Published in final edited form as:

Eur J Pharmacol. 2013 April 15; 706(0): 4–10. doi:10.1016/j.ejphar.2013.02.030.

Postnatal manganese exposure does not alter dopamine autoreceptor sensitivity in adult and adolescent male rats

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Abstract

Administering manganese chloride (Mn) to rats on postnatal day (PD) 1–21 causes long-term reductions in dopamine transporter levels in the dorsal striatum, as well as persistent increases in D₁ and D₂ receptor concentrations. Whether dopamine autoreceptors change in number or sensitivity is uncertain, although D_{2S} receptors, which may be presynaptic in origin, are elevated in Mn-exposed rats. The purpose of this study was to determine if early Mn exposure causes long-term changes in dopamine autoreceptor sensitivity that persist into adolescence and adulthood. To this end, male rats were exposed to Mn on PD 1–21 and autoreceptor functioning was tested 7 or 70 days later by measuring (a) dopamine synthesis (i.e., DOPA accumulation) in the dorsal striatum after quinpirole or haloperidol treatment and (b) behavioral responsiveness after low-dose apomorphine treatment. Results showed that low doses (i.e., “autoreceptor” doses) of apomorphine (0.06 and 0.12 mg/kg) decreased the locomotor activity of adolescent and adult rats, while higher doses increased locomotion. The dopamine synthesis experiment also produced classic autoreceptor effects, because quinpirole decreased dorsal striatal DOPA accumulation; whereas, haloperidol increased DOPA levels in control rats, but not in rats given the nerve impulse inhibitor γ -butyrolactone. Importantly, early Mn exposure did not alter autoreceptor sensitivity when assessed in early adolescence or adulthood. The lack of Mn-induced effects was evident in both the dopamine synthesis and behavioral experiments. When considered together with past studies, it is clear that early Mn exposure alters the functioning of various dopaminergic presynaptic mechanisms, while dopamine autoreceptors remain unimpaired.

Keywords

Manganese; Apomorphine; DOPA accumulation; Autoreceptors; Ontogeny; Dorsal striatum

1. Introduction

Manganese chloride (Mn) is a complex trace mineral that preferentially accumulates in the basal ganglia and associated brain structures of humans and other animals (Takeda, 2003; Aschner et al., 2005). Prolonged Mn exposure causes extrapyramidal motor impairments in humans (Aschner et al., 2007), whereas less intense Mn exposure may predispose children to attention deficit hyperactivity disorder (ADHD). More specifically, it has been suggested

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that the high levels of Mn found in some soy-based infant formulas may cause neural changes that lead to ADHD-like symptoms (Tran et al., 2002b; Crinella, 2012). Studies using a variety of species suggest that Mn alters the functioning of multiple neurotransmitter systems, including dopamine, glutamate, and GABA (Fitsanakis et al., 2006; Stanwood et al., 2009), but actions at the dopaminergic presynaptic terminal are very prominent (Guilarte et al., 2006, 2008). Notably, exposing rats to Mn on postnatal days (PD) 1–21 (i.e., prior to weaning) causes significant reductions in dopamine transporter levels and protein expression that can persist into adulthood (McDougall et al., 2008; Kern et al., 2010). Consistent with these findings, the velocity of dopamine uptake is reduced in the dorsal striatum of Mn-exposed rats and monkeys (Chen et al., 2006; McDougall et al., 2008). Dopamine release is also impacted by Mn (Guilarte et al., 2006, 2008; Khalid et al., 2011), since acute Mn treatment decreases K⁺-stimulated dopamine efflux in adult rat striatum (Vidal et al., 2005), while administering Mn on PD 1–21 causes long-term reductions in cocaine-induced dopamine efflux (McDougall et al., 2008).

In addition to affecting presynaptic terminals, early Mn exposure causes long-term changes in postsynaptic dopamine receptors. Using immunohistochemistry techniques, Kern and Smith (2011) found that administering Mn on PD 1–21 significantly increased D₁ and D₂ receptor densities in the nucleus accumbens and prefrontal cortex, whereas 25 mg/kg Mn caused a nonsignificant enhancement of D₁ and D₂ receptor levels in the striatum. Treating adult rats and mice with Mn also increases the number of D₂ binding sites in the dorsal striatum (Seth et al., 1981) as well as D₂ receptor protein levels and mRNA expression (Nam and Kim, 2008). In general agreement with these past studies, we reported that exposing rats to Mn during early ontogeny increased [³H]-spiperone binding in the dorsal striatum, while enhancing D₂L and D₂S protein expression in the same brain structure (McDougall et al., 2011). These dopamine receptor changes have behavioral impact, because low doses of cocaine and amphetamine stimulate greater locomotor activity in Mn-exposed adult rats than controls (Reichel et al., 2006; Kern and Smith, 2011), whereas high doses produce more intense stereotypy in Mn-treated rats (McDougall et al., 2008).

Considering that early Mn exposure affects postsynaptic receptors, it is perhaps not surprising that Mn has been reported to alter the functioning of presynaptic autoreceptors, an important neuronal mechanism regulating dopamine synthesis and release. For example, apomorphine transiently loses its ability to inhibit K⁺-evoked dopamine release in rats given a two week exposure to Mn in adulthood (Cuesta de Di Zio et al., 1995). Moreover, dorsal striatal D₂S receptor levels, which may be presynaptic in origin (Lindgren et al., 2003; De Mei et al., 2009), are elevated in Mn-treated adult rats. Therefore, the purpose of this study was to determine whether exposing rats to Mn on PD 1–21 would alter the sensitivity of dopamine autoreceptors when examined during early adolescence (PD 28) and adulthood (PD 91). Autoreceptor sensitivity was assessed by measuring locomotor responding after low-dose apomorphine treatment as well as autoreceptor-mediated alterations in dopamine synthesis.

2. Materials and methods

2.1. Animals

Subjects were 276 male rats ($n = 7–9$ per group) of Sprague-Dawley descent (Charles River, Hollister, CA), born and raised at California State University, San Bernardino (CSUSB). Litters were culled to ten pups on PD 1 (day of parturition is PD 0). Rats were weaned on PD 25 and group housed with same-sex littermates. Standard rodent chow (Harlan-Tekland #8604) and water were freely available. The colony room was kept at 21–23°C, with a 12:12 h light/dark cycle. Behavioral testing was conducted in separate experimental rooms during the light phase of the cycle. Subjects were cared for according to the “Guide for the Care

and Use of Laboratory Animals” (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

2.2. Apparatus

Locomotor activity was measured in commercially available (Coulbourn Instruments, Allentown, PA) activity monitoring chambers, consisting of acrylic walls, a plastic floor, and an open top. Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine distance traveled. The position of each rat was determined every 100 msec. To somewhat control for differences in body size, PD 28 rats were tested in smaller sized chambers (26 × 26 × 41 cm) than PD 90 rats (41 × 41 × 41 cm). In all other regards, the different-sized chambers were identical to each other.

2.3. Drugs

Haloperidol was dissolved in a minimal amount of glacial acetic acid and diluted with saline; whereas, *R*(–)-apomorphine hydrochloride hydrate, (–)-quinpirole hydrochloride, and 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015) were dissolved in saline. γ -Butyrolactone was purchased at an initial density of 1.12 g/ml. All drugs were injected intraperitoneally (i.p.) at a volume of 1 ml/kg and were purchased from Sigma-Aldrich (St. Louis, MO).

2.4. In vivo Mn administration

On PD 1–21, rats were given daily oral supplements (via micropipette) of 0 or 750 μ g Mn chloride (Sigma-Aldrich) dissolved in 25 μ l of a 10% sucrose solution (Tran et al., 2002a, b; Reichel et al., 2006; McDougall et al., 2008, 2011). This dosing paradigm was similar to that used by Tran and colleagues (2002a, b), and is purported to approximately mimic the amount of Mn consumed by human infants fed milk and soy formulas. An outcome of this dosing paradigm is that rats received more Mn, relative to body weight, during the neonatal period than at later ages. At the conclusion of Mn dosing (i.e., on PD 21), dorsal striatal Mn levels are approximately three-fold greater than control values (Reichel et al., 2006).

2.5. Behavioral procedures

On PD 28 or PD 90, young ($N = 80$) and adult ($N = 105$) rats were injected with apomorphine (0, 0.03, 0.06, 0.12, or 0.6 mg/kg, i.p.) and immediately placed in activity chambers for 35 min. Additional groups of adult rats were tested after receiving 1.2 mg/kg apomorphine. The first 5 min of the testing session served as a habituation phase, after which distance traveled (a measure of horizontal locomotor activity) was measured for an additional 30 min.

2.6. DOPA accumulation protocol

On PD 28 ($N = 91$) and PD 91 ($N = 105$; these are the same adult animals that were tested in the behavioral experiment), rats were injected with saline, the D_2/D_3 agonist quinpirole (0.5 mg/kg, i.p.), or the D_2 antagonist haloperidol (0.5 mg/kg, i.p.). After 25 min, groups were further subdivided with rats being injected with distilled water or the nerve impulse inhibitor γ -butyrolactone (PD 28, 625 mg/kg; PD 91, 750 mg/kg, i.p.). Although 750 mg/kg γ -butyrolactone is commonly administered to adult rats (Walters and Roth, 1976; Svensson et al., 1991; Baldessarini et al., 1994), a lower dose of the drug was given to PD 28 rats because 750 mg/kg γ -butyrolactone, when combined with haloperidol, frequently causes respiratory failure in younger animals. After an additional 5 min, all rats were injected with the DOPA decarboxylase inhibitor NSD-1015 (100 mg/kg, i.p.). Rats were killed by rapid decapitation 30 min after NSD-1015 treatment, and their dorsal striata were removed on an ice-cold dissection plate and stored at -80°C until assay. A total of 7–8 adolescent rats and

8–9 adult rats were assigned to each group. Because adult rats had been injected with apomorphine 24 h earlier, counterbalancing procedures were used to assign subjects to the DOPA accumulation experiment. Specifically, adult rats treated with saline and the various doses of apomorphine were approximately equally dispersed among the individual groups of the DA accumulation experiment.

DOPA accumulation was determined using high-performance liquid chromatography. Frozen tissue samples were sonicated in 10 volumes of 0.1 N HClO₄ and centrifuged at 20,000 × *g* for 20 min at 4°C. The supernatant was then filtered through a 0.22 μm centrifugation unit at 2,000 × *g* for 5 min at 4°C. Twenty microliters of the resulting extract was assayed for DOPA using a Breeze™ HPLC system (Waters, Milford, MA), with a Coulochem II electrochemical detector (ESA, Chelmsford, MA). The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM ethylenediamine tetracetic acid, and 10% acetonitrile (pH 3.1) and was pumped at a rate of 0.5 ml/min.

2.7. Data analysis

Litter effects were minimized by assigning no more than one subject from each litter to a particular group (for a discussion of litter effects, see Zorrilla, 1997). When this procedure was not possible (i.e., analysis of body weights), a single litter mean was calculated from multiple littermates assigned to the same group (Holson and Pearce, 1992; Zorrilla, 1997). Body weight data from PD 1–21 were analyzed using a two-way (Pretreatment Condition × Day) within-subject analysis of variance (ANOVA); whereas, body weights on PD 28 and PD 90 were analyzed using related *t*-tests (Pretreatment Condition). In these analyses, litter was used as the unit of analysis (Zorrilla, 1997). With this statistical model each litter, rather than each rat, is treated as an independent observation (i.e., a within analysis using one value/condition/litter). When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Greenhouse-Geisser epsilon statistic was used to adjust degrees of freedom (Geisser and Greenhouse, 1958). Corrected degrees of freedom were rounded to the nearest whole number.

For the behavioral experiments, distance traveled data were analyzed using separate two-way (Pretreatment Condition × Test Drug) between-subject ANOVAs at each age. Additionally, ED₅₀ values were obtained using a four-parameter sigmoid curve fit model (Motulsky and Christopoulos, 2003) as described in Assié et al. (2010). For curve fitting, only “autoreceptor” doses (0.03–0.12 mg/kg) were examined and data were transformed to percent of the maximal effective dose. Confidence intervals (95%) for ED₅₀ values were calculated, with non-overlapping confidence intervals indicating a statistically significant effect (Julious, 2004). In one case, the curve did not converge and the ED₅₀ value could not be estimated. In the dopamine synthesis experiments, DOPA accumulation (ng/mg wet weight tissue) was analyzed using separate three-way (Pretreatment Condition × Test Drug × Impulse Inhibitor) between-subject ANOVAs at each age. Post hoc and planned comparisons were done using Tukey tests and independent *t*-tests, respectively ($P < 0.05$).

3. Results

3.1. Effects of postnatal Mn exposure on body weight

Body weights increased in a progressive manner from PD 1 to PD 21 (Table 1) [^aDay main effect, $F(2, 52)=1941.92$, $P < 0.001$]. The weight gain of Mn-treated rats was depressed relative to vehicle controls [Pretreatment Condition main effect, $F(1, 33)=17.50$, $P < 0.001$], with this effect being apparent from PD 3 to PD 21 [^aPretreatment Condition × Day interaction, $F(2, 54)=13.53$, $P < 0.001$]. Mn-exposed rats ($\bar{x} = 98.8$ g, ± 2.3) continued to weigh less than vehicle controls ($\bar{x} = 106.8$ g, ± 1.6) on PD 28 (i.e., 7 days after cessation of

Mn treatment) [$t(21)=4.06$, $P < 0.01$]. Body weights of vehicle-treated ($\bar{x} = 505$ g, ± 6.9) and Mn-treated ($\bar{x} = 496$ g, ± 10.1) rats did not differ significantly on PD 90.

3.2. Effects of postnatal Mn exposure on dopamine autoreceptor-modulated locomotor activity

Analysis of the drug main effect showed that apomorphine differentially affected the locomotor activity of PD 28 rats depending on dose (upper graph, Fig. 1) [Test Drug main effect, $F_{(4, 70)} = 25.43$, $P < 0.001$]. Planned comparisons indicated that autoreceptor-selective doses of apomorphine (0.03, 0.06, and 0.12 mg/kg) caused a significant decrease in the distance traveled scores of PD 28 rats, whereas a higher dose of apomorphine (0.6 mg/kg) increased locomotor activity. Exposing rats to Mn on PD 1–21 did not alter responding to apomorphine on PD 28 [Pretreatment Condition main effect, $F_{(1, 70)} = 0.31$, $P = 0.58$; Pretreatment Condition $\times$ Test Drug interaction, $F_{(4, 70)} = 0.56$, $P = 0.69$]. Indeed, with the exception of the 0.12 mg/kg apomorphine group, the patterns of locomotor activity exhibited by vehicle- and Mn-pretreated rats were almost identical (upper graph, Fig. 1). For rats treated with 0.12 mg/kg apomorphine, a separate statistical analysis indicated that Mn and vehicle pretreatment did not differentially affect locomotor responsiveness (Tukey test, $P > 0.05$). Overall, the ED_{50} value for vehicle-pretreated PD 28 rats was 0.029 mg/kg apomorphine (confidence interval, 0.01–0.08), while the ED_{50} for Mn-pretreated rats could not be estimated.

Rats tested on PD 90 showed approximately the same pattern of effects as the younger age group (lower graph, Fig. 1), because 0.06 and 0.12 mg/kg apomorphine significantly decreased the distance traveled scores of rats on PD 90, while 1.2 mg/kg apomorphine increased locomotion [Test Drug main effect, $F_{(5, 84)} = 11.62$, $P < 0.001$]. When compared to the control group, neither 0.03 nor 0.6 mg/kg apomorphine affected the locomotor activity of PD 90 rats. Importantly, exposing rats to Mn from PD 1 to PD 21 did not significantly impact the autoreceptor-mediated behavior of rats tested on PD 90 [Pretreatment Condition main effect, $F_{(1, 70)} = 2.00$, $P = 0.16$; Pretreatment Condition $\times$ Test Drug interaction, $F_{(4, 70)} = 0.65$, $P = 0.66$]. Consistent with this finding, the ED_{50} values of PD 90 rats did not differ according to pretreatment condition. Specifically, the ED_{50} value for Mn-treated rats was 0.033 mg/kg apomorphine (confidence interval, 0.02–0.06), whereas the ED_{50} for vehicle-treated rats was 0.047 mg/kg apomorphine (confidence interval, 0.02–0.11).

3.3. Effects of postnatal Mn exposure on dopamine autoreceptor-modulated DOPA accumulation

When assessed on PD 28, the Test Drug and Impulse Inhibitor variables interacted to affect DOPA accumulation in the dorsal striatum (Fig. 2). Among control rats (i.e., those not receiving γ -butyrolactone), quinpirole significantly decreased DOPA accumulation, whereas haloperidol caused a dramatic increase in DOPA levels (upper graph, Fig. 2) [Test Drug $\times$ Impulse Inhibitor interaction, $F_{(2, 79)} = 112.54$, $P < 0.001$]. The nerve impulse inhibitor γ -butyrolactone ($\bar{x} = 3.73$ ng/mg, ± 0.12) produced almost a three-fold increase in DOPA accumulation relative to saline-treated rats given a control injection of distilled water ($\bar{x} = 1.33$ ng/mg, ± 0.04). Among the γ -butyrolactone groups, quinpirole significantly reduced DOPA accumulation, but haloperidol was unable to potentiate the actions of γ -butyrolactone in PD 28 rats [Test Drug $\times$ Impulse Inhibitor interaction, $F_{(2, 79)} = 112.54$, $P < 0.001$]. In terms of the latter result, dorsal striatal DOPA accumulation in rats given haloperidol alone was significantly greater than in rats treated with both γ -butyrolactone and haloperidol (Tukey tests). None of the interactions involving the pretreatment variable (Mn vs. vehicle) reached statistical significance.

The pattern of dopamine synthesis data gathered during adulthood are essentially identical to the adolescent data. Among adult rats tested on PD 91, quinpirole decreased dorsal striatal DOPA accumulation in both the distilled water and γ -butyrolactone conditions (Fig. 3); whereas, the D₂ antagonist haloperidol increased DOPA accumulation in control rats, but not in rats injected with 750 mg/kg γ -butyrolactone [Test Drug $\times$ Impulse Inhibitor interaction, $F(2, 93)=45.20$, $P < 0.001$]. The same analysis showed that DOPA accumulation in the dorsal striatum of γ -butyrolactone-treated adult rats ($\bar{x} = 3.13$ ng/mg, ± 0.15) was substantially greater than in control rats given a distilled water injection ($\bar{x} = 1.29$ ng/mg, ± 0.04). Exposing rats to Mn on PD 1–21 did not alter the sensitivity of synthesis-modulating autoreceptors, because the DOPA accumulation values of vehicle- and Mn-exposed rats did not differ on PD 91.

4. Discussion

The purpose of the present study was to determine whether postnatal Mn exposure would alter the sensitivity of dopamine autoreceptors in young and adult rats. To this end, apomorphine-induced locomotor activity and autoreceptor-mediated alterations in dopamine synthesis were assessed during early adolescence and adulthood. Dopamine autoreceptors have a 5 to 10 fold greater sensitivity to dopamine agonists than do postsynaptic receptors (Cooper et al., 2003). For this reason, low doses of a dopamine agonist (e.g., apomorphine and quinpirole) preferentially stimulate autoreceptors, whereas higher doses also stimulate postsynaptic receptors (Lappalainen et al., 1990; Lin and Walters, 1994; Dziedzicka-Wasylewska and Rogóz, 1997; Tammimäki et al., 2006). This pattern of effects was clearly observed in the present study as low doses of apomorphine (0.06 and 0.12 mg/kg) significantly decreased the locomotor activity of adolescent (PD 28) and adult (PD 90) rats, whereas higher doses of apomorphine (i.e., “postsynaptic” doses) increased locomotor activity at both ages. Importantly, exposing rats to Mn on PD 1–PD 21 did not alter behavioral responding to low-dose apomorphine treatment. Autoreceptor-mediated functioning is typically very sensitive to pharmacological manipulation (Spampinato et al., 1988; Harsing et al., 1992; Dziedzicka-Wasylewska and Rogóz, 1997; Moreno et al., 2003; Der-Ghazarian et al., 2010), thus the absence of perturbations in apomorphine-induced locomotor activity suggests that Mn-exposure did not cause short-term (7 days) or long-term (69 days) alterations in dopamine autoreceptor sensitivity.

In the present study, synthesis-modulating autoreceptors were assessed using both an “open-loop” and “closed-loop” model. In the “open-loop” model, it is possible for dopaminergic drugs to modify dopamine synthesis via long-loop feedback pathways; whereas, γ -butyrolactone is used in the “closed-loop” model to inhibit nerve impulse flow, thus dopamine agonists and antagonists can only modify dopamine synthesis through autoreceptor-mediated actions (Walters and Roth, 1976). As expected, γ -butyrolactone, when administered alone, increased dopamine synthesis in the dorsal striatum of adolescent and adult rats (see also Walters and Roth, 1976; Shalaby et al., 1981; Baldessarini et al., 1994; Andersen et al., 1997). Regardless of the model employed, stimulating D₂ autoreceptors with quinpirole inhibited dopamine synthesis in both age groups (Svensson et al., 1991; Andersen et al., 1997; Heidbreder and Baumann 2001; Der-Ghazarian et al., 2010). A different pattern of effects was apparent with D₂ receptor blockade, because haloperidol only increased DOPA accumulation using the “open-loop” model (Carlsson et al., 1976; Svensson et al., 1991, 1993; Iñiguez et al., 2008), but not when haloperidol was co-administered with γ -butyrolactone (Magnusson et al., 1988; Yoshida et al., 2006; but see Iñiguez et al., 2008). Importantly, the present results clearly show that administering Mn on PD 1–21 did not modify the sensitivity of synthesis-modulating autoreceptors in adolescent or adult rats. The absence of Mn-induced effects was apparent in control and γ -butyrolactone-treated rats that were tested either 7 or 70 days after cessation of Mn

treatment. The inability of early Mn exposure to alter dopamine synthesis was unexpected, because other treatments known to affect dopamine autoreceptor sensitivity (e.g., reserpine, long-term aripiprazole exposure, etc.) alter the functioning of synthesis-modulating autoreceptors (Reches et al., 1985; Der-Ghazarian et al., 2010).

Despite this result, it is well established that Mn affects various presynaptic mechanisms in the dorsal striatum of adult rats. Some of the most significant changes include Mn-induced reductions in dopamine transporter levels (Reichel et al., 2006; McDougall et al., 2008) and persistent declines in dorsal striatal dopamine concentrations (Gianutosos and Murray, 1982; Komura and Sakamoto, 1992; Ingersoll et al., 1995; Tran et al., 2002a, b; Aschner et al., 2005). We previously speculated that these Mn-induced reductions in dopamine levels may, in turn, cause an up-regulation of postsynaptic D₂ receptors in the dorsal striatum and a dysregulation of cAMP and Akt signaling pathways (McDougall et al., 2011). Prolonged changes in dopamine levels are known to impact autoreceptor functioning (Reches et al., 1985; Pucak and Grace, 1991), therefore we also anticipated that early Mn exposure would cause long-term alterations in autoreceptor sensitivity. The expected pattern of results was not obtained, with results from the present study strongly suggesting that early Mn exposure does not affect autoreceptor sensitivity when assessed in early adolescence or adulthood. Interestingly, Cuesta de Di Zio et al. (1995) reported that apomorphine transiently loses its ability to inhibit dopamine release in adult rats given a two-week, but not an eight-week, exposure to Mn. Therefore, it remains possible that acute Mn treatment causes temporary alterations in dopamine autoreceptors, but that autoreceptor functioning returns to normal with more sustained Mn exposure (Cuesta de Di Zio et al., 1995) or after cessation of Mn treatment (present study).

A notable feature of the present results is that the patterns of apomorphine-induced locomotor activity and autoreceptor-mediated dopamine synthesis were nearly identical in adolescent and adult rats. After the discovery of dopamine autoreceptors there was debate about when these receptors become functionally mature. Older neurochemical and behavioral studies suggested that dopamine autoreceptors do not become mature until the fourth or fifth postnatal week (Shalaby and Spear, 1980; Arnt, 1983; Hedner and Lundborg, 1985); however, it is now clear that synthesis- and release-modulating autoreceptors are functional by PD 5 or earlier (De Vries et al., 1992; Andersen and Gazzara, 1993, 1994; Andersen et al., 1997), and that they are capable of mediating adult-like behavioral effects sometime between PD 10 and PD 21 (Lin and Walters, 1994; but see Sobrian et al., 2003). In the present study, the similar autoreceptor profiles of young and adult rats are consistent with the idea that dopamine autoreceptors are functionally mature by early adolescence or before. In contrast, a “postsynaptic” dose of apomorphine (0.6 mg/kg) appeared to stimulate more locomotor activity in adolescent rats than adults. Consistent with these data, Frantz and Van Hartesveldt (1999) reported that the D₂/D₃ agonist quinpirole produces greater behavioral activation during adolescence than in adulthood.

In conclusion, early Mn exposure is characterized by long-term reductions in dopamine transporter levels (McDougall et al., 2008; Reichel et al., 2006) as well as persistent increases in D₁ and D₂ receptor concentrations and protein expression (Kern and Smith, 2011; McDougall et al., 2011). It is uncertain whether dopamine autoreceptor numbers are also elevated after early Mn treatment (dorsal striatal D₂S protein expression is enhanced), but results from the present study strongly suggest that exposing rats to Mn on PD 1–21 does not alter autoreceptor sensitivity when measured during early adolescence or adulthood.

Acknowledgments

This research was supported by NIGMS training grant GM083883 (R.J.L.) and NIDA research training grants DA025319 (AMY) and DA033877 (AMY).

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Fig. 1.

Mean distance traveled scores ($\pm$ SEM) of apomorphine-treated rats during the 60-min behavioral testing session on PD 28 ($n = 8$ per group) and PD 90 ($n = 8-9$ per group). Rats had received oral infusions of vehicle (left panels) or 750 μ g Mn (middle panels) on PD 1–21. The right panels show mean distance traveled scores collapsed across the pretreatment condition.

* Significantly different from same aged rats receiving 0 mg/kg apomorphine (Test Drug main effect, $P < 0.05$).

Fig. 2.

Mean dorsal striatal DOPA accumulation of PD 28 rats treated with saline, quinpirole (0.5 mg/kg), or haloperidol (0.5 mg/kg, i.p.) 25 min before an injection of distilled water (upper graph) or γ -butyrolactone (lower graph) on PD 28 ($n = 7-8$ per group) and PD 91 ($n = 8-9$ per group). Rats had received oral infusions of vehicle or 750 μ g Mn on PD 1–21.

* Significantly different from rats receiving distilled water and saline (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

† Significantly different from rats receiving γ -butyrolactone and saline (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

‡ Significantly different from similarly-treated rats in the distilled water condition (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

Fig. 3.

Mean dorsal striatal DOPA accumulation of PD 91 rats treated with saline, quinpirole (0.5 mg/kg), or haloperidol (0.5 mg/kg, i.p.) 25 min before an injection of distilled water (upper graph) or γ -butyrolactone (lower graph) on PD 28 ($n = 7-8$ per group) and PD 91 ($n = 8-9$ per group). Rats had received oral infusions of vehicle or 750 μ g Mn on PD 1-21.

* Significantly different from rats receiving distilled water and saline (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

† Significantly different from rats receiving γ -butyrolactone and saline (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

‡ Significantly different from similarly-treated rats in the distilled water condition (Test Drug $\times$ Impulse Inhibitor interaction, $P < 0.05$).

Table 1

Mean ($\pm$ S.E.M.) body weights (g) of rats exposed to vehicle ($N=139$) or manganese ($N=137$) on PD 1–21.

Treatment	Postnatal Day (PD)										
	PD 1	PD 3	PD 5	PD 7	PD 9	PD 11	PD 13	PD 15	PD 17	PD 19	PD 21
Vehicle	7.8 ±1	10.4 ±2	14.3 ±3	18.9 ±4	24.0 ±5	29.1 ±6	34.1 ±6	39.1 ±8	44.4 ±8	51.2 ±1.0	61.3 ±1.1
750 µg Mn	8.0 ±1	9.8 ±2 ^a	13.1 ±3 ^a	17.6 ±4 ^a	22.0 ±5 ^a	27.1 ±6 ^a	31.9 ±7 ^a	36.6 ±9 ^a	41.3 ±1.0 ^a	48.1 ±1.1 ^a	57.1 ±1.3 ^a

^aSignificantly different from rats exposed to vehicle.