012; Blondeau and Dellu-Hagedorn 2007; D'Amour-Horvat and Leyton 2014; Navarra et al. 2008). The impaired inhibitory control seen in the Mn-exposed animals (in the vehicle condition), as well as the differential effects of MPH in the Mn and control animals may be explained by the following two premises: (1) the relationship between catecholaminergic activity and inhibitory control follows an inverted-U shaped dose response function, with either too little or too much catecholaminergic activity impairing impulse control; and (2) the impulse control dysfunction of the Mn-exposed animals is due to hypo-catecholaminergic activity.

Similarly, for the measures of focused attention (accurate responses, omission errors), MPH treatment significantly impaired performance of the Mn-exposed animals but had no effect on controls (Figure 3A, B, Figure 4; Table 2). This latter finding regarding the control animals is consistent with prior animal studies showing that low doses of MPH had little to no effect on measures of attention in control animals tested in the 5-CSRTT (Bizarro et al. 2004; Navarra et al. 2008). While the reason(s) for the lack of therapeutic efficacy of MPH on attention is unclear, this pattern of findings provides support that catecholaminergic activity is altered by postnatal Mn exposure. It is likely that the optimal catecholaminergic activity varies in the different brain regions subserving these different functional domains, and that a different dose of the drug may achieve efficacy in the domain of attentional function.

In the present study, we also investigated whether Mn exposure and/or MPH treatment affected spine density of mPFC pyramidal and dorsal striatal spiny neurons, as a measure of neuronal plasticity, and whether the drug effect differed in the Mn-exposed and control animals. This analysis did not reveal an effect of Mn-exposure on spine density in either brain region. However, it provided further evidence for a differential drug response in Mn-exposed versus control animals. Others have reported that chronic MPH treatment increased medium spiny neuron spine density in the nucleus accumbens (Kim et al. 2009), and that acute Mn exposure led to spine degeneration on striatal medium spiny neurons (Milatovic et al. 2009), but none have investigated MPH treatment effect on neuronal plasticity in chronically Mn-exposed animals as presented here.

The findings from this study showing that MPH treatment affected the Mn-exposed animals differently than controls for impulse control and attentional functions indicates that postnatal Mn exposure causes lasting alterations of the catecholaminergic neurobiology underlying these functional domains. They also suggest that the therapeutic index/window of MPH efficacy is different for the neurosystems underlying impulse control and attentional dysfunction in Mn-exposed animals. These inferences are consistent with evidence from previous studies suggesting that catecholaminergic systems in the fronto-striatal circuit play an important role in Mn neurotoxicity. For example, Mn has been shown to target dopaminergic systems modulating executive and motor functions (Aschner et al. 2005; Erikson et al. 2007; Eriksson et al. 1987; Guilarte et al. 2006; Kern and Smith 2011; Pappas et al. 1997; Stanwood et al. 2009), and there is some evidence suggesting effects on noradrenergic systems as well (Anderson et al. 2009). In particular, studies in animal models have shown that early postnatal oral Mn exposure reduces striatal dopamine release (Beaudin et al. 2015; McDougall et al. 2008; Reichel et al. 2006), dopamine-1 (D1) receptor and dopamine transporter (DAT) protein expression (Kern et al. 2010; Kern and Smith 2011; McDougall et al. 2008; Reichel et al. 2006) in the striatum and nucleus accumbens, and increases D2 receptor levels in the PFC (Calabresi et al. 2001; Kern et al. 2010; Kern and Smith 2011).

The fronto-striatal circuit plays a critical role in a variety of cognitive and executive function processes shown to be affected by developmental Mn exposure, such as regulating arousal state, attention and behavioral flexibility, impulse control, and memory for motor responses (Arnsten 2006; Beaudin et al. 2017, 2013; Chudasama and Robbins 2006; Golub et al. 2005; Kern et al. 2010; Kern and Smith 2011). PFC regulation of these processes is achieved through norepinephrine and dopamine release, with the relationship between catecholaminergic activity and these functional endpoints following an inverted U-shaped curve, such that either too much or too little neurotransmitter release weakens cognitive control of behavior and attention (Arnsten 2009, 2010; Arnsten and Pliszka 2011; Brennan and Arnsten 2008; Dalley et al. 2004). Consistent with this, studies have shown that PFC lesions produce a profile of distractibility, forgetfulness, poor planning, and motor deficits (Brennan and Arnsten 2008), and that selective depletion of norepinephrine in the PFC in rats produces deficits in focused/sustained attention (Milstein et al. 2007), similar to the pattern of deficits produced by developmental postnatal Mn exposure in our studies (Beaudin et al. 2015, 2017, 2013). Moreover, neuropsychological and imaging studies in children have shown that ADHD (and attentional dysfunction more broadly) is generally

associated with hypo-functioning of catecholaminergic systems within the cortico-striatal loop (Arnsten 2010; Brennan and Arnsten 2008). Furthermore, studies have shown that the behavioral response to a variety of catecholaminergic drugs used to treat attentional dysfunction, such as MPH (a DAT and NET inhibitor) and atomoxetine (a selective NET inhibitor) also follows an inverted-U dose-response function, whereby moderate doses significantly improve performance, while higher doses impair performance (Arnsten 2011; Arnsten and Dudley 2005; Levy 2009; Newman et al. 2008).

Conclusions

This study shows that chronic postnatal Mn exposure from birth impairs impulse control and selective attention in young adulthood. We also showed that a therapeutically relevant oral MPH dose alleviated the Mn dysfunction in impulse control, but not selective attention, and that under some conditions MPH treatment produced detrimental effects on focused attention in the Mn animals. More broadly, the Mn-exposed rats responded differently than controls to MPH treatment across a range of behavioral and neural measures, including impulsivity, compulsivity, attention, and dendritic spine density. These findings provide strong evidence that Mn exposure alters catecholaminergic neurobiology underlying impulse control and attentional processes. These findings also suggest that MPH may hold promise for treating the behavioral dysfunction caused by developmental Mn exposure, though further research is needed with multiple MPH doses to determine the dose-response effects of MPH on measures of impulse control and attention, and to more fully understand the cellular basis for the differential response of Mn-exposed animals to MPH. Collectively, these studies will better inform the therapeutic potential for MPH or other catecholaminergic agonists in the treatment of behavioral disorders associated with elevated Mn exposure in humans.

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Abbreviations

MPH methylphenidate

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Highlights

- Chronic Mn exposure impaired impulse control and selective attention
- MPH alleviated the impulse control but not selective attention deficits due to Mn
- MPH impaired focused attention in the Mn group.
- mPFC spine density was differentially altered in control versus Mn animals treated with MPH
- MPH may hold promise for treating the behavioral dysfunction from Mn exposure

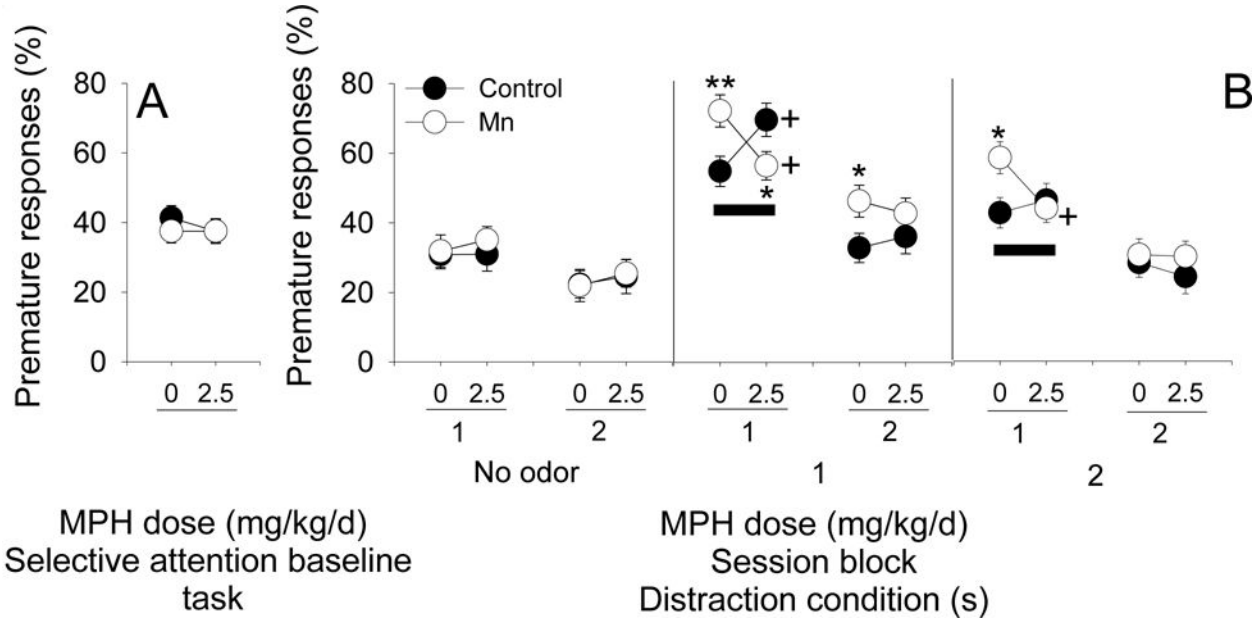

Figure 1. Mn exposure increased distractor-induced impulsivity in the selective attention task, and MPH treatment alleviated the Mn effect while increasing impulsivity in rats never exposed to Mn

Mean percent premature responses (± SE) for the control and Mn-exposed groups in (A) the baseline attention task, as a function of MPH dose, and in (B) the selective attention task, as a function of MPH dose, session block (three test session days/block), and distractor condition (n=10/group). * and ** indicate significant differences between the Mn and control groups at $p \leq 0.05$ or $p \leq 0.01$, respectively, for each of the 0 or the 2.5 mg MPH/kg/d treatment conditions. + indicates significant differences between the MPH and vehicle-treated groups at $p \leq 0.05$ for each of the control or the Mn-exposed conditions. The full line in (B) indicates no difference between the Mn+MPH group and the Control+Veh groups, reflecting the therapeutic effect of MPH treatment in Mn-exposed rats. The dotted line in (B) indicates a significant difference between the Mn+MPH and the Control+Veh groups at $p \leq 0.05$, reflecting the absence of a therapeutic MPH effect on Mn-exposed rats.

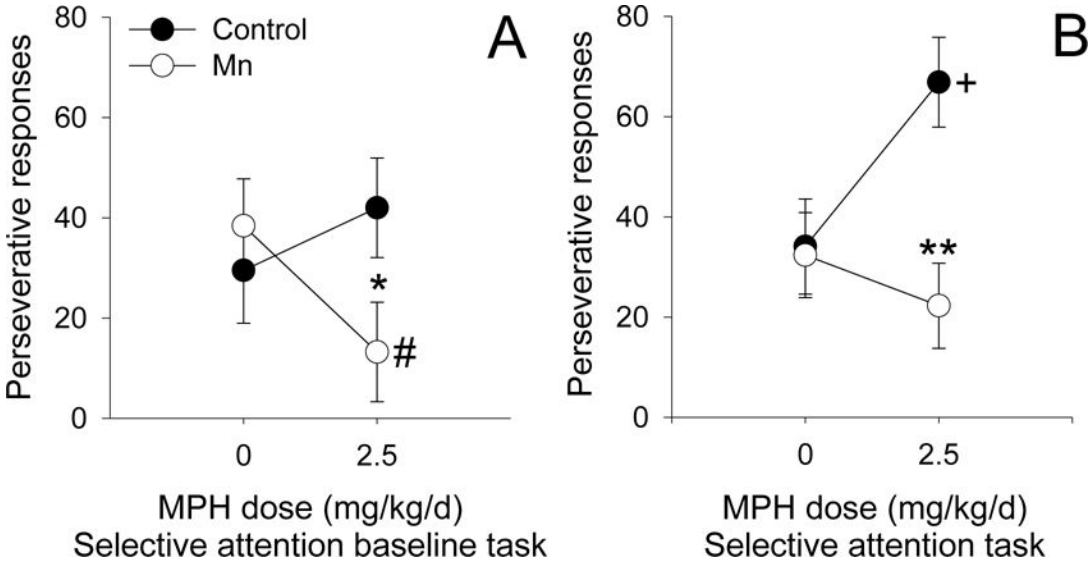

Figure 2. Mn exposure did not affect compulsive perseveration, but Mn-exposed and control rats responded differently to MPH treatment for perseverative responses

Mean number of perseverative responses (± SE) for the control and Mn-exposed groups in (A) the baseline attention task and in (B) the selective attention task, both as a function of MPH dose (n=10/group). + indicates a significant difference between the MPH and vehicle-treated groups at $p \leq 0.05$ for the control condition in (B), and # indicates a trending significant difference between the MPH and vehicle-treated groups at $0.05 < p \leq 0.01$ for the Mn exposure condition in (A). * and ** indicate significant differences between the Mn + MPH and control + MPH groups at $p \leq 0.05$ or $p \leq 0.01$ in (A) and (B) respectively.

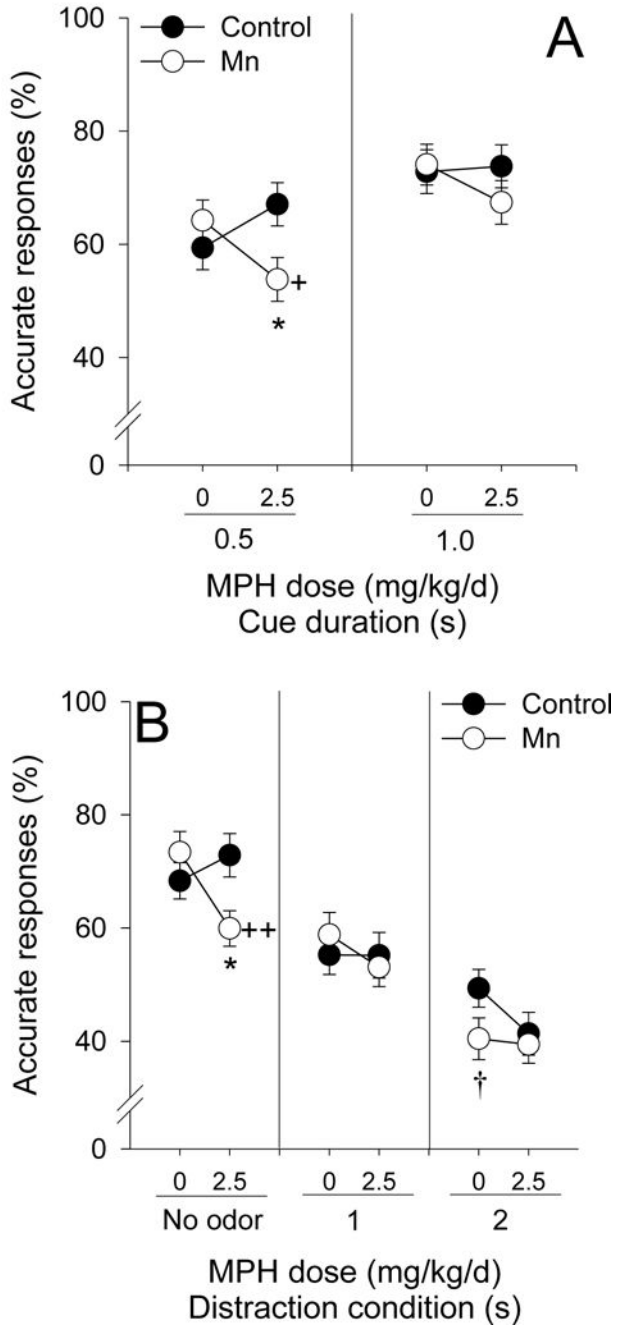

Figure 3. MPH did not alleviate the Mn-induced impairment in selective attention and impaired focused attention in the Mn rats only

Mean percent accurate responses (± SE) for the control and Mn-exposed groups in (A) the baseline attention task, as function of MPH dose and duration of the visual cue, and in (B) the selective attention task, as function of MPH dose and distractor condition (n=10/group). † indicates a trending significant difference between the Mn+Veh and Control+Veh groups at $0.05 < p \leq 0.10$. + and ++ indicate significant differences between the Mn+MPH and Mn +Veh groups at $p \leq 0.05$ and $p \leq 0.01$ in (A) and (B), respectively. * indicates a significant difference between the Mn+MPH and Control+MPH groups at $p \leq 0.05$. The dotted line in

(B) indicates a significant difference between the Mn+MPH and the Control+Veh groups at $p \leq 0.05$, reflecting the absence of a therapeutic effect of MPH on Mn-exposed rats.

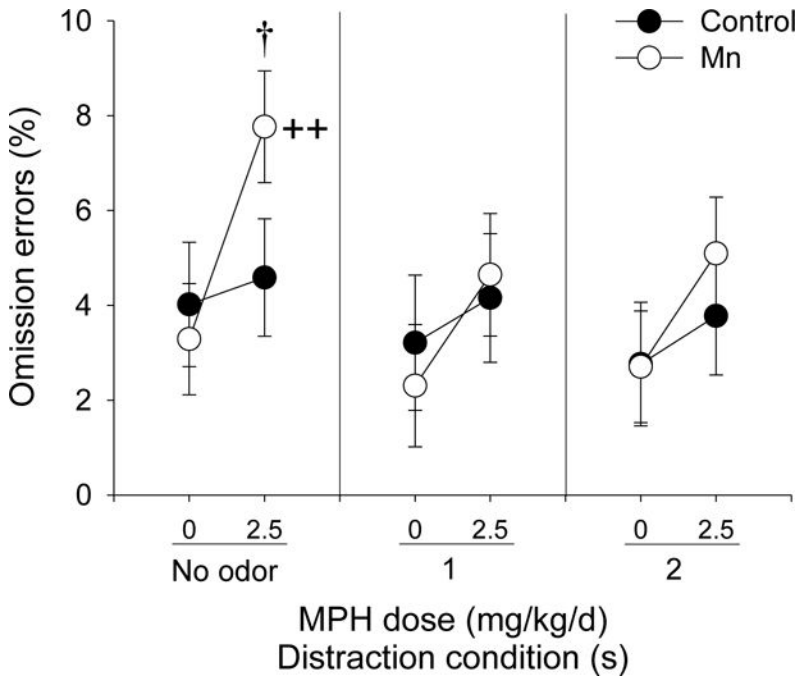

Figure 4. Mn-exposed rats committed more omission errors under MPH treatment in the selective attention task

Mean percent omission errors (± SE) for the control and Mn-exposed groups in the selective attention task, as function of MPH dose and distractor condition (n=10/group). ++ indicates a significant difference between the Mn+MPH and Mn+Veh groups at $p \leq 0.01$, and † indicates a trending significant difference between the Mn+MPH and the Control+MPH groups at $0.05 < p \leq 0.10$.

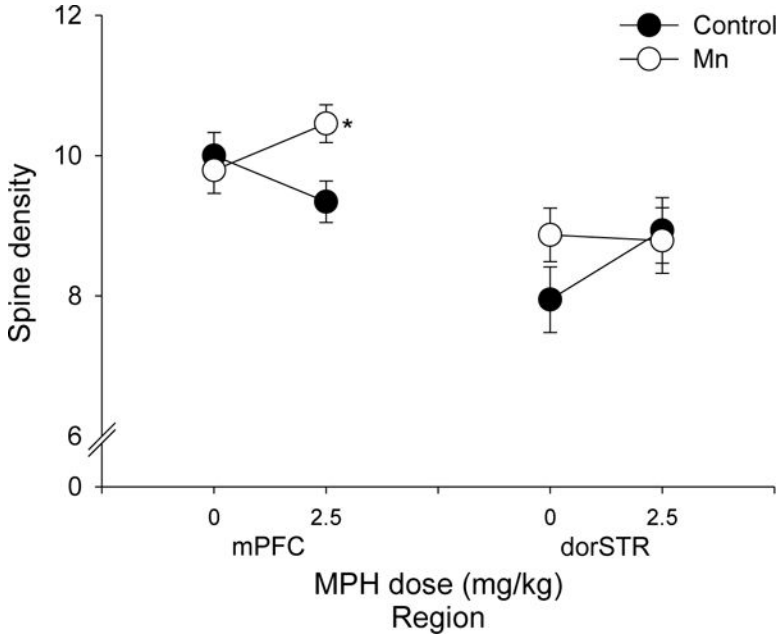

Figure 5. Spine density of PFC pyramidal, but not striatal spiny neurons is higher in Mn versus control rats treated with MPH

Mean spine density (spines/10 μm dendrite length, ± SE) for the control and Mn-exposed groups, as function of MPH dose and brain region (n=5–6/group). * indicates a significant difference between the Mn+MPH and Control+MPH groups at $p \leq 0.05$.

Table 1

Blood Mn concentrations in the PND 145 study animals following behavioral testing. Also shown are blood and brain Mn from PND 24, 66 and ~490 male rats from our prior study (Beaudin et al. 2017).

Treatment	PND 145*	PND 24		PND 66		PND ~490	
	Blood Mn	Blood Mn	Brain Mn	Blood Mn	Brain Mn	Blood Mn	Brain Mn
Control+Veh	6.34 ±0.36 (10)^{A,a}	24.2 ± 0.79 (11)^{A,b}	3.77 ±0.19 (11)^{A,a}	9.51 ±0.36 (14)^{A,c}	2.12 ±0.031 (14)^{A,b}	5.76 ±0.28 (16)^{A,a}	1.95 ±0.063 (13)^{A,b}
Control+MPH	6.33 ±0.49 (10)^{A}	NA	NA	NA	NA	NA	NA
Mn+Veh	10.8 ±0.46 (9)^{B,a}	247 ±23 (15)^{B,b}	12.8 ±1.64 (14)^{B,a}	19.4 ±1.2 (13)^{B,c}	2.53 ±0.047 (14)^{B,b}	15.2 ±1.14 (20)^{B,ac}	2.63 ±0.085 (17)^{B,b}
Mn+MPH	9.52 ±0.75 (10)^{B}	NA	NA	NA	NA	NA	NA

*PND = postnatal day. Data are mean ± standard error (n); blood Mn in ng/mL, brain Mn in μg/g dry weight. A, B, etc. superscripts: within an age group and tissue, treatment groups with different upper case letter superscripts are statistically different from one another ($p<0.05$), based on Tukey's post hoc test. Lower case a, b, etc.: within a treatment group and tissue, values across ages with different lower case superscripts are statistically different from one another. Main effect statistics on $\log_{10}$ transformed data are: Blood Mn, age $F(3,102) = 234$, $p<0.0001$, Mn treatment $F(1,102) = 265$, $p<0.0001$, age × Mn treatment $F(3,102) = 28.7$, $p<0.0001$; Brain Mn, age $F(2,76) = 224$, $p<0.0001$, Mn treatment $F(1,76) = 179$, $p<0.0001$, age × Mn treatment $F(2,76) = 40.0$, $p<0.0001$).

Table 2

Summary of the differential effects of MPH treatment on control and Mn-exposed animals across the range of behavioral responses in the selective attention baseline and selective attention tasks grouped under measures of impulsivity/compulsivity and attention. Arrow indicates direction of significant MPH effect (i.e., $p \leq 0.05$) to increase (↑) or decrease (↓) the specific response type, followed by whether the effect may be considered beneficial (pos) or detrimental (neg) in parentheses. Lateral arrows (←→) indicate no measurable effect. See text for details.

	MPH effect on behavioral responses							
	Impulsive/compulsive				**Attention**			
Treatment	**Premature**		**Perseverative**		**Accuracy**		**Omissions**	
Control	↑	(neg)	↑	(neg)	←→		←→	
Mn-exposed	↓	(pos)	↓	(pos)	↓	(neg)	↑	(neg)